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Physical Methods in ORGANIC CHEMISTRY

edited by

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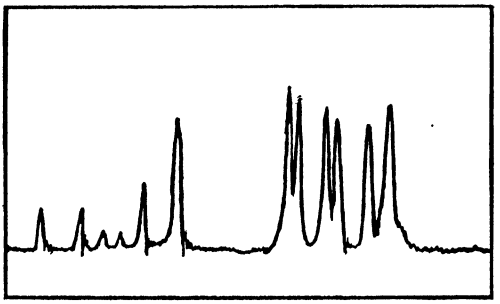

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EDITOR'S PREFACE

Physical methods for the determination of structure now play a vital part in the daily work of the organic chemist. This book is intended to provide an introduction to these methods, primarily for undergraduate and post-graduate students, but also for more senior chemists who wish to assess the relevance of these modern techniques to their own work.

The basic principles are presented in simple terms and the scope of each method is illustrated by examples of structural problems to which it can be applied. Important analytical applications are also mentioned. The aim has been to provide a readable introduction to the various techniques rather than an exhaustive survey of data and applications; references are given to monographs and reviews which provide more detailed information. Methods which are of outstanding importance (particularly infrared spectroscopy) are discussed in greater detail than the others. In the opening chapter the methods are assessed as a whole.

The Oxford University Press and Pergamon Press Ltd. are thanked for providing the line blocks for the Figures on p. 298 and p. 309. Permission to copy diagrams from other sources mentioned in the text is also gratefully acknowledged. Finally the authors and the editor wish to express their special thanks to the many colleagues and students who have stimulated the writing of this book and who have read part or all of the manuscript.

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CONTENTS

		Page
EDITOR'S PREFACE		v
Chapter		
1. INTRODUCTION	*J. C. P. Schwarz*	1
Historical		2
The 'theoretical' and 'empirical' approaches		3
The electromagnetic spectrum		4
A survey of the methods		7
Reference works		19
2. ULTRAVIOLET, VISIBLE AND INFRARED SPECTROSCOPY	*P. Bladon and G. Eglinton*	22
General introduction		22
The origin of light absorption		23
The measurement of light absorption		24
Applications of ultraviolet, visible and infrared spectroscopy		31
3. INFRARED AND RAMAN SPECTROSCOPY ..	*G. Eglinton*	35
Introduction		35
PART I. INFRARED SPECTROSCOPY—THEORY AND PRACTICE		36
Molecular vibrations and their interaction with infrared radiation		36
Instruments and sampling methods		42
The applications of infrared spectroscopy		44
The correlation and interpretation of infrared spectra		48
Factors affecting group frequencies		55
Physical state and group frequencies		57
Intramolecular factors and group frequencies		62
Prediction of approximate values for group frequencies		73

PART II. INFRARED SPECTROSCOPY—EXAMPLES OF APPLICATIONS 78
1. Solvents 80
2. Functional group absorptions (i) In simple alicyclic systems 80
3. Functional group absorptions (ii) In the 5α-cholestane skeleton 84
4. Straight- and branched-chain hydrocarbons 89
5. The gem-dimethyl group and double bond absorptions 90
6. Polynuclear aromatic hydrocarbons 93
7. A reaction study. The photochemistry of verbenone 95
8. Long-chain acids and esters 98
9. Synthetical confirmation of a natural product structure 101
10. Chelate systems 104
11. Ethers, ketals and carbohydrates 106
12. Amines, amine salts and alkaloids 108
13. Carboxylic acid salts and amino-acids 110
14. The total synthesis of griseofulvin 113

PART III. RAMAN SPECTROSCOPY 116
Origin of Raman spectra 116
Apparatus and sampling 120
Applications 121

4. ULTRAVIOLET AND VISIBLE SPECTROSCOPY .. *P. Bladon* 12(
Theoretical aspects 12(
Experimental technique 129
The absorptions of some common structural types 132
Absorption due to carbon-carbon multiple bonds 133
Absorption due to carbonyl groups 138
Absorption of compounds containing nitrogen or sulphur 144
Light absorption of saturated compounds containing heteroatoms 145
Absorption due to aromatic chromophores 146
Applications of ultraviolet and visible spectra 155
The literature of electronic spectra 164

5. MAGNETIC RESONANCE SPECTROSCOPY .. *L. M. Jackman* 168
Introduction 168
The spectroscopic method (N.M.R.) 170
Proton nuclear magnetic resonance 173
The chemical shift 173
Time-dependent effects 178
Types of shielding 179
Spin-spin coupling 183
Examples of applications 193
Electron spin resonance 20

OPTICAL ROTATION .. .. .. *J. C. P. Schwarz* 210
Introduction 210
The measurement of optical rotations 212
The theory of optical rotation 217
The correlation of structure and optical rotation 220
The presence or absence of optical activity 222
Cotton effect curves 223
Circular dichroism 232
Plain curves 234
Rotations at a single wavelength 235
Summary 242

7. THE DETERMINATION OF MOLECULAR WEIGHT .. .. *C. T. Greenwood* 244
Introduction 244
Molecular weights of up to 2,000 248
Molecular weights in the range 2,000–20,000 259
Molecular weights above 20,000 262

. DIFFRACTION METHODS .. .. .. *G. A. Sim* 276
Introduction 276
The crystalline state 278
X-Ray diffraction: experimental procedure 285
X-Ray diffraction: structure analysis 292
X-Ray diffraction: applications 300

9. MASS SPECTROMETRY .. .. .. *R. I. Reed* 306
Theory and operation 306
Applications of isotopic abundance measurements 310
Thermochemistry and cracking patterns 312
Structural applications of mass spectrometry 313

DIPOLE MOMENTS .. .. *B. L. Shaw* 323
Introduction 323
General results 326
Contributions to the electronic theory of organic chemistry 327
Problems in stereochemistry 331
Organometallics 334

INDEX OF COMMON GREEK SYMBOLS 338

INDEX 339

Chapter 1

J. C. P. Schwarz

INTRODUCTION

Methods based on relationships between structural features and physical properties are now making an ever-increasing contribution to organic chemistry, not only in the elucidation of structure, but also in analytical and preparative work. The advantages of these methods are numerous: (i) they can give much more precise information about molecular structure than a classical chemical approach, (ii) the amount of sample required is small and it can generally be recovered, (iii) the information is usually obtained rapidly, often in the form of a permanent record on a chart, (iv) many of the methods provide analytical techniques of high selectivity and sensitivity, invaluable in the analysis of complex mixtures and in the detection of impurities, (v) continuous analysis is often possible, facilitating kinetic work and the automatic control of process variables in industry, (vi) systems can be examined without disturbing their composition, an obvious advantage over chemical analysis in the study of tautomeric, conformational and other equilibria. It is therefore not surprising that the growing availability of physical methods has altered both the scope and pace of organic chemistry. Many recent successes in the determination of the structures of complex natural products would have remained out of reach without them, and their contribution in widening our understanding of the reactivity of organic compounds can hardly be overestimated.

The various physical techniques of greatest interest to the organic chemist are discussed individually in the following chapters. The aim of this introduction is to provide a broad survey of the methods and of their applications, particularly in the determination of the structures of complex molecules, e.g. natural products. The first few pages are devoted to general considerations, and then the salient features of the various methods will be presented, largely in tabular form. Details of the principles and applications of the methods must be sought in the

individual chapters, and cross-references for this purpose are provided.

Historical. Physical properties have always played an important part in the practice of organic chemistry. They form the basis of separation techniques, both classical and modern, and also provide criteria of purity and identity which are indispensable in laboratory work. Furthermore, two physical methods—molecular weight determination and the detection of molecular asymmetry by optical rotation—were of great importance in the early growth of structural theory. Otherwise the classical approach to the elucidation of structure depended almost entirely on the systematic investigation of chemical reactions. Although there were many attempts, even in the nineteenth century, to relate physical properties and structure, these yielded little of interest to the organic chemist; in fact, readily determined properties such as melting point, boiling point, density and refractive index give little direct information about structural features.

During the first three decades of the present century, advances in physics began to make major contributions to the concept of molecular structure. It became apparent that certain physical properties (e.g. spectra, X-ray diffraction, dielectric behaviour, etc.) could provide numerical data about the relative positions of the atoms in a molecule and the nature of the bonds between them. This led to a much more precise picture of molecular structure than could be obtained by purely chemical means and laid the basis of modern theories of reaction mechanisms.

At the same time it was found that some physical properties, particularly absorption spectra, could be related empirically to the presence of certain functional groups, even though the rigorous theoretical basis of the relations was not fully understood. Methods based on these properties began to be used in both structural and analytical work, and by 1930 the fundamentals of most of the techniques discussed in this book were well established. Nevertheless, the impact of these methods on the laboratory practice of organic chemistry was still comparatively small. With the apparatus available at the time, the measurement of physical properties such as absorption spectra was tedious and required considerable skill. For this reason, most of the methods remained in the hands of specialists, who were usually more interested in the fundamental basis of the effects and in the fine structure of simple molecules than in the investigation of the more complex molecules with which the average organic chemist has to deal.

The fact that physical methods now play such an important part in the work of the organic chemist is due only in a minor degree to further

knowledge of their fundamental theory. Advances in instrumentation, many of which were stimulated by military needs during the second world war, were probably the most important factor in establishing physical methods as routine tools. These advances came at a time when the need for new methods of approach was widely felt. Complex natural products (e.g. steroid hormones), often available only in very small quantities, were providing structural problems of a new order of complexity. Developments in the chemical industry underlined the need for rapid and powerful analytical methods. Moreover, an increasing interest in reaction mechanisms and in the interpretation of relative reactivities inevitably drew attention to physical methods of investigation. Organic chemists were therefore quick to take advantage of the new methods which had come within their reach. Economic factors also played a part in these developments; the increasing financial support for scientific research, both academic and industrial, stimulated the instrument manufacturer—a vital link between the physicist and the organic chemist—to undertake the development and production of the costly equipment required for the routine exploitation of many of the physical techniques.

The 'theoretical' and 'empirical' approaches. Many of the physical methods discussed in this book can be considered from two different, though not unrelated, points of view. In the first approach, structural information is obtained by interpreting the experimental data in terms of fundamental physical theories. This 'theoretical approach' can provide detailed information, not only about molecular geometry, but also about electron distributions, polarisabilities, molecular energetics and other aspects of fine structure.[1-3] In general this approach is limited to fairly simple molecules (e.g. ethane, benzene, etc.) and its exploitation belongs to the realms of physical chemistry and physics, although the organic chemist may often profit by the results.

The second approach may be called the 'empirical approach', as it depends on the empirical correlation of certain physical properties with structural features. It is particularly successful with spectroscopic methods, and its general principles are explained and illustrated in Chapter 3 (pp. 48 and 80). It depends on two related assumptions: (i) that the physical characteristic in question can be associated with a particular portion of the molecule, and (ii) that it is more or less unaffected by changes in other parts of the molecule. The utility of a particular physical property thus depends greatly on the extent to which it can be regarded as 'localised'. Highly localised physical properties (e.g. characteristic infrared absorption peaks) can be used to detect the

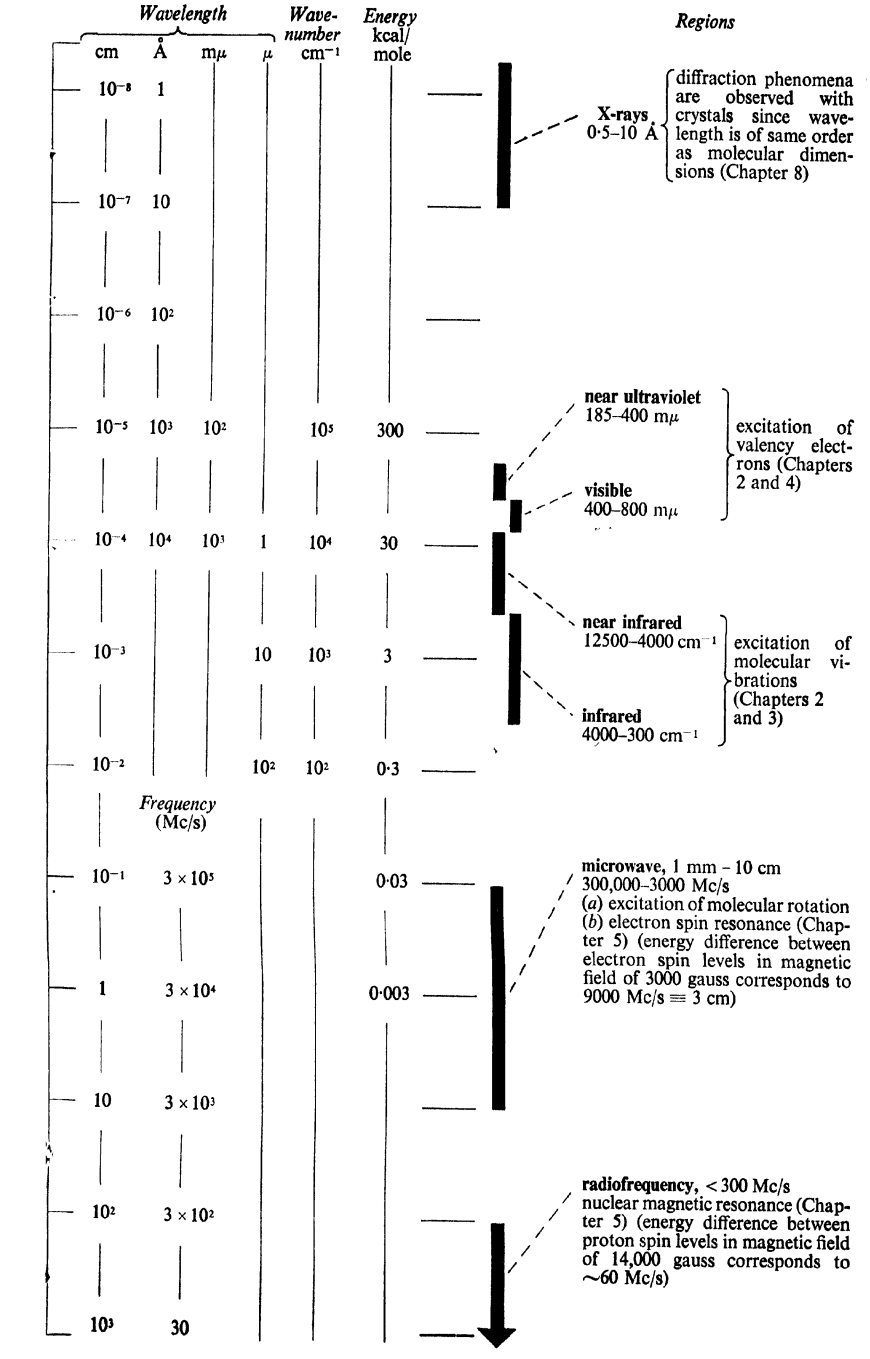
presence of functional groups even in molecules of completely unknown structure. By contrast, methods depending on less localised physical properties (e.g. optical rotation) can seldom be used profitably unless a considerable amount of structural information is already available. (Often they are restricted to the problem of deciding between two or three alternative structures, the physical behaviour of which can be predicted from that of closely analogous compounds; cf. p. 220.)

The application of physical methods to organic chemistry is largely based on the empirical approach because, even for molecules of only moderate complexity, a more fundamental approach is usually impossible. Moreover, the arguments used in the empirical approach are congenial to the organic chemist since they are rather similar to those employed in deducing structure from chemical evidence. The theoretical approach is usually more demanding and specialised; it is therefore unprofitable to adopt it unless otherwise inaccessible information about fine structure is sought. However, even when a largely empirical approach is used, considerations based on fundamental theory often play an important part; they provide a rational background for the collection and qualitative interpretation of empirical data, and frequently lead to useful predictions about the physical behaviour of compounds for which direct analogies are not available. For example, the effect of structural environment on the light absorption characteristics of a group can often be predicted on theoretical grounds (cf. p. 62).

In the diffraction methods (Chapter 8) a simple empirical approach is not possible, and the theoretical evaluation of the results is rather complex and time-consuming. The diffraction methods have therefore remained in the hands of specialists. However, since the structural information given by these methods (particularly by X-ray diffraction) is of great importance to organic chemistry, they have nevertheless been included in this book. Thanks to the development of electronic computers, X-ray diffraction may now provide a quicker route to the structure of a complex organic compound than a chemical approach, especially if the chemistry is complicated by rearrangements or if the material is difficult to obtain in quantity.

The electromagnetic spectrum.† Many of the physical methods which are of greatest value to the organic chemist depend on the interaction of matter with electromagnetic radiation of various wavelengths. The most familiar type of electromagnetic radiation is visible light. Early

† Readers who are familiar with the fundamentals of the absorption and diffraction processes characteristic of the various regions of the electromagnetic spectrum (Fig. 1) can omit this section and proceed to the section beginning on p. 7.


Wavelength
cm
Å
mμ
μ
Wave-number
cm⁻¹
Energy
kcal/mole
Regions
10⁻⁸ 1
10⁻⁷ 10
10⁻⁶ 10²
10⁻⁵ 10³ 10² 10⁵ 300
10⁻⁴ 10⁴ 10³ 1 10⁴ 30
10⁻³ 10 10³ 3
10⁻² 10² 10² 0·3
Frequency (Mc/s)
10⁻¹ 3 × 10⁵ 0·03
1 3 × 10⁴ 0·003
10 3 × 10³
10² 3 × 10²
10³ 30
X-rays 0·5–10 Å
diffraction phenomena are observed with crystals since wavelength is of same order as molecular dimensions (Chapter 8)
near ultraviolet 185–400 mμ
visible 400–800 mμ
excitation of valency electrons (Chapters 2 and 4)
near infrared 12500–4000 cm⁻¹
infrared 4000–300 cm⁻¹
excitation of molecular vibrations (Chapters 2 and 3)
microwave, 1 mm - 10 cm 300,000–3000 Mc/s
(a) excitation of molecular rotation
(b) electron spin resonance (Chapter 5) (energy difference between electron spin levels in magnetic field of 3000 gauss corresponds to 9000 Mc/s ≡ 3 cm)
radiofrequency, < 300 Mc/s
nuclear magnetic resonance (Chapter 5) (energy difference between proton spin levels in magnetic field of 14,000 gauss corresponds to ~60 Mc/s)

in the nineteenth century it was found that the spectrum extends outside the visible region, and the adjacent parts of it were named the ultraviolet and infrared. It is now known that these regions form part of a continuous spectrum embracing microwaves and radiowaves at one end, and X-rays and γ-rays at the other. The boundaries of most of the regions are not rigidly defined; those of greatest interest to the organic chemist are indicated approximately in Fig. 1.

Units. The electromagnetic spectrum covers a very wide range of wavelengths, and different units are therefore used in the different regions. For convenience, the units used in the later chapters are briefly summarised here (see also Fig. 1).

X-rays have wavelengths of the order of 10^{-8} cm and in this region the Ångström (1 Å $= 10^{-8}$ cm) is the preferred unit of wavelength. In the visible and ultraviolet regions, wavelengths are usually expressed either in Ångströms or in millimicrons† (1 mμ $= 10^{-7}$ cm $= 10$ Å). In the infrared region, wavelengths are expressed in microns (1 μ $= 10^{-4}$ cm). An alternative convention, commonly used in the infrared region and less frequently in the ultraviolet and visible, is to give the number of waves per cm. This is known as the *wavenumber* and is expressed in cm^{-1}. It is clear that

$$\text{wavenumber } (cm^{-1}) = \frac{1}{\text{wavelength in cm}} = \frac{10^4}{\text{wavelength in } \mu}$$

A wavelength of 4 μ ($=4\times10^{-4}$ cm) therefore corresponds to a wavenumber of 2500 cm^{-1}.

Many infrared spectroscopists loosely refer to the wavenumber as 'frequency' and represent it by the symbol ν (the symbols $\tilde{\nu}$ or σ are preferable, but less common among organic chemists). The term 'frequency' and the symbol ν are therefore used in this sense in Chapters 2 and 3. The true frequency, also represented by ν, is given by

$$\text{frequency} = \frac{\text{velocity of light}}{\text{wavelength}}$$

and is preferred in microwave and radiofrequency spectroscopy (see Chapter 5), the usual unit being megacycles per second (1 Mc/s $= 10^6$ cycles per second). A wavelength of 1 cm (in vacuo) corresponds to a frequency of *ca.* 30,000 Mc/s.

Types of interaction. Interaction of radiation with matter may occur in a number of ways. X-ray diffraction (see Chapter 8) depends on

† The term 'nanometer' (1 nm = 1 mμ) is also used, particularly in the German literature.

elastic scattering of the radiation—there is usually no permanent transfer of energy between the radiation and the molecules. Many of the other methods are based on interactions which involve an exchange of energy. The molecule may either emit radiant energy at the expense of its internal energy, or it may absorb radiant energy, being promoted to an excited state. The spectroscopic methods of greatest interest to the organic chemist all depend on the latter process.

According to the quantum theory, the energy content of a molecule cannot change continuously, but is confined to certain discrete values (*energy levels*). Furthermore, a molecule will absorb radiation only when the (true) frequency (ν) of the radiation is related to the energy difference (ΔE) between two energy levels by the equation $\Delta E = h\nu$, where h is Planck's constant. Since the relative positions of the energy levels depend characteristically on the molecular structure, absorption spectra (i.e. observations on the intensity with which a compound absorbs at various frequencies) provide subtle tools for structural investigation.

A molecule can become excited in a variety of ways, corresponding to absorption in different regions of the spectrum. The various processes and the approximate energy differences involved are summarised in Fig. 1; details may be found in the individual chapters. The practical applications of the various absorption methods and of the other physical techniques are compared and contrasted below. Rotational spectra (see Fig. 1) are not considered further; although they provide an accurate method for the determination of the geometry of simple molecules, they are of little direct interest to organic chemists. Quantised absorption of X-rays can occur and involves the excitation of electrons in the inner shells of atoms, but this process is also of little significance in the determination of organic structures (see however p. 304).

A survey of the methods. The effective and economic use of physical tools in organic chemistry clearly depends on an appreciation of the theoretical and practical capabilities and limitations of the various methods. The following chapters are intended to provide a basis on which such an appreciation can be built. Unfortunately, since the methods differ widely both in principle and practice, it is difficult to discuss them as a whole in a systematic way; indeed even the sequence in which the various methods are presented in this book is determined more by their relative importance than by any more logical physical or chemical criteria. Nevertheless, an attempt to survey some outstanding features will now be made.

Factors which determine the choice of the methods to be used in any particular problem are (i) the information required and the type and complexity of the compound under investigation, (ii) the amount of relevant empirical data already in the literature and/or the availability of relevant reference compounds, (iii) the quantity of sample available, (iv) certain physical characteristics of the sample, e.g. its solubility in the permissible solvents, (v) the importance of the problem, i.e. the amount of time and effort which it justifies and (vi) the accessibility and cost of the apparatus required. A full appreciation of these factors

TABLE 1. A survey of

1. *Method*	2. *Principle*	3. *Time required*†‡	4. *Approximate amount of sample*	5. *State of sample*‡
INFRARED SPECTROSCOPY (Chapters 2 and 3; see Tables 1, 2 and 3, Chapter 2 for comparison with ultraviolet spectroscopy)	quantised absorption of infrared radiation leading to vibrational excitation of molecules (pp. 23 and 36)	**mins, hrs**	1–10 mg	solid (usually mull), liquid film, dilute solution in suitable solvent (not, in general, water), alkali halide disc or *vapour* (see p. 42)
RAMAN SPECTROSCOPY (Chapter 3, p. 116)	scattering of visible or ultraviolet light with quantised abstraction of *some* of the energy, leading to vibrational excitation of molecules (p. 116)	mins, **hrs**	50 mg–10 g	fairly concentrated solution, clear and colourless (p. 120)

† Time required for experimental observations (including preparation of solutions, etc.) on one compound and for their immediate interpretation (see p. 16); mins = 5 minutes to 1 hour, hrs = 1 hour to 1 day, days = 1 day to 1 month, months = 1 month to 1 year, years = more than one year.

‡ Bold type signifies 'much more common', italic type means 'relatively rare'.

necessitates a basic knowledge of the individual methods (see the following chapters) and some practical experience in their use. However, it is hoped that Table 1, which summarises the salient features of the methods, will prove useful as a preliminary guide. Cross-references to the following chapters are provided for many of the entries and the Table can be used later for revision purposes and to gain perspective. It must be emphasised that the Table does not cover every circumstance and should be regarded only as a general guide; many of the statements are not without exceptions.

[*discussion of Table 1 continued on p. 16*

the physical methods

6. *Approximate cost of apparatus*	7. *Availability*§	8. *Information obtained and applications*
£1,500–5,000	bench, **service**	Presence and environment of functional groups, esp. those containing X – H bonds (e.g. O – H) or multiple bonds (e.g. C=O) (see p. 38 *et seq.* and p. 48 *et seq.*). Diagnosis of finer structural detail, e.g. conformation (p. 70), intramolecular hydrogen bonding (pp. 66, 76 and 105), *cis-trans* isomerism (pp. 72 and 101), etc. Analytical applications include qualitative and quantitative analysis (e.g. pp. 32, 45 and 46), investigation of reaction rates and equilibria (including intermolecular hydrogen bonding, p. 61), examination of crude reaction products (p. 95), detection of impurities (p. 45), industrial production control (p. 46), etc.
£2,500 (photographic)–6,000 (photoelectric) –18,000	specialist, *service*	As for infrared but less widely applicable, mainly because of sampling restrictions. However, aqueous solutions can be used (not easy in infrared). Functional groups which have weak infrared absorption often give strong Raman spectra and vice versa (p. 119)

§ Bench = apparatus suitable for operation after only brief instruction is widely available, **service** = available as a service in most laboratories, service = available as a service in many laboratories, *service* = available as a service in relatively few laboratories, specialist = to a large extent in the hands of specialists.

TABLE 1. A survey of

1. *Method*	2. *Principle*	3. *Time required*†‡	4. *Approximate amount of sample*	5. *State of sample*‡
ULTRAVIOLET AND VISIBLE SPECTROSCOPY (Chapters 2 and 4; see Tables 1, 2 and 3, Chapter 2 for comparison with infrared spectroscopy)	quantised absorption of ultraviolet or visible radiation leading to electronic excitation (pp. 23 and 126)	**mins,** hrs	0·1–10 mg	dilute solution in transparent solvent (p. 130)
NUCLEAR MAGNETIC RESONANCE (Chapter 5)	quantised absorption of radiation giving rise to transitions between different spin orientations of nuclei (commonly protons) in a magnetic field (p. 168)	mins, hrs, *days* (for analysis of complex spin-spin coupling)	*ca.* 0·5 ml of 0·1–1M soln., i.e. *ca.* 5–100 mg	fairly concentrated solution, commonly in CCl_4, $CDCl_3$ or D_2O
ELECTRON SPIN RESONANCE (electron paramagnetic resonance) (Chapter 5, pp. 168 and 203)	quantised absorption of radiation giving rise to transitions between opposite spin orientations of unpaired electrons in a magnetic field (pp. 168 and 203)	mins, hrs, days (for analysis of complex spectra)	10^{-11} mole of radicals detectable, but larger quantity required for structural studies	solution or solid
OPTICAL ROTATION (Chapter 6) (*a*) at a single wavelength, e.g. sodium D line	rotation of the plane of plane polarised light by asymmetric molecules in solution (theory is complex, p. 217)	mins	1–200 mg (depending on apparatus and specific rotation)	solution

the physical methods—*cont.*

6. *Approximate cost of apparatus*	7. *Availability*§	8. *Information obtained and applications*
£700 (non-recording)–1,500 (recording)–5,000	bench, service	Presence and nature of unsaturation, particularly conjugated double bonds (p. 133 *et seq.*) and aromatic rings (p. 146 *et seq.*). Stereochemistry of conjugated systems (pp. 135 and 159). Detection of certain (relatively few) functional groups, e.g. ketonic or aldehydic C=O (pp. 138 and 155). Analytical applications: similar to infrared, except that (*a*) restricted mainly to conjugated and aromatic compounds (*b*) aqueous solutions can be used (*c*) often more sensitive, i.e. very dilute solutions can be examined. (pp. 32, 152 and 160)
£10,000–20,000	service, specialist	Environment of nuclei (esp. protons), hence unambiguous detection of certain functional groups (p. 193) and information about the environment of many others (p. 174 *et seq.*). Counting of protons of various types in a molecule (p. 173). Determination of proton sequences (e.g. p. 195) and hence of relative points of attachment of functional groups. Assignment of configuration and conformation (p. 191). Can be used for quantitative analysis including study of reaction rates and equilibria, but requires more material than u.v. or i.r. spectroscopy—however sometimes applicable when these fail (an advantage is that the intensity of the signal from a nucleus is independent of its molecular environment). Study of fast reactions (pp. 178 and 191) and of hydrogen bonding (pp. 178 and 196).
£5,000–10,000 (depends on resolution)	specialist	Detection and estimation of free radicals and diagnosis of their structure and electron distribution (p. 205). Applications include study of free radicals in irradiation products and in complex biological material. Organometallic compounds with unpaired electrons can also be studied.
£150 (visual)–500 (photoelectric)–1,500	bench, *service*	Determination of relative and absolute configurations of asymmetric centres (p. 236). Location of functional groups in certain types of compound (e.g. steroids, p. 240). Information about conformation. Criterion of purity and identity esp. valuable when m.p. and spectra are indefinite (e.g. carbohydrates).

TABLE 1. A survey of

1. *Method*	2. *Principle*	3. *Time Required*†‡	4. *Approximate amount of sample*	5. *State of sample*‡
OPTICAL ROTATION (Chapter 6)—*cont.* (*b*) rotatory dispersion	variation of optical rotation (see above) with wavelength	mins, hrs	0·5–20 mg	solution (in solvent transparent over the required wavelength region)
CIRCULAR DICHROISM (Chapter 6, p. 232)	difference in intensity of absorption of right- and left-circularly polarised light (shown only if absorbing function is in asymmetric environment, p. 218)	mins, hrs	1–15 mg	solution (in transparent solvent)
X-RAY DIFFRACTION (Chapter 8)	interference between scattered X-rays (scattering caused by electrons of atoms)	months–years (structure analysis), hrs–days (M. Wt. detn. or powder photograph)	1–10 mg	**single crystal** (structure analysis, M. Wt. detn.), crystalline powder (identification only, p. 290), *orientated fibre* (p. 289)
ELECTRON DIFFRACTION (Chapter 8)	interference between scattered electron beam 'wavelets' (scattering caused by electrostatic field—depends on distribution of both nuclei and electrons)	months	~1–10 mg	**vapour,** very thin single crystal

the physical methods—*cont.*

6. *Approximate cost of apparatus*	7. *Availability*§	8. *Information obtained and applications*
£2,000–10,000	specialist, *service*	Same as (*a*) but more powerful method, esp. when rotation can be measured at wavelengths at which one of the functional groups absorbs; this is usually possible for ketonic carbonyl groups (p. 223) and certain other groups (p. 231). Sensitive method for detection of these groups. Conformation of macromolecules (p. 235). The 'Octant Rule' (p. 228) greatly facilitates determination of configuration and conformation of substituted cyclohexanones (e.g. steroids, terpenes).
£2,000–10,000	specialist (at present)	Applications similar to those of optical rotation, see (*a*) and (*b*) above; even more powerful than rotatory dispersion (p. 233). However, unlike optical rotation it is restricted to compounds which absorb at wavelengths longer than ~220 mμ (ketonic carbonyl is suitable absorbing function; for many other functions the dichroism may not be measurable even though the absorption is strong).
£2,000–10,000 and access to electronic computer	specialist	Determination of complete molecular structure and stereochemistry (but location of hydrogen atoms is difficult); such 'structure analysis' gives bond lengths and angles, as well as distances between non-bonded atoms in crystals (p. 300). Presence of atom of high atomic number generally desirable for structure analysis (p. 297). Molecular weight determination (p. 291). Investigation of fibres (p. 289). Powder photographs (p. 290) useful for identification purposes, esp. when i.r. and u.v. spectra are not characteristic (e.g. for di- and tri-saccharides).
£2,000–10,000 and access to electronic computer	specialist	Determination of complete molecular structure (bond lengths and angles) of fairly simple molecules. Indicates preferred conformation in gas phase (p. 277). Location of hydrogen atoms easier than with X-ray diffraction.

TABLE 1. A survey of

1. *Method*	2. *Principle*	3. *Time required*†‡	4. *Approximate amount of sample*	5. *State of sample*‡
NEUTRON DIFFRACTION (Chapter 8)	interference between scattered neutron beam 'wavelets' (scattering caused by atomic nuclei)	**months**–years	0·1–1 g	large single crystal
MOLECULAR WEIGHT DETERMINATION (Chapter 7)	various (see Chapter 7)	*mins*, **hrs**, days (esp. for polymers)	2–20 mg for micro methods, more for polymers, less for mass spectrometry	**solution**, vapour (pp. 248 and 255), crystalline solid (p. 254)
MASS SPECTROMETRY (Chapter 9)	determination of the mass/charge ratio and relative abundance of the ions (molecular and fragment) formed on electron bombardment of the sample (pp. 306 and 312)	mins, hrs	10 μg (!)–1 mg	vapour (compounds must usually be volatile without decomposition below ~350°)
DIPOLE MOMENTS (Chapter 10)	determination of the permanent electric polarisation (charge separation) of the molecule, generally by a combination of dielectric constant, refractivity and density measurements	hrs	1–100 mg depending on size of moment	**solution** (in non-polar solvent) or vapour

the physical methods—*cont.*

6. *Approximate cost of apparatus*	7. *Availability*§	8. *Information obtained and applications*
requires access to atomic pile and to electronic computer	specialist	Particularly useful for the location of hydrogen atoms in a molecule.
£1 (Rast)–7,000 (ultracentrifuge)	varies, often specialist (esp. for high polymers)	Required for determination of molecular formulae. Vital for characterisation of polymers; useful for characterisation of certain industrial materials (e.g. petroleum fractions).
£1,500 ($M < 100$)–15,000–25,000 (double focusing)	specialist, service	Accurate molecular weight determination (p. 315). Determination of molecular formulae (p. 315). Elucidation of structure (functional groups, length of side chains, nature of skeleton, etc., p. 317; in favourable cases complete structure can be deduced). Isotopic abundance measurements (tracer studies, isotope dilution analysis, p. 310). Quantitative analysis (including detection of impurities, p. 313). Ionisation potentials and bond dissociation energies (p. 312).
~£400	specialist	Demonstration and measurement of electron displacements (e.g. inductive and mesomeric effects, p. 327). Configuration and conformation of fairly simple molecules containing more than one polar bond (e.g. geometrical isomers, dihalides, haloketones, esters, pp. 327 and 332). Especially valuable for organometallic compounds (p. 334).

The principles on which the methods are based (Column 2) are discussed in detail in the individual chapters, and the summaries of applications (right-hand pages) are also best read in conjunction with the later text. The remaining columns deal with practical considerations —these may be all-important in determining the choice of method. Thus Column 3 indicates the approximate time usually required for experimental observations on one compound and their immediate interpretation (the recognition of the relevance of a particular result within the framework of a larger problem may of course take longer). The time factor depends to a great extent both on the type of problem and on the degree of sophistication of the apparatus; bold type indicates that a particular time requirement is much more common than the others and italics distinguish one which is relatively rare. The amount of sample material required also depends on the type of problem and the apparatus. The quantities given in Column 4 are only a rough guide—in some cases, particularly if micro-attachments are available, smaller amounts may be sufficient; in others, the requirements may be greater.

Column 5 gives the physical states in which the sample can be presented to the apparatus.† Certain limitations are obvious from this column, e.g. nuclear magnetic resonance cannot be used in the manner discussed in Chapter 5 if the sample is insufficiently soluble in the permissible solvents, and X-ray structure analysis is excluded unless satisfactory single crystals can be obtained.

The cost (Column 6) generally increases with the accuracy, flexibility and degree of automation of the apparatus; the price ranges given are only approximate and are subject to inflation. Column 7 indicates the extent to which the method is available in organic chemical laboratories. 'Bench' signifies that apparatus suitable for operation after only brief instruction is widely available; the use of such instruments is often included in the teaching for degree courses. More elaborate and expensive apparatus usually requires trained technicians for routine operation; this is indicated by **'service'**, 'service' or *'service'* depending on whether the apparatus is available in most laboratories, in many laboratories or only in a few. The entry 'specialist' indicates that the method is to a *large* extent in the hands of specialists, either because the instrumentation or the interpretation of the results is particularly complicated or because the method is rather limited in scope. Fortunately, specialists

† This column applies only to the more usual applications in organic chemistry. Physical chemists often examine samples in other states; for example, the nuclear magnetic resonance and ultraviolet absorption of *solids* and the X-ray diffraction behaviour of *liquids* can give important, though specialised, information.

usually have a missionary interest in the application of their method and are therefore remarkably patient with organic chemists who approach them with a worthwhile problem.

The relevance of the various physical methods to some typical chemical problems is summarised in a rather different way in Table 2. This supplements and sometimes duplicates Table 1, and is subject to the same reservations. However, it is hoped that it may prove useful both as a general guide and as an index. A rough indication of the relative significance of the methods listed for each application is given by the use of different type (see heading).

TABLE 2. Applications of the physical methods

Problem	*Methods applicable* (bold type = convenient and very widely applicable; ordinary type = often applicable; italic type = less frequently used; ir = infrared spectroscopy, uv = ultraviolet and visible spectroscopy, nmr = nuclear magnetic resonance, Raman = Raman spectroscopy)
Detection of functional groups	**ir** (p. 48 *et seq.*), **nmr** (p. 193), uv (p. 155), mass spectrometry (p. 317), Raman (p. 121), *rotatory dispersion* (p. 139).
Structural environment of functional groups (i.e. position of group with respect to other functional groups, branch points, rings or unsaturation; see also next entry)	**nmr** (p. 174 *et seq.*), **ir** (p. 48 *et seq.*), **uv** (particularly for elucidation of extent of conjugation, p. 132), mass spectrometry (p. 317), Raman (p. 121), rotatory dispersion.
Location of functional groups in steroid, triterpene or similar skeleton	**ir, optical rotation** (esp. rotatory dispersion, p. 226; also circular dichroism), nmr, Raman, mass spectrometry.
Configuration about double bonds (geometrical isomerism)	**nmr** (p. 191), **ir** (p. 72), Raman, *uv* (p. 160), *dipole moments* (small polar molecules only).
Absolute configuration of asymmetric molecules	**optical rotation** (esp. rotatory dispersion, p. 220; also circular dichroism), *X-ray diffraction* (p. 303).
Relative configuration of two or more asymmetric centres in a single molecule (e.g. *erythro* or *threo* ?, *cis* or *trans* ring junction ?)	**optical rotation** (esp. rotatory dispersion, p. 220; also circular dichroism), **nmr**, *ir* and *Raman, dipole moments* (simple polar molecules).

Table 2—*cont.*

Problem	*Methods applicable*
Conformational problems	**nmr, optical rotation** (esp. rotatory dispersion, pp. 229 and 239; also circular dichroism), **X-ray diffraction** (solid state conformation only), **electron diffraction** (small molecules in gas phase, p. 277), dipole moments (simple polar molecules, p. 332), ir and Raman (e.g. p. 70), *uv* (p. 138).
Complete molecular structure, including bond lengths and angles (embracing all of the above problems except, in general, absolute configuration)	**X-ray diffraction,** *electron diffraction* (small molecules only).
Study of electronic effects (e.g. inductive and mesomeric electron displacements, etc.)	**dipole moments** (simple molecules only, p. 327), nmr (p. 179), ir and Raman (e.g. pp. 42 and 68), uv (p. 150), *mass spectrometry* (p. 317).
Criterion of purity	**ir** (p. 45), **uv, mass spectrometry** (p. 313), optical rotation (including rotatory dispersion), molecular weight (indispensable for characterisation of polymers).
Identification by direct comparison or by reference to literature compilations	**ir** (p. 45), mass spectrometry (p. 313), X-ray diffraction (powder photograph, p. 290), nmr, optical rotation (including rotatory dispersion), uv.
Quantitative analysis (including laboratory and industrial control of reactions, kinetic studies and investigations of equilibria, e.g. tautomerism and ionisation)	**uv** (e.g. pp. 32 and 160), **ir** (e.g. pp. 32 and 45), nmr, optical rotation (e.g. p. 216), mass spectrometry (gas phase only, p. 313), *Raman* (p. 121).
Investigation of hydrogen-bonding	**ir** (pp. 61 and 66), **nmr** (p. 178), Raman, neutron diffraction (location of H-atom, in crystal), *uv*.

Two general features, apparent from these Tables, deserve special comment. In the first place, it is abundantly clear that the methods differ greatly in scope and significance. Infrared spectroscopy is probably the most important. It can be applied profitably at nearly all stages of an investigation, e.g. as a guide in synthesis and purification, in the recognition of gross structural features and in the diagnosis of finer details. Ultraviolet and visible spectroscopy and nuclear magnetic resonance are also of great general importance, though the use of the latter is somewhat restricted by the complexity and expense of the apparatus and by the rather more rigid sample requirements. Together

with the methods for the measurement of molecular weights, these three methods are the chief routine tools of the organic chemist. In work on natural products they are supplemented by optical rotation measurements; these are invaluable both as a criterion of purity and for the investigation of stereochemical features. The routine application of mass spectrometry is at present restricted to a few fortunate laboratories, but in the future this technique will probably be used to a much greater extent. The other methods are more specialised.

Secondly, it may be seen that the information obtained by the various methods is often complementary. For example, infrared spectroscopy is valuable for the detection of functional groups but less useful in the investigation of conjugation, while the reverse is true of ultraviolet and visible spectroscopy. The different methods are therefore best used in conjunction; often the results obtained by one technique can be interpreted profitably only in the light of information obtained by the others or by chemical means. The independent confirmation which may be obtained when a particular structural feature can be detected by more than one method is also valuable. In many cases, physical evidence about structure is suggestive rather than conclusive—chemical evidence, together with the physical examination of transformation products, is therefore usually essential and always desirable.

The joint use of physical and chemical methods is illustrated by some of the examples in the later chapters (e.g. pp. 96, 195 and 200) and further examples may be found in refs. 4–13; some of these are quite complex and they are best consulted after the reader has become familiar with the individual methods. Attention is also drawn to a valuable book[25] which provides an extensive collection of examples and problems illustrating the use of infrared, ultraviolet and mass spectrometry and nuclear magnetic resonance.

Reference works. References to monographs which may be consulted for further information about the individual methods will be found in the later chapters. In addition, there are general reference works (often expensive) which survey a number of the methods. These are quoted in refs. 14–26, usually with some indication as to their content.

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Chapter 2

P. Bladon and G. Eglinton

ULTRAVIOLET, VISIBLE AND INFRARED SPECTROSCOPY

General Introduction

The main purpose of this chapter is to outline the common features of the ultraviolet, visible and infrared spectroscopic methods as applied to organic chemistry (detailed discussions of the individual methods are given in chapters 3 and 4). The spectral regions† covered (see p. 5) are 185–400 mμ (ultraviolet), 400–800 mμ (visible) and 800 mμ (0·8 μ) to 30 μ (infrared). Serious experimental difficulties preclude routine measurements at wavelengths shorter than 185 mμ and longer than 30 μ.

The absorption in the visible spectrum which is responsible for the colour of certain organic compounds, for example chlorophyll, haemoglobin and dyestuffs, has been widely used for purposes of identification and estimation since the beginning of this century when simple spectroscopes and colorimeters were first developed. Work in the visible region was extended into the ultraviolet when quartz spectrographs, in which the spectrum is recorded photographically, became available. In the 1920's and 1930's, quartz spectrographs were much used in several fields of organic chemistry, notably in connection with Vitamins A and D. During World War II, the first photoelectric spectrophotometers began to appear (Cary and Beckman). The commercial production of the Beckmann DU and similar instruments (e.g. Unicam SP 500, Hilger Uvispek) resulted in the widespread use of ultraviolet and visible spectrometry. Such instruments are manually

† The units of wavelength are *microns* (μ), *millimicrons* (mμ) and Ångströms (Å); 10,000 Å = 1000 mμ = 1 μ = 10^{-3} mm. When absorptions are reported as 'frequencies', the units are of wavenumber (ν), measured in waves per cm (*reciprocal centimetres*, cm^{-1}). ν (cm^{-1}) $\equiv 10^4/\lambda$ (in μ) e.g. 5 $\mu \equiv 2000$ cm^{-1}. The term 'light' is used for the whole region, 185 mμ to 30 μ, in chapters 2, 3 and 4.

operated (i.e. spectra are obtained by the measurement of dial readings) and further developments have concentrated on providing recording spectrophotometers, in which the spectra are continuously recorded on paper charts.

Infrared spectroscopy has had a rather different history. A great deal of fundamental work was done about 1905 by the American, Coblentz, using a non-recording spectrometer, but his work went largely unnoticed. It was only during the decade 1940–50 that recording instruments were commercially developed. These instruments eliminated the extremely tedious point-by-point plotting previously necessary for the determination of the numerous peaks of the typical infrared spectrum, e.g. Fig. 3, p. 28. (Ultraviolet and visible spectra are generally much less complicated, e.g. Fig. 4, p. 158.) Progress has since been very rapid.

The Origin of Light Absorption[1]

When light is absorbed by a molecule, the energy is utilised in one or more of three main ways:

(1) In raising the electrons forming the chemical bonds to higher energy levels (*excited states*). This *electronic excitation* requires quite high energy quanta (*ca.* 100 kcal./mole) and thus corresponds to the absorption of light of relatively short wavelength (high frequency)—i.e. in the ultraviolet and visible regions.

(2) In increasing the *vibrational energy* of the chemical bonds. The energies which are required to increase the amplitude of vibration of the atomic nuclei in the bonds are of the order 5 kcal./mole and are provided by radiation in the near infrared (0·8–2 μ, 12,500–5000 cm^{-1}) and infrared regions (2–30 μ, 5000–333 cm^{-1}).

(3) In increasing the *rotational energy* of the molecule as a whole (and of parts of it about the chemical bonds). Here the energy quanta are very small (*ca.* 0·001 kcal./mole); the radiation is of long wavelength (low frequency) and is in the far-infrared and microwave regions (30–10,000 μ, 333–1 cm^{-1}). Pure rotational spectra are difficult to observe; they have little practical application in organic chemistry, and are not discussed further.

The ultraviolet and visible spectra of most molecules in solution consist of very broad absorption bands (e.g. Fig. 4, p. 158). For any electronic state of a polyatomic molecule, there is a large number of vibrational states, each of which can be further subdivided into rotational levels (see p. 128). Transitions between two electronic levels consequently give rise to a family of lines which are so closely spaced

that they generally merge into a broad band. In some cases vibrational 'fine structure' may remain discernible (Fig. 1, p. 128) but more often a single smooth band is obtained, especially in solution where the lines are broadened by intermolecular effects. For most molecules, the number of electronic transitions which give rise to absorption in the experimentally accessible region is very small and consequently the typical ultraviolet or visible spectrum consists of a few, broad bands.

Transitions between two vibrational levels (infrared spectra) would also be expected to give rise to a family of lines, since a number of rotational sub-levels are available for each vibrational level. Rotational 'fine structure' is in fact observed in the infrared spectra of simple molecules in the gas phase. However, in solution the fine-structure is merged into a smooth-contoured vibrational-rotational band (Fig. 1, p. 36) which is, of course, much narrower and sharper than the electronic-vibrational-rotational bands of the ultraviolet or visible regions. Many different vibrations are possible in a polyatomic molecule and consequently the complete infrared spectrum consists of a large number of comparatively sharp absorption peaks (Fig. 3, p. 28).

The Measurement of Light Absorption[2]

The Laws. There are two laws which describe the absorption of light by matter. *Lambert's law* states that: The proportion of monochromatic light absorbed by a homogeneous medium is independent of the intensity of the incident light, and each successive unit layer absorbs an equal fraction of the light incident upon it. *Beer's law* states that: The fraction of the incident light absorbed by a solute in a transparent solvent depends only on the quantity of solute in the light path, i.e. it is independent of the concentration, provided that the path length is adjusted so that the total quantity of solute in the light path remains the same. Combined, these lead to the expression

$$E = \log_{10}(I_0/I_t) = kcl$$

where E is the optical density (absorbance) of the solution.†

I_0 „ intensity of the incident radiation.
I_t „ „ „ transmitted „
c „ concentration of the absorbing substance.
l „ path length of the solution.
k „ extinction coefficient of the absorbing substance (a constant).

† The Joint Committee on Nomenclature in Applied Spectroscopy has published the following recommended nomenclature: Absorbance (A) instead of optical density (E), molar absorptivity (ε) instead of molar extinction coefficient (ε), absorptivity (a) instead of extinction coefficient (k). See *Analyt. Chem.*, 1952, **24**, 1349.

In practice, the quantities actually measured are the relative intensities of the radiation beam transmitted by a cell full of pure solvent, and by an identical cell full of the solution. When these intensities are taken as I_0 and I respectively (Fig. 1), the resulting optical density is that of the dissolved solute only. In manual (ultraviolet)

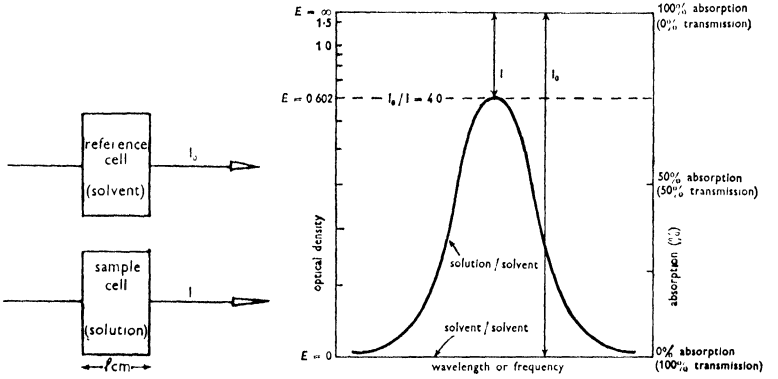


FIG. 1. Measurement of optical density, *E*. The right-hand diagram represents an absorption band as recorded by a double-beam spectrophotometer (p. 29). $E = \log_{10}(I_0/I)$.

single-beam spectrophotometers (see p. 29) the optical density scale is first 'zeroed' with the solvent blank alone. The solvent blank is then replaced by the cell containing the solution and the optical density read off. Recording instruments (mainly infrared) frequently provide only a linear scale of *percent transmission* [$100\ I/I_0$; 'transmittance'] or *percent absorption* [$100\ (1-I/I_0)$].

The extinction coefficient, k, at a given wavelength is a *constant*, characteristic of the substance under examination (it may show some dependence on the solvent and temperature). When l is expressed in centimetres and the concentration as a percentage, k is called $E\ {}^{1\%}_{1cm}$ ('ee one one'; the optical density for a 1% w/v solution in a 1 cm cell). When the molecular weight is known, the concentration can be expressed in g.moles/litre and k is then called the *molar extinction coefficient*, ε (epsilon; optical density for a molar solution in a 1 cm cell). It follows that:

$$\varepsilon = \frac{E \times \text{M.Wt.}}{c \times l} \quad [c \text{ in g/litre (or mg/ml)}, l \text{ in cm}].$$

The salient features of the spectrum of a substance are the position and intensity of the absorption maxima (and minima). These are conveniently reported in the forms:

λ_{max} (in EtOH) 275 mμ (ε 4700), i.e. the substance, in ethanol solution, has a maximum absorption of 4700 molar extinction units (litre mole^{-1} cm^{-1}) at a wavelength of 275 mμ in the ultraviolet region;
ν_{max} (in CCl_4) 1666s cm^{-1}, i.e. there is an infrared absorption maximum (strong) at a 'frequency' of 1666 waves per cm [alternatively, λ_{max} (in CCl_4) 6·0 μ (6 microns)].

The Lambert-Beer law does not hold exactly. Thus, any self-association of the solute molecules may make the extinction coefficient dependent on concentration ('true deviation' from Beer's law). Further, infrared, but not ultraviolet, spectrometers pass a rather wide range of wavelengths in comparison with the widths of the usual absorption bands, and the extinction coefficients measured with them are therefore somewhat dependent on slit width and other instrument conditions ('apparent deviation'). Molar extinction coefficients for infrared bands are hence regarded as 'apparent' and are designated ε^a.

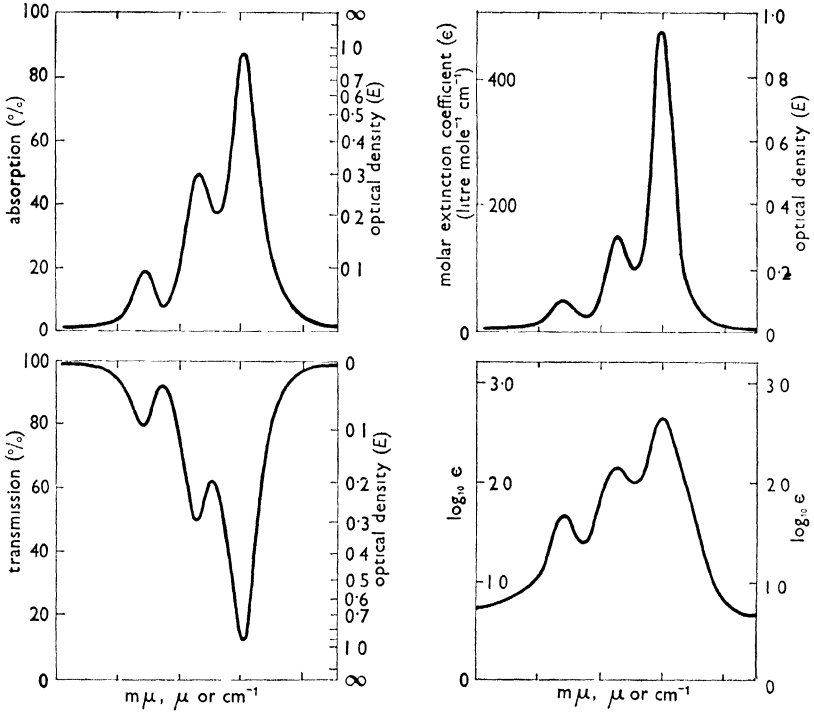


FIG. 2. Different presentations for the absorption curve of a substance (substance of molecular weight 200 at a concentration of 8·0 g/litre in a cell of path length 0·05 cm)

Presentation of Spectra. Spectra are generally presented as plots of the intensity of absorption of the substance (% transmission, % absorption, optical density, ε or log ε) on the vertical (ordinate) scale, against the wavelength (Å, mμ, or μ) or 'frequency' (cm^{-1}) of the light on the horizontal (abscissa) scale (plotted short to long wavelength from left to right). Fig. 2 illustrates the effect of the various intensity presentations. The linear optical density or ε plot gives the clearest impression of the relative intensities of the peaks. $\text{Log}_{10}\,\varepsilon$ is often used for ultraviolet spectra when peaks of widely differing intensities must be shown on the same diagram.

The recommended[3] presentation (% transmission and linear cm^{-1}) for automatically recorded infrared spectra is as shown in Fig. 3. The expansion of the scale below 2000 cm^{-1} prevents undue crowding of the many bands in that region; viewed in another way, the contraction of the scale above 2000 means that the comparatively empty, though significant, region 2000–5000 cm^{-1} does not occupy an excessive length of the chart. However, it is slightly easier to design instruments with a linear wavelength scale (μ) and the spectra 1 to 52, given in Chapter 3, have the latter scale. Many ultraviolet spectra are obtained by manual plotting of optical density readings and are commonly presented as plots of optical density or ε against wavelength (mμ, not Å, since 1 mμ is about the limit of accuracy).

Design of spectrophotometers.[2] A spectrophotometer† consists of a radiation source, a means of dispersing the radiation into its component wavelengths (the monochromator) and a detector. Most modern instruments, especially in the infrared field, have the additional feature of automatically recording the spectrum on a paper chart. Typical individual components are given in Table 1.

TABLE 1. Components of spectrophotometers

Component	*Ultraviolet and Visible*	*Infrared*
Source	185–350 mμ: hydrogen discharge lamp 320–1000 mμ: ordinary tungsten lamp	Glowing ceramic rod (approx. 1700°C, e.g. Nernst filament)
Monochromator	Silica prism (glass usable in certain regions) or diffraction grating Mirrors, silica lenses and windows	NaCl prism (LiF, CaF_2 and KBr in some regions) or diffraction grating Mirrors and NaCl windows
Detector	Photoelectric cell or photomultiplier	Thermocouple, bolometer or Golay pneumatic cell

† Spectrophotometer (implying accurate intensity measurement) and spectrometer are used more or less indiscriminately at the present time. The term spectrograph usually implies photographic recording.

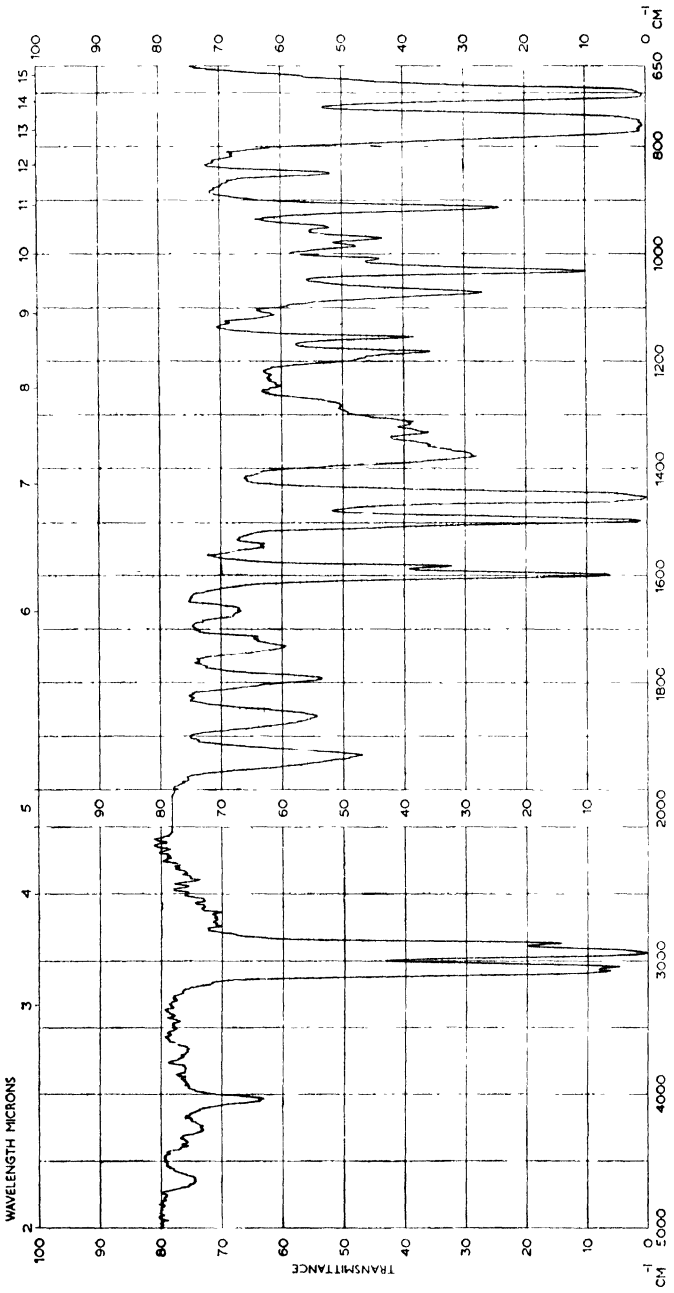


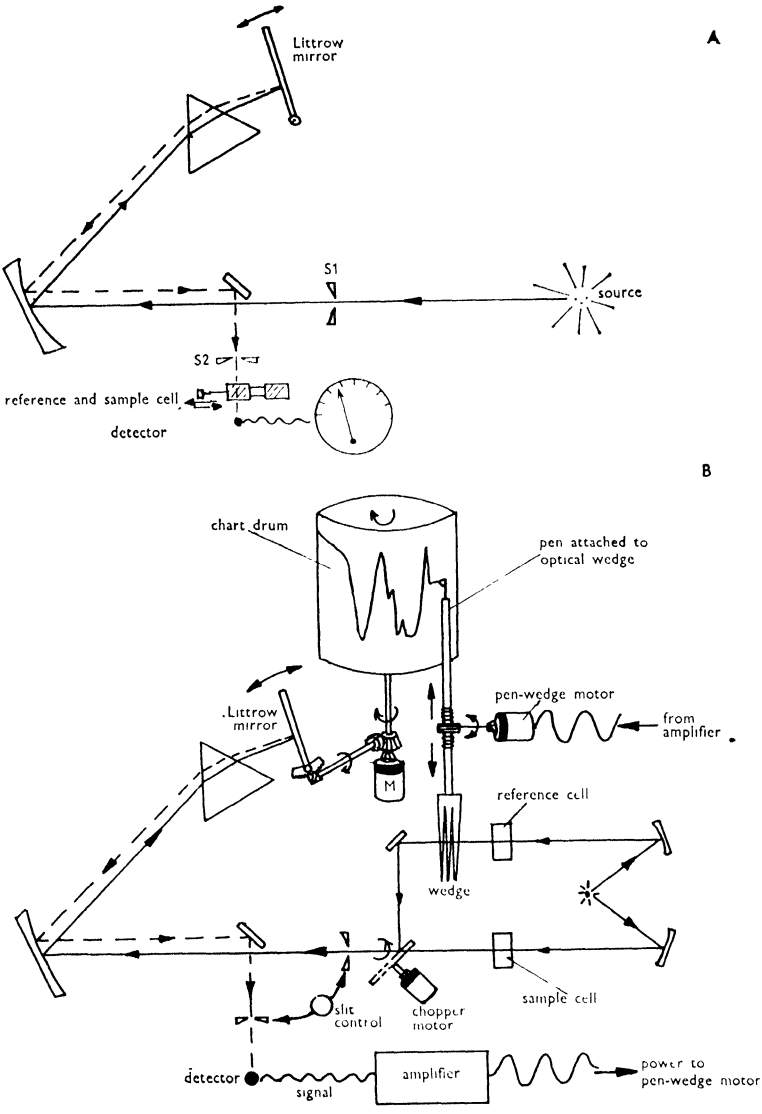
FIG. 3. Infrared spectrum of polystyrene, 0·002″ film; Unicam SP.200 spectrophotometer, recording time 10 mins (by courtesy of Unicam Instruments Ltd.).

The two main types of spectrophotometer, single-beam and double-beam, are briefly described below and represented diagrammatically in Fig. 4.

Single-beam non-recording ultraviolet spectrophotometer. The radiation from the source enters the monochromator through the entrance slit (S1), is dispersed by two passages through the prism, leaves the monochromator through the exit slit (S2), passes through the sample compartment, and then impinges on the detector. The wavelength of the light being transmitted is read off on a rotatable dial which is connected directly to the Littrow mirror or to a movable prism.

Two identical cells, one containing pure solvent and the other a solution of the compound under examination, may be slid into the light beam in turn, the solvent 'blank' being used to set the zero for the optical density scale at each wavelength examined. When the solution cell is in the light beam, the response of the detector is less than that when the solvent cell is in the beam, and the resulting difference in e.m.f. is balanced by a potentiometer, which is connected to the optical density scale. The sample is placed *after* the monochromator as photodecomposition is minimised by exposing the sample only to the weak beam of 'monochromatic' radiation. (This precaution is unnecessary with infrared radiation and in this case it is usual to take the easier course of introducing the sample into the full, undispersed beam.)

Double-beam recording spectrophotometer. In double-beam recording spectrophotometers, the sample absorption is determined directly from the difference in energy between two beams, one of which passes through the solution while the other passes through a solvent blank. This energy difference is usually measured by either the optical null or the ratio-recording method. In the *optical null* method, a wedge is pushed automatically (servo system) into the reference beam until the two beams give signals of equal strength. The energy for the wedge-movement comes from the difference between the beams and is therefore zero when the beams are equal. The movement of the wedge is accompanied by a simultaneous and proportional movement of the recorder pen and hence gives a percent transmission or percent absorption recording. The time needed for a full-scale pen movement is usually about 1 second. The *ratio-recording* method employs a self-balancing potentiometric recorder to compare the sample- and reference-beam signals directly. Most ultraviolet instruments are ratio-recording and most infrared instruments optical null recording. In both double-beam methods a light-chopping device is introduced (e.g. a semicircular



(M = motor driving Littrow and chart drum)

FIG. 4. Simplified diagrammatic representation of spectrophotometers:

A. Single-beam non-recording ultraviolet spectrophotometer.

B. Double-beam recording infrared spectrophotometer (optical null).

mirror cutting the beam at approximately 10 cycles per second). The amplifier is so designed that only oscillating (10 c/s) signals are amplified. The sample and reference beams can be distinguished since they impinge on the detector alternately. The spectrum is scanned by rotating the Littrow mirror (a mechanical cam is necessary to provide a linear frequency or wavelength scale). The dispersed radiation moves across the exit slit, only a narrow band of wavelengths passing through on to the detector. The narrower the range allowed through, the better the resolving power of the instrument (resolving power = ability to separate two, closely-spaced spectral lines). However, the slits must not be too narrow, as sufficient radiation has to arrive at the detector to give an adequate signal for the amplifier-recorder system. The widths of the slits are controlled by a slit programme (a cam on the wavelength drive, or an electrical device which monitors the energy in the reference beam) and an approximately constant level of radiation is maintained in the reference beam in spite of big variations in the source intensity with wavelength.

Instrument performance. The main criteria are:

(i) Constancy and accuracy of the wavelength or frequency scale. The instrument should be calibrated with standards, e.g. a polystyrene film (see Fig. 3) for the infrared region.

(ii) Constancy and accuracy of the percent transmission or optical density scale (photometric accuracy). Calibration curves can be drawn using solutions of a standard substance at known concentrations and thicknesses. Accurate intensity measurements are then possible.

(iii) Good resolving power. A standard substance having two closely spaced peaks is usually employed to check this, e.g. the degree of separation of the sharp peak on the side of the main one at 1600 cm^{-1} in Fig. 3 is a common test for the infrared region. For routine purposes a compromise giving a reasonable level of resolution together with rapid recording and little noise (random fluctuation of the pen due to electronic and other disturbances) is generally adopted.

Applications of Ultraviolet, Visible and Infrared Spectroscopy

Studies in all these spectroscopic regions basically depend on the selective absorption of the radiation by groupings (or atoms) within the individual molecules. Most of the applications rely on empirical correlations between the observed absorption and the presence of these groupings. Table 2 summarises the spectral absorption characteristics of a limited range of chemical groups. Most of the groups give

rise to absorption in the infrared, whereas strong absorption in the readily accessible part (above 210 mμ) of the ultraviolet region occurs only when conjugated systems of double or triple bonds are present.

TABLE 2. Absorptions due to simple chromophores*

Chromophores†	*Ultraviolet*		*Infrared*	
(a) *Non-conjugated systems*	λ_{max} (mμ) (> 180 mμ)	ε	ν_{max} (cm^{-1})	Approx. relative intensity
C – C	—	—	*ca.* 1000	w
C – H	—	—	3300–2700	m
			1450–1360‡	m
C – O	185	1000	1300–900	s
C – N	195	3000	*ca.* 1000	w
C – S	210	2000	*ca.* 700	w
C – Hal	180–260	500	1000–500	m or s
O – H	—	—	3600 (unbonded)	m
N – H	—	—	~3400	m
C = C	185	8000	1650	w to m
C = N	190	5000	1600	m to s
C = O	280¶	50	1800–1600	s to vs
C ≡ C	175	8000	2150	vw
C ≡ N	—	—	2200	m
(b) *Conjugated systems*				
C = C – C = C	220–250	5000 to 20,000	1650–1550 (two bands)	w to m
C = C – C = O	220–260	10,000 to 20,000	1680	s
			1630	m
C = C – C ≡ C	225	14,000	2150	m to s
			1600	m
(unconjugated aromatic system)	250	200 to 1000	1600 1570 1500	w to m

§ The absorption is really a property of the heteroatom and not the bonds (see p. 127).

Comments on Table 2

* Only certain highly significant absorption bands are given here. The frequency, wavelength and intensity values are very approximate. Designation of infrared bands: vs = very strong ($\varepsilon^a > 500$), s = strong, m = medium, w = weak, vw = very weak ($\varepsilon^a < 10$).

† For definition of chromophore see p. 127.

‡ Frequency of bending mode; all others are for stretching modes.

¶ Ketones and aldehydes only. Acids, esters, etc., do not absorb here.

The main purpose of the two following chapters is to outline the use of spectroscopy in structure determinations. However, both the ultraviolet and infrared spectrophotometric methods owe their development to their value in quantitative analysis, and probably most spectrometers are used for this purpose. This type of application can be

important to the organic chemist engaged in synthetical and degradative work for what might be termed 'experiment control'. For instance, the course of a reaction may often be followed by spectroscopic analysis, impurities may be detected in a product, and materials may be purified to constant spectral properties as an alternative to the more usual melting point criterion. Exact matching of two infrared spectra in the so-called 'fingerprint region' (1400–650 cm^{-1}) is considered the proof *par excellence* of identity of the substances (it is not infallible, however). The ultraviolet spectra of acids or bases often depend on the pH of the solution, and it is possible to determine the ionisation constants of such compounds by spectroscopic means (see p. 161). The simple procedure of using spectroscopy to follow the course of a reaction can be extended to a full-scale investigation of the kinetics. Some of the more important aspects of the ultraviolet, visible and infrared methods are compared and contrasted in Table 3.

TABLE 3. Comparison of ultraviolet, visible and infrared spectroscopic methods

	Ultraviolet and visible	*Infrared*
Units, wavelength (λ)	mμ or Å (10 Å = 1 mμ)	μ
Units, wavenumber (ν)	cm^{-1}	cm^{-1}
Energy	95 kcal./mole at 300 mμ	4·9 kcal./mole at 1700 cm^{-1}
Lightwaves per sec	10^{15}/sec	10^{12} to 10^{13}/sec
Origin of spectra	electronic-vibrational-rotational transitions	vibrational-rotational transitions
Typical intensities (molar extinction coefficient, ε)	100,000 (polyacetylenes) 10,000 (unsaturated ketones) 50 (saturated ketone)	1300 (isocyanate group) 400 (carbonyl group) 2–50 (olefines)
Typical concentrations used	10^{-4} to 10^{-3} molar	10^{-2} to 10^{-1} molar
Lower limits of detection (for chromophores of average intensity)	0·003 g/litre	0·1 g/litre
Usual state of sample	solution	solid, liquid or solution
Quantitative accuracy	good	moderate to good
Principal solvents used	H_2O, C_2H_5OH, C_6H_{14}	CCl_4, $CHCl_3$, CS_2
Cell materials	silica	sodium chloride
Common cell dimensions	1 mm–1 cm	0·025–5 mm
Prism materials	silica, glass (visible)	NaCl, CaF_2, LiF, KBr
Cost of routine recording instrument	*ca.* £1500	*ca.* £1500

TABLE 3—*Cont.*

	Ultraviolet and visible	*Infrared*
Principal applications		
Identification of individual substances	very limited	extensively used
Detection of chromophores	X=Y and X≡Y; especially conjugated functions	X–Y, X=Y and X≡Y; non-conjugated and conjugated
Measurement of physical constants e.g. dissociation constants, molecular weight	useful	little used
Quantitative analysis, reaction kinetics Qualitative analysis and reaction control	Both methods extensively used. Choice depends on the chromophores present	

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Chapter 3

G. Eglinton

INFRARED AND RAMAN SPECTROSCOPY

INTRODUCTION

Today, infrared spectroscopy is a standard research and analytical tool in nearly all academic and industrial chemical laboratories. With its aid most of the functional groups present either in a pure compound or in a mixture can be both detected and determined within a few minutes. Furthermore, only a few milligrams of the sample are required and even these are recoverable. Similar analyses by the older chemical procedures might require many days and the loss of several grams of valuable material. The whole pace of organic chemistry has quickened and the method of approach to problems changed, as a result of the widespread use of 'infrared'.†

It is now essential that the organic chemist should have a working knowledge of the infrared method. In this chapter, Part I gives a brief introduction to the theory and practical aspects of infrared measurements and their interpretation. Part II consists of an extensive series of illustrated examples which should show what can, and what cannot, be readily achieved with the method. This seems the best approach to a predominantly visual subject since successful interpretation of spectra depends largely on the worker's grasp of the relative significance of bands of varied position, shape and size.

In Part III, the subject of Raman spectroscopy is briefly reviewed. The organic chemist makes little use of this method at present and it has accordingly been given only a few pages.

† Important descriptive texts which will repay study are cited in refs. 1–14. Reference collections of spectra are also available.[15–19]

PART I. INFRARED SPECTROSCOPY—THEORY AND PRACTICE

MOLECULAR VIBRATIONS AND THEIR INTERACTION WITH INFRARED RADIATION

Electromagnetic radiation of the near-infrared and infrared regions (0·8–30 μ) is absorbed to various extents by all substances, the energy so absorbed eventually appearing as heat. The absorption process involves excitation of the molecule to higher vibrational states and it is therefore quantized. Even at 0°K the atomic nuclei are vibrating about the bonds which bind them together. The vibrations increase in amplitude when electromagnetic radiation (infrared) of the same frequency reaches the molecule and raises it to the next vibrational level. The infrared spectrometer measures this absorption at each frequency in turn and so produces an infrared spectrum. The availability of numerous rotational levels for each vibrational level gives rise to the 'rotational fine structure' observed when small molecules are examined

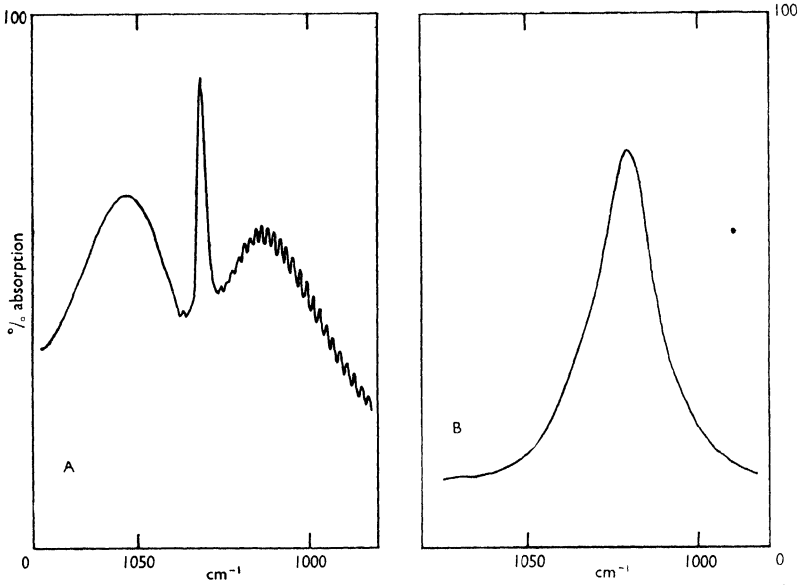


FIG. 1. A vibration-rotation absorption band (νC – O) of methyl alcohol as recorded with a Unicam SP.130 grating spectrophotometer for:

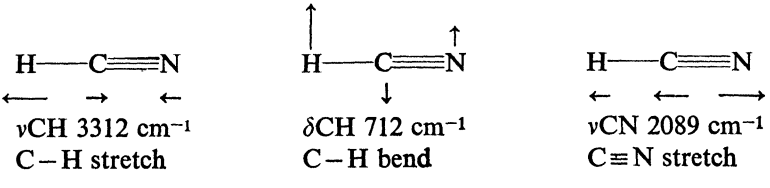
(A) the vapour state (10 cm cell).

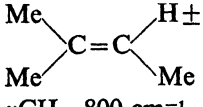
(B) a dilute solution in carbon tetrachloride (0·05 cm cell).

In the vapour spectrum the rotational fine structure of the P-branch is clearly resolved.

in the vapour state (Fig. 1). Mathematical treatment of the frequency spacings gives the moments of inertia and hence the bond lengths and angles of the molecule.[1] However, for the average complex organic molecule in solution or in the solid state, these rotational lines are so close together and blurred by collision and other interactions that they cannot be distinguished. The result for a single vibrational band is a smooth peak of about 5–30 cm^{-1} half-band width (Fig. 1 and p. 41).

Group frequencies. In addition to the above quantum-mechanical treatment, the derivation of vibrational spectra can also be discussed from the more classical standpoint of simple harmonic motion of the nuclei. As an analogy, one can consider a molecular model composed of balls connected by springs. The whole model vibrates when it is struck. However, if it is illuminated by a flashing light (stroboscope) of variable frequency, a particular ball will appear stationary when the frequency of the light flashes matches its frequency of vibration. Thus the apparently random motion of the molecular model can be broken down into a number of simpler modes of vibration. These, the *fundamental* or *normal modes*, require that there must be no movement of the centre of gravity of the molecule, and that the nuclei move in straight lines and in phase. They are of two types, *stretching* (ν) and *bending* (δ, γ etc.) and correspond in the quantum treatment to the first vibrational transition from the zeroth vibrational level to the first, $v_0 \rightarrow v_1$ (at room temperature most molecules are in the zeroth level). The transitions 0→2 and 0→3 are the first and second *overtones* of the fundamental and require light of twice and three times its frequency; in the average spectrum they are very much weaker than the fundamental.





γCH ~ 800 cm^{-1}
C–H out-of-plane bend

The three fundamentals for the HCN molecule are given above. For the stretching mode, νCH, and bending mode, δCH, of the H–C bond

the motion is almost entirely in the light hydrogen atom. Movement of a hydrogen atom above and below the plane of a double bond is depicted as shown for 2-methylbut-2-ene. For any molecule of n atoms there are generally $3n$-6 degrees of freedom and $3n$-6 fundamental modes. However, some of these will not absorb infrared radiation (they are said to be *'forbidden'* or *'infrared inactive'*) since absorption of energy can only occur when the vibrations of the nuclei in the bond are accompanied by changes in dipole moment. Thus νC=C of ethylene is inactive as the bond is symmetrically substituted. The *selection rules* for the appearance of Raman lines are different (p. 119).

Although, rigorously, a fundamental mode must be considered as involving all the atoms of a molecule, experience has shown that the absorption frequencies of many groups are largely independent of the rest of the molecule, i.e. the vibrational motion is concentrated within the group. These vibrations are referred to as *specific* or *localised group frequencies*, and their values are determined by the masses of the vibrating atoms, the molecular geometry, and the restoring forces holding the atoms together. Structural information is generally obtained from a spectrum by assigning the observed absorption bands to specific group frequencies. The exact position, intensity, and shape of a band may then give further information as to the environment of the particular group. This fortunate relationship between spectral absorptions and the classical functional group concept of organic chemistry has been the main factor in ensuring the success and popularity of the empirical infrared approach. Assignment of all, or even most of, the bands in a spectrum is rarely possible. In some cases the assignments can only be tentative but, in general, the empirical assignments arrived at for complex molecules have received considerable support from the measure of agreement between the theoretical and empirical approaches in the small molecule field.

Position of group frequency. In a diatomic molecule, such as hydrogen chloride, there is a single fundamental mode—the periodic stretching of the bond. For a simple harmonic oscillation following Hooke's Law, the vibrational frequency (cm^{-1}) is dependent on f, the force constant of the bond, and on the masses (m) of the atoms.

$$\nu = \frac{1}{2\pi c}\left(\frac{f}{\bar{m}}\right)^{\frac{1}{2}}$$

c = velocity of light
$\bar{m}$ = reduced mass
$= m_x m_y/(m_x + m_y)$

In complex molecules, the stretching vibration of a C–H bond consistently behaves as if largely localised between the two atoms. It absorbs close to 3000 cm^{-1} which agrees well with 3040 cm^{-1} calculated as above for a diatomic system C–H. In general, the group frequencies with the narrowest range (i.e. those least affected by the rest of the molecule) are those which are at much higher frequencies than the numerous skeletal modes (*ca.* 1000 cm^{-1}). 'Coupling' or 'mixing' (p. 40) is then at a minimum. Such groupings are of two types:

(*a*) The X–H type, e.g. C–H, N–H and O–H, where the mass of the hydrogen is small in comparison with that of the X atom. These absorb at the high frequency end of the spectrum (approximately 3000 cm^{-1}) and the vibratory motion is almost entirely confined to the H atom.

(*b*) The X=Y (e.g. C=C, C=N, C=O) and X≡Y type (e.g. C≡C, C≡N) where the high force constants result in high stretching frequencies. The force constants for single, double and triple bonds are approximately 5, 10 and 15×10^5 dynes/cm, respectively. The stretching frequencies of a series X–Y, X=Y and X≡Y rise in more or less the steps to be expected from the increasing force constants. For νC–C, νC=C and νC≡C the values are approximately 1100, 1650 and 2200 cm^{-1} respectively.

Extensive coupling (the vibration being no longer localised; see below) takes place between adjoining C–C, C–N and C–O bonds in a molecule and these skeletal absorptions consequently occur over a wide range and are generally difficult to assign. As regards the hydrogen bending vibrations, the in-plane bending bands (δ) occur in fairly narrow ranges while the out-of-plane bends (γ) absorb over a wider range and at lower frequencies. Less energy is required to bend a bond out of the plane of a double bond than to deform the in-plane bond angle.

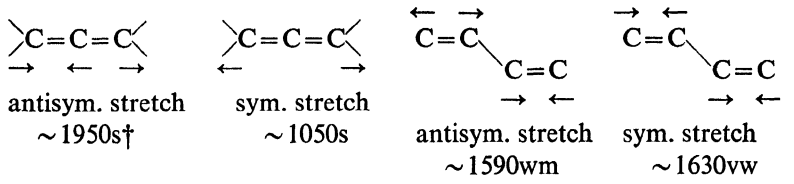
Overtones, combination bands, mechanical coupling and Fermi resonance. In addition to the fundamental stretching and deformation modes the following types of absorption are observable:

Overtones. Absorption of radiation at almost exactly twice, three, etc. times the fundamental frequency. For example, for the carbonyl fundamental νC=O at 1700 cm^{-1}, the first overtone 2νC=O will be close to 3400 cm^{-1}. Usually only the first overtone need be considered and the absorption is generally very weak ($\frac{1}{10} - \frac{1}{100}$ of the intensity of the fundamental, see spectra 7 and 8, p. 82).

Combination bands. These occur when radiation of frequency $\nu_1 + \nu_2$ is capable of exciting the fundamentals ν_1 and ν_2 simultaneously.

More than two fundamentals can take part. Difference modes are also possible, i.e. $\nu_1 - \nu_2$. They are normally weak. The diagnostically-useful aromatic patterns between 2000 and 1600 cm^{-1} are made up of overtones and combination modes of the γCH modes in the 900–650 cm^{-1} region.

Coupling. Two group vibrations are said to be coupled when they are no longer localised within the individual group. Some degree of coupling seems present in most vibrations but it is at a maximum when groupings having similar vibrational frequencies are linked together. The allenic grouping is a case where one atom is common to both groups. Instead of a single absorption near 1650 cm^{-1}, *two* bands, separated by some 900 cm^{-1}, appear as a result of the coupling, all three carbon atoms being involved. In conjugated dienes, where a single bond separates the doubly bonded carbons the coupling is very much weaker, the separation between the two absorptions being only some 40 cm^{-1}.



Fermi resonance. Fermi resonance occurs to some extent in nearly all infrared spectra. When an overtone or combination tone falls within a few cm^{-1} of a fundamental, a sharing of intensity occurs and the fundamental loses some of its intensity to the other band. There are certain requirements to be met; thus, vibrations must be of the same symmetry and must originate from atoms close to one another in the molecule. Several types of monocarbonyl compound, e.g. butenolides, coumarins and cyclopentanones, have two or more bands in the carbonyl region, which have been ascribed to Fermi resonance. Twin bands can also have their origin in conformational equilibrium, vibrational coupling and in solvent and solid-state effects. It is not always easy to distinguish these phenomena.

Intensities. The intensity of an infrared band is a measure of the probability of the transition to the next vibrational level and is dependent on the change of dipole moment occurring during the vibration. If there is no dipole moment change then the electromagnetic radiation cannot interact with the vibration and there is no absorption; the band

† See p. 50 for details of the abbreviations.

is *inactive* or *forbidden*. Thus $\nu C{\equiv}C$ absorption does not appear in the spectra of symmetrically substituted dialkylacetylenes, $RC{\equiv}CR$. Bonds having only small dipole moment changes absorb weakly, e.g. 2-methylbut-2-ene has $\nu C{=}C$ with an ε of about 5. Carbonyl and similar highly polar groupings absorb strongly, $\varepsilon \sim 100$–1000. But even the intensities of the most polar groups are two or three factors of ten smaller than those of the most intense electronic transitions.

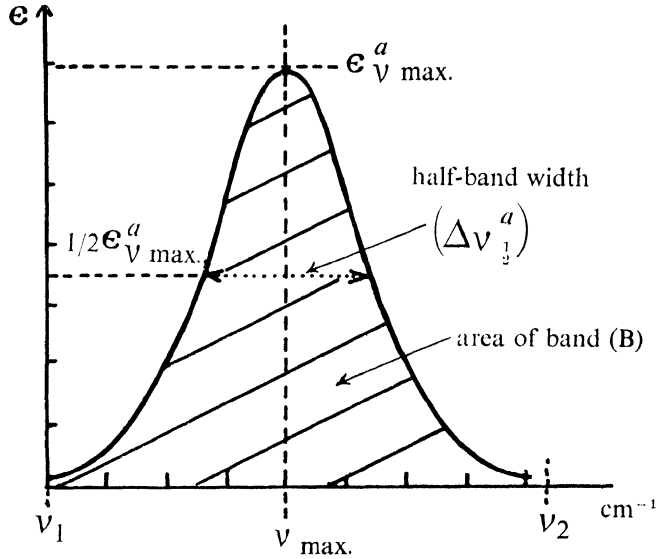


FIG. 2. The measurement of an absorption band

Bands vary in width as well as in extinction coefficient (ε) so that it is frequently necessary to quote the half-band width ($\Delta\nu_{\frac{1}{2}}^{a}$). This is the width of the band at half the maximum extinction (see Fig. 2). The absolute measure of intensity, the *band area*, encompasses the variations in the width and shape of the bands and is the fundamental quantity of the radiation absorption process. For most purposes the *apparent*† *integrated absorption intensity* (B) is adequate: $B = 2{\cdot}303 \int_{\nu_2}^{\nu_1} \varepsilon_\nu^a d\nu$ (Fig. 2). Jones[2] defined 10^4 of the above B units as 1 integrated intensity unit; many bands have values of 1–5 units. There has been some success in using area values diagnostically and for correlation with other physical measurements.

Relationships of group frequencies with other properties. A considerable effort has been made to correlate the group frequencies with

† *The true integrated absorption intensity*, A, is defined as $A = 2{\cdot}303 \int_{\nu_2}^{\nu_1} \varepsilon_\nu d\nu$ and represents the integration of the *true* molar extinction coefficient (p. 26).

other molecular properties which are largely determined by the same functional groups. Thus quantitative relationships with dissociation constants, polarographic and redox potentials, chelate stabilities and bond lengths (X-ray) have been established. Correlations with the reactivities of molecules are of particular interest and significance: the Hammet σ values for the substituents in substituted anilines, phenols and benzoic acids often give straight-line relationships with the functional group frequencies, i.e. νNH_2, νOH and νCO. It is clear that the group frequency (and also the intensity in certain cases) is a sensitive measure of the inductive (I) and mesomeric (M) effects† which mainly control the reactivity of the group concerned.

Instruments and Sampling Methods

The basic design of infrared (and also ultraviolet) instruments has been dealt with on p. 27. A simplified layout of the small bench-type spectrometer used to record most of the spectra in this chapter is given on p. 30. More expensive instruments[7] of higher resolving power and greater accuracy (e.g. a grating instrument) are necessary for the investigation of the finer points of group-frequency studies. Cross[6] gives an excellent review of the available infrared instruments and of the factors which have to be considered when recording a spectrum.

The radiation beam is usually 2 mm wide by 10 mm high at the sampling position, and approximately 0·5 mg of sample evenly occupying this area gives a reasonable spectrum. Most cells are rather larger in area and consequently 1–3 mg of a substance is commonly required [special microcells and microscope systems are available to deal with very small quantities (~ 10 μg)]. The transparent portions of cells are usually made from large sodium chloride crystals, cut and polished; cells may be quite expensive ($\sim$£20 each). Gases, solids, liquids and solutions can all be dealt with, and a single sample may, of course, be examined in more than one state. Thorough drying of samples and solvents is a wise precaution. Organic chemists rarely need to examine substances in the gas state (a typical path length for a gas cell is 10 cm). The sampling methods for solids, liquids and solutions are given briefly below:

Solid state. There are three main methods for examining solids:

(*a*) *Nujol mull.* More spectra are recorded as Nujol mulls than in any other form. This is because a mull is extremely simple to prepare and mount, and because the method is suitable for all solids. The

† The terms, −I and −M, used later, refer to electron *withdrawal* and +I and +M to electron *supply*.

substance, 1–3 mg, is ground for about 2 minutes in one drop of medicinal liquid paraffin (Nujol) and the resulting paste is pressed between two salt plates and placed in the beam. The Nujol serves to immerse the minute particles of the substance in a transparent medium of similar refractive index and so prevents the severe scattering of radiation which would otherwise occur (the particles, though small, still represent many unit cells). One disadvantage is that Nujol, being a mixture of paraffin hydrocarbons, has strong absorption near 2900, 1465 and 1380 cm^{-1} (Spec. 1, p. 81). These bands must first be located and marked off on the spectrum to avoid misinterpretation. If information is required from the CH regions, a perhalogenocarbon oil (e.g. Florube–3) which has C–C and C–Hal links only, may be used, but this absorbs intensely in the region below 1400 cm^{-1}. Another disadvantage of the mulls is the general one that solid state spectra may be difficult to interpret (see p. 57).

(*b*) *Melts.* If the substance is a gum or has a melting point less than 150°, it is frequently possible to melt it between two salt plates and examine the resulting solid or molten film. One difficulty is that the crystalline sheet may be in one of several polymorphic forms and is probably uniformly orientated. Direct comparison with a Nujol mull, which is a more or less randomly orientated suspension of crystallites, may well reveal considerable differences.

(*c*) *Halide disc.* A finely ground mixture of 1 mg of substance and 300 mg of anhydrous potassium chloride or potassium bromide powder is subjected to considerable pressure for about 5 minutes in an evacuated metal die. The transparent alkali halide disc containing the dispersed substance usually gives an excellent spectrum free from other absorption and with little scatter. It is difficult to ensure complete absence of water and peaks at *ca.* 3500 cm^{-1} must be viewed with suspicion. Other snags, such as polymorphism, can arise as a result of the grinding with the halide and there are sometimes changes on keeping (especially with substances containing OH or NH). This method can be made semi-quantitative in favourable cases.

Liquid state. Pure liquids are very easily examined in a qualitative fashion; a drop is merely placed between two plates ('capillary film') or is inserted into a thin 'cell' (0·025–0·1 mm thick; two plates of sodium chloride separated by a spacer). Interactions, such as hydrogen bonding, between molecules may necessitate additional measurements in dilute solution in an inert solvent.

Solution spectra. Solution spectra may not always be the most easily obtained but they give the most precise information. Detailed com-

parisons between related compounds cannot be made with confidence unless the measurements are for solutions in the same solvent, as the environment of the solute molecules is then constant. A *solvent-compensated* spectrum may be recorded as follows, using a double-beam spectrophotometer: A cell containing pure solvent is placed in the reference beam, and an identical cell containing the solution placed in the sample beam. The absorption due to the solvent is thus cancelled out and not recorded. Useful spectral data for the solute are obtained only in the 'window regions' where solvent absorption does not exceed 95%. Typical requirements for a monocarbonyl compound, molecular weight *ca.* 200, $\varepsilon_{C=O}$ *ca.* 450, might be a 1% w/v solution (0·5 ml) in a 0·5 mm cell.

To the sorrow of the biochemists, water is virtually the exact opposite of the ideal infrared solvent as it rapidly dissolves the most convenient window material (sodium chloride) and absorbs intensely over nearly the entire spectrum! Only very concentrated aqueous solutions can be handled and these only over certain regions in special cells. The three commonly used infrared solvents are CCl_4, CS_2 and $CHCl_3$. Although all absorb strongly in parts of the spectrum, they have large window regions (see Chart VIII, p. 54 and Spec. 2, 3 and 4, p. 81). Complete coverage can be obtained by running the compound in more than one solvent; CCl_4 and CS_2 make a good pair. Chloroform is a more powerful solvent but has stronger and more widespread absorption of its own. Solution spectra are more reliable than solid state spectra since it is possible to exercise control over concentration and sample thickness, and also over the environment of the solute molecules. Further, there are no problems of partial orientation or polymorphism.

Summary. Choose the sampling method carefully, bearing in mind possible interactions and the absorption due to the sampling medium. More than one state and/or solvent or concentration should be investigated for the best results. Trace impurities can be studied by using thick cells and high concentrations.

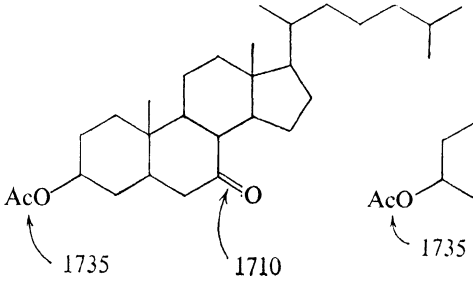
The Applications of Infrared Spectroscopy

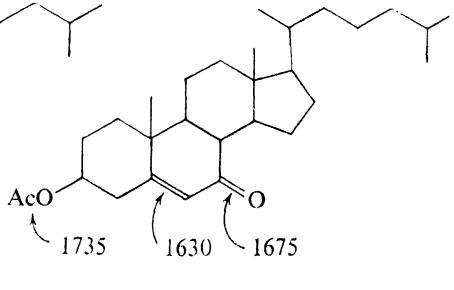
The numerous applications of infrared spectroscopy in organic chemistry are not amenable to rigid classification and the arrangement below is consequently somewhat arbitrary. *Structure determination*, the application with which this chapter is chiefly concerned, is discussed separately under 'The correlation and interpretation of infrared spectra' p. 48.

Identification of a substance. The identity of a substance can often be established by the comparison of its infrared spectrum with that of an authentic specimen. There is no need to assign the absorptions to specific groupings; the whole process may take less time than a mixed melting point and can be more informative. The large number of peaks usually found in the region 1400–650 cm^{-1} provide a characteristic pattern ('fingerprint region'). Optical isomers (enantiomers) give identical spectra but racemates may show differences in the solid state which disappear entirely in solution. Libraries [15–19] and bibliographies[20,21] of spectra facilitate the identification of compounds by spectral comparison. Punched-card sorting equipment can now handle infrared spectra, holes being punched to match the absorptions and functional groups present.

Detection of impurities. The spectrum clearly reveals the presence of impurities only when they absorb fairly strongly in positions where the main component does not. For instance, cyclohexanone is readily detected in cyclohexanol on account of the intense carbonyl peak (Spec. 6 and 7, p. 83). Very impure compounds often give blurred, indistinct spectra. The *difference method* can be useful in revealing the actual spectrum of an impurity; when the impure substance is placed in the sample beam, and the same thickness of pure sample placed in the reference beam, the spectrum of the impurity is recorded.

Control of reactions and separations. Infrared spectroscopy can be used to follow the progress of a reaction. Often examination of only a small portion of the spectrum may be sufficient to indicate whether the desired product is formed and to show when the reaction has gone to completion. For example, the Clemmensen reduction of a ketone ($>C=O \rightarrow >CH_2$) is accompanied by the disappearance of the carbonyl peak. The separation of a mixture may be followed similarly. Fig. 3 illustrates the chromatographic separation of the two steroid acetates shown below. The mixture (2% in CCl_4; 1 mm cell) prior to separation has the carbonyl bands due to both substances. The spectra of the





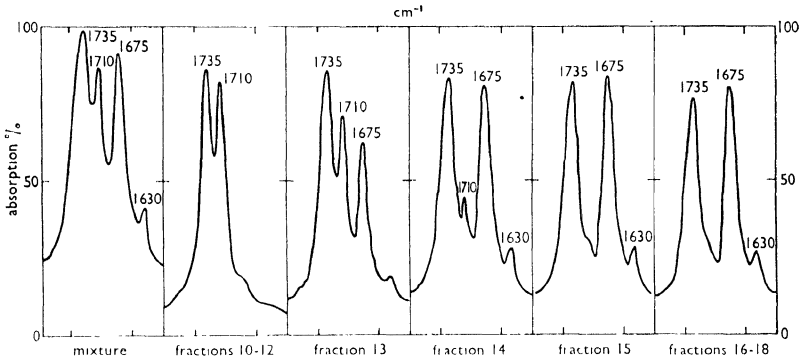


FIG. 3. Chromatographic separation of 5α-cholestan-3β-ol-7-one acetate and cholest-5-en-3β-ol-7-one acetate. Carbonyl-region absorption of the fractions (by courtesy of Dr P. Bladon).

evaporated fractions (1% in CCl_4; 1 mm cell) show clearly that fractions 10–12 are almost entirely the saturated ketoacetate; fractions 13–15 are mixtures and fractions 16–18 are the pure conjugated ketoacetate.

Quantitative Analysis. The percentage composition of a mixture is obtained by standard spectrometric procedures. If Beer's Law is obeyed the spectrum of a mixture is that resulting from the superposition of the spectra of the pure components. A unique band is chosen for each component and its intensity measured in the pure state or in mixtures of known composition. The actual procedure is illustrated in the example below. Quantitative infrared analysis is of great importance in the chemical industry, for example in the refining and cracking of petroleum. Continuous monitoring at fixed frequencies can be used to provide completely automatic production control, e.g. in the manufacture of acrylonitrile from acetylene and HCN. When excessive or insufficient absorption occurs, electronic devices automatically alter the flow controls to bring the absorption—and hence the product composition—back to the correct level.

Example of quantitative analysis. Commercial xylene contains the *ortho-*, *meta-*, and *para*-isomers. Separation by distillation is virtually impossible (complete separation by gas–liquid chromatography is not easy), and the ultraviolet spectra of the isomers hardly differ. However, considerable differences occur in the infrared spectra, especially in the region where the out-of-plane hydrogen bending modes, γCH, occur and quantitative analysis of the mixture can be simple and accurate: (*a*) The 'analytical' (i.e. unique) bands are first chosen. The significant portions of the liquid film spectra of the individual isomers are shown

in Fig. 4, from which it is easily seen that the commercial xylene is a mixture of all three isomers (the samples of the individual isomers are also not quite pure; for example, a trace of *meta-* can definitely be discerned in the *ortho*-isomer). The chosen bands are: *ortho* 741, *meta* 690 and *para* 794 cm^{-1}. (*b*) Mixtures of known composition are next recorded and 'working curves' (optical density against % content

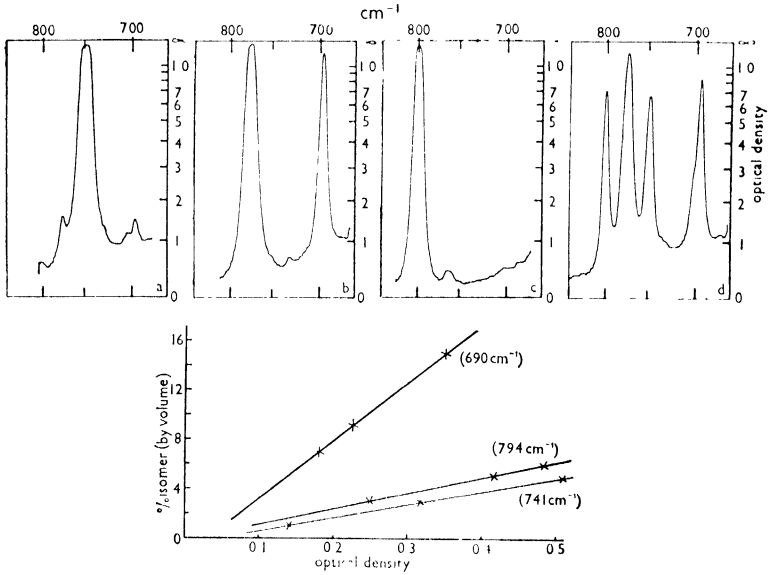


FIG. 4. Quantitative analysis of commercial xylene. Spectra are (a) *o*-xylene (b) *m*-xylene (c) *p*-xylene and (d) commercial xylene, all as liquids in a 0·025 mm cell.

The graph represents plots of optical density against % content of the individual isomers in mixtures of known composition. The optical densities are for the 741 cm^{-1} (*o*-isomer), 690 cm^{-1} (*m*-isomer) and 794 cm^{-1} (*p*-isomer) bands.

of the isomer) drawn for the 741, 690 and 794 cm^{-1} bands. In the present case Beer's Law is evidently obeyed since the working curves are straight lines. (*c*) The optical density values for the commercial xylene yield the % composition directly from the working curves.

A similar analysis is commonly used for oleic and elaidic acids and their esters (Example 8, p. 98). Which band might be chosen? Only one component need be estimated in a simple binary mixture.

The Correlation and Interpretation of Infrared Spectra

For over 20 years chemists have intensively developed the correlations between infrared absorption frequencies and functional groups, for it is from the presence or absence of characteristic frequencies that structural information is chiefly derived. The usual empirical procedure has been to examine the spectra of some 20 or 30 compounds having a common functional group, to establish the similarities and then to check them by examination of similar series bearing different functional groups. The main correlations in the region 4000–650 cm^{-1} have been made and some of them are summarised in the correlation charts (pp. 51–54). Attention is now centred on establishing the nature of the factors which determine the exact absorption frequency and intensity of a group as the molecular environment is changed. An idea of the empirical approach can be gathered from Spec. 5–14, pp. 80–87. The introduction of the OH and CO functions into the cyclohexane ring (Spec. 5–8) results in the appearance of the corresponding absorptions. The same behaviour is seen in Spec. 9–14; although the cyclohexane ring is then part of a much larger carbon skeleton, the specific group absorptions are more or less independent of the rest of the molecule.

The correlation charts and examples which follow reflect the emphasis on natural products in current teaching and research in organic chemistry. The principles of the methods discussed here can, of course, be applied in other fields. The aim has been to present a simplified picture rather than an exhaustive one. Approximate positions for absorption frequencies are considered adequate for most of the discussion. For greater detail comprehensive surveys (refs. 2–14) should be consulted.

It is suggested that at this stage it is advisable to memorise Chart I and to study the others. A knowledge of these approximate frequency positions together with that of the modifying factors (pp. 55–78) will be invaluable. Part II should provide adequate experience in the interpretation of actual spectra.

Interpretation procedure. The infrared method is not an absolute one giving the exact structure of a complex molecule in the way that X-ray studies can. The information derived from it, though often correct, is always tentative and requires confirmation by chemical or other means. Its great value lies in the *guidance* it can give to the probable constitution of the sample. It is not feasible to lay down definite procedures for the interpretation of spectra, as much depends

on the type of compound examined, its state, and on the knowledge (both of infrared and of chemistry!) of the observer. The initial approach is usually to examine the region 3650 to 1500 cm^{-1} where characteristic absorptions (e.g. OH, NH, C=O and C=C stretching) are readily spotted. Most compounds have only a few strong bands between 3650 and 2750 cm^{-1} and/or between 1800 and 1500 cm^{-1}. The region 2750–1800 cm^{-1} is frequently barren. The fact that bands *are* present in the appropriate places is *no guarantee* that particular groups are present and confirmation should be sought in other regions of the spectrum; e.g. an aliphatic acetate should absorb not only near 1740s but *also* near 1250s cm^{-1}. Interference by the fundamentals or overtones of other groups can sometimes cause confusion. Even the absence of a band, e.g. in the normal carbonyl region, is not absolutely conclusive; powerful interactions, e.g. chelation, can adversely affect the intensity of a group frequency or move the band well away from the expected position. All the possible structures should be tried by predicting their regions of absorption for comparison with the actual spectrum.

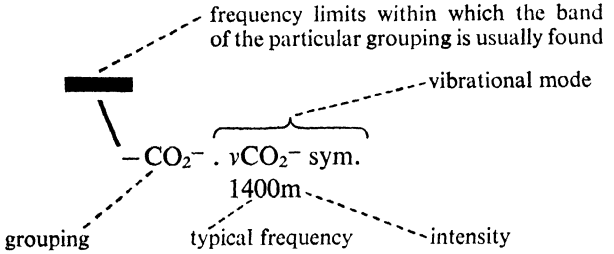
Absorption in the 1500–650 cm^{-1} region is generally much more complex and difficult to interpret than that between 3650 and 1500 cm^{-1}. Assignment of all the bands is not only almost impossible but also inadvisable, as most of the correlations would be extremely unreliable.

Although satisfactory conclusions can often be drawn by using generalised charts or tables, it is always best to make direct comparisons with the spectra of closely related compounds (e.g. to compare a diterpene hydrocarbon with other diterpene hydrocarbons). Some correlations are limited to a particular class of compound but within this class they may still be of considerable assistance.

A further approach is to modify the molecule and then examine the spectra of the resulting derivatives or degradation products. Thus hydrogenation, deuteration (of CH, NH or OH), esterification, methylation, salt formation, or acetylation should effect predictable changes in the specific group absorptions. Often, a change of state or of concentration can be very informative and it is normal practice to record the spectrum of an unknown under a variety of conditions. The extent of intermolecular association, if any, can be examined in different solvents and at different concentrations (p. 61). A recent introduction is the method of '*solvent shifts*' whereby the nature of a functional group can sometimes be inferred from the shift in frequency from one solvent to another (pp. 58–61). [*continued on p. 55*

Notes on correlation charts and abbreviations used in text

The charts give a presentation which is linear in wavelength, but they are marked in cm^{-1} (cf. Part II). All data are in cm^{-1}. The intensities given are very approximate and only of much significance within a particular table. Some group frequencies (Charts IV, V, VI and VII) are markedly affected by physical state (hydrogen-bonding effects, etc.), though they are usually to be found in the ranges given. In Chart I, the OH and the NH values are those in the absence of hydrogen-bonding. Most of the correlations are given in the form:



Where several groupings superpose (e.g. $\nu C=O$ in Chart IV) the top one has the highest average frequency and the bottom one the lowest. Correlations already given in one chart (e.g. CH vibrations) are not repeated in later charts.

Vibrational modes

ν = stretch	γ = out-of-plane bend	τ = twist
δ = in-plane bend	ρ = rock	ω = wag
sym. = symmetrical	antisym. = antisymmetrical	

(ν is also used for quoting an absorption frequency, e.g. ν_{max} 1050 cm^{-1} etc. Other representations for vibrational modes are current in the literature, e.g. ν(OH) and ν_{OH}.)

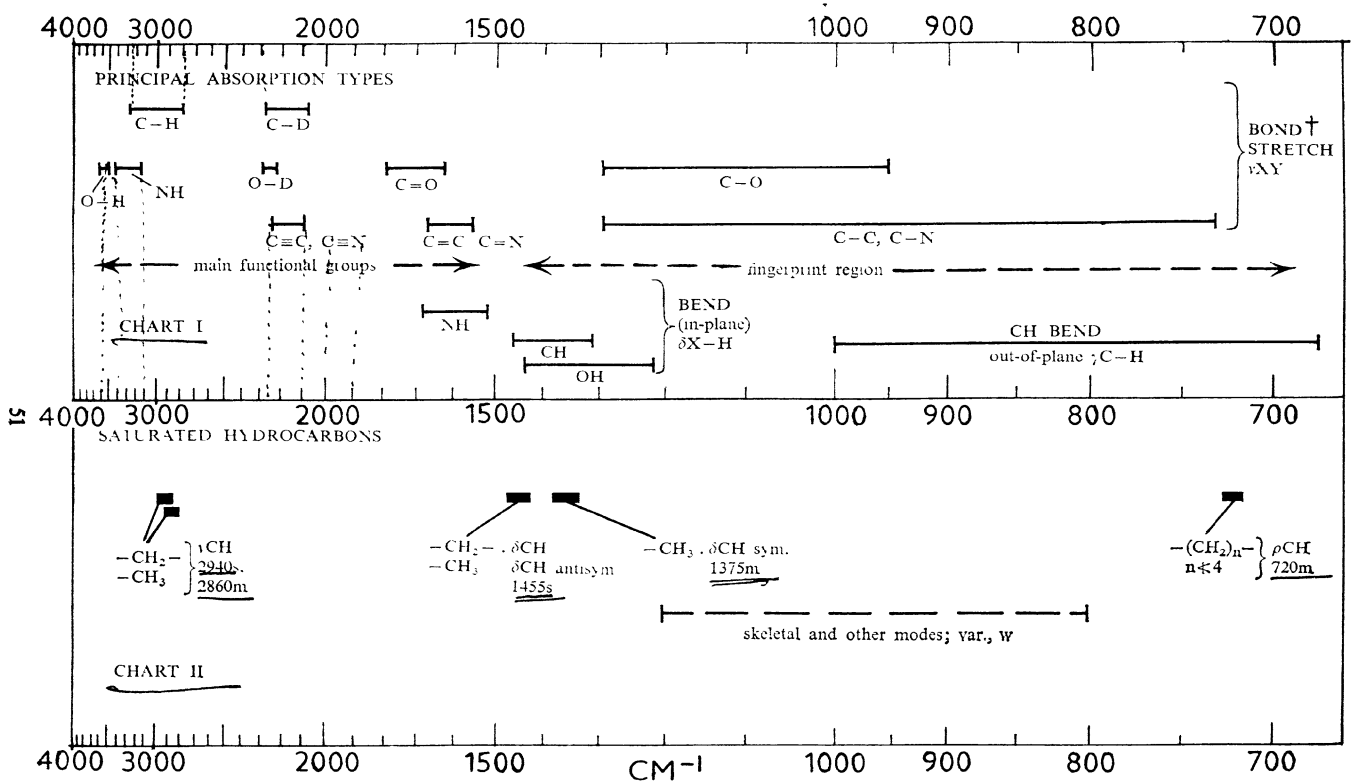
Symbols denoting intensity of absorption

w = weak	vw = very weak	wm = weak to medium
m = medium	vs = very strong	ms = medium to strong
s = strong		

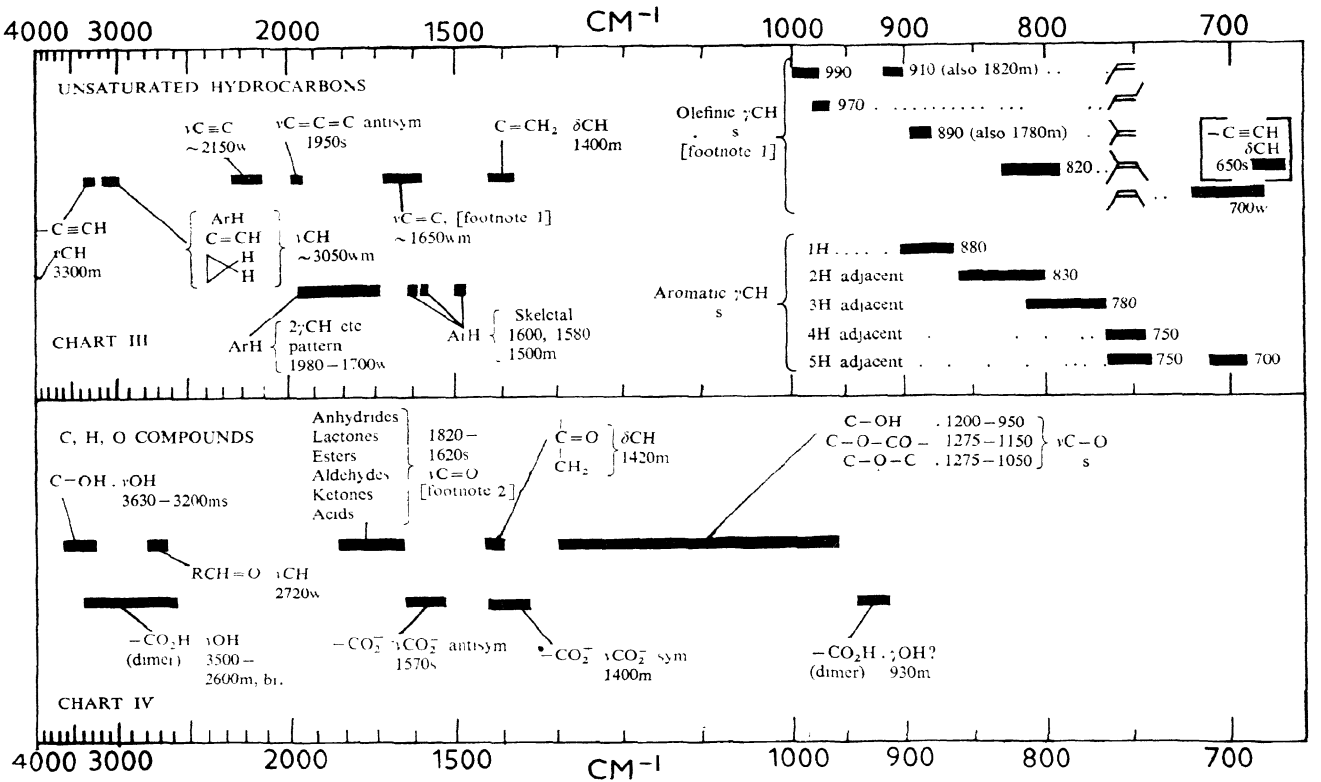
Other abbreviations

adj. = adjacent	abs. = absorption	br. = broad
var. = variable (in frequency)		
R = alkyl or aryl	Alk = alkyl	Ar = aryl

Example. νOH (in CCl_4) 3300wm, br. signifies an OH stretching mode which gives a broad absorption band in CCl_4 of weak to medium intensity at 3300 cm^{-1}.

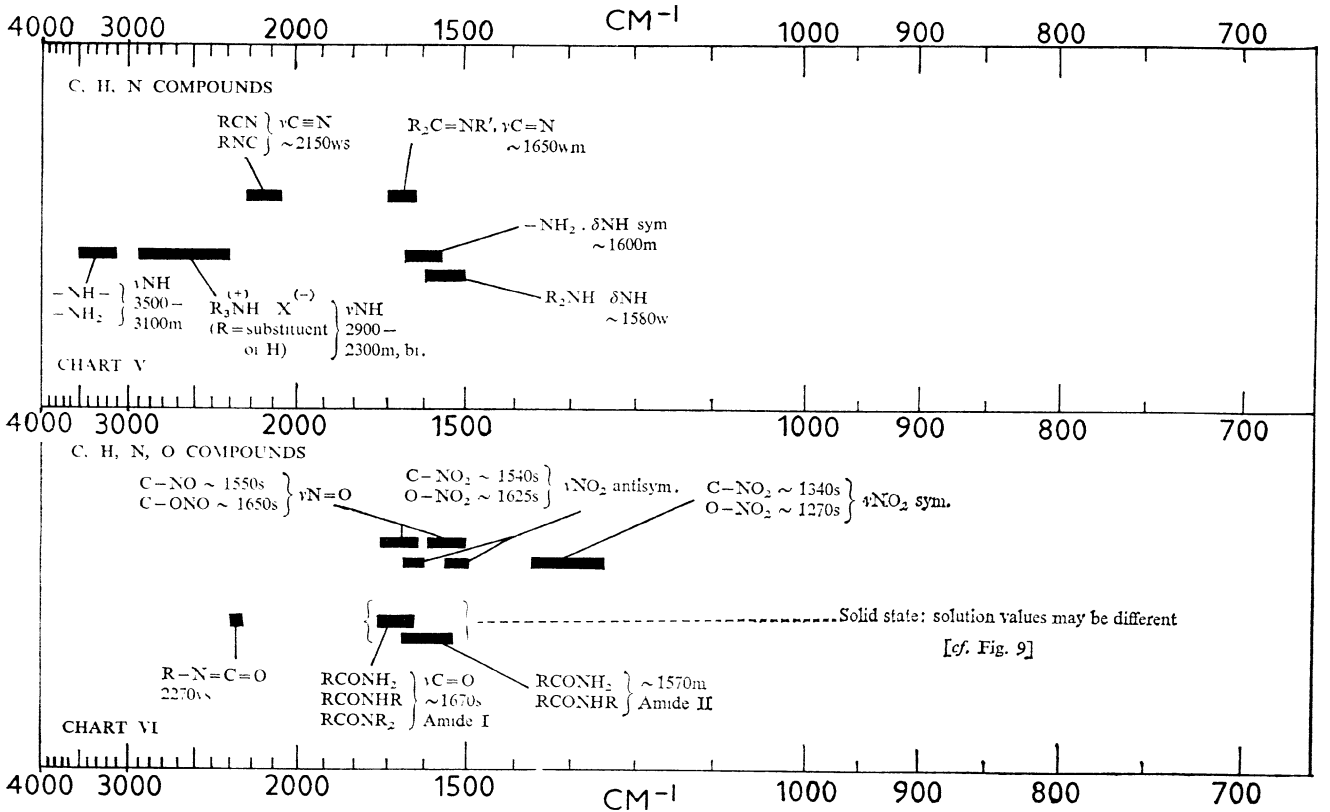


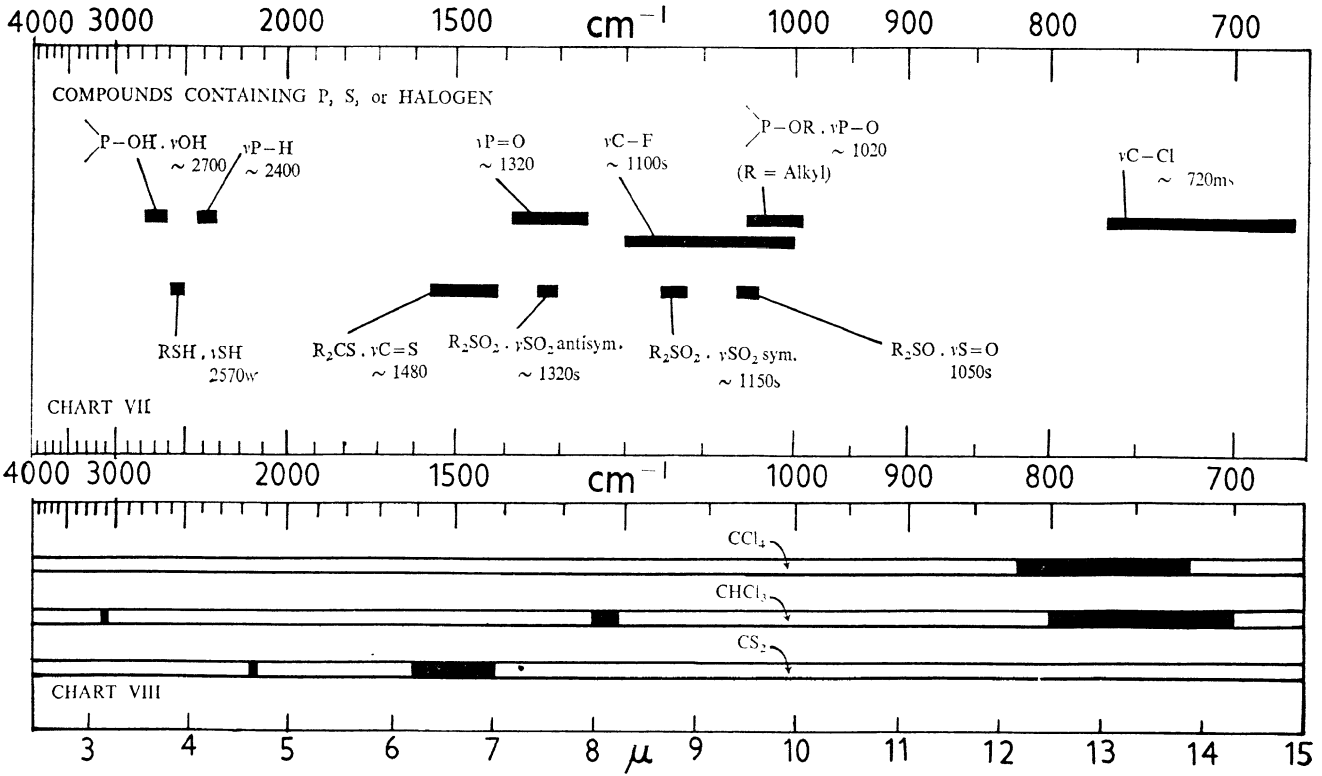
† This refers to all the systems to the left of the bracket.



[1] see pages 64, 73 and 76.

[2] see page 73 and Fig. 9, p. 75.


4000 3000 2000 1500 CM⁻¹ 1000 900 800 700
C, H, N COMPOUNDS
RCN } νC≡N
RNC } ~2150ws
R₂C=NR', νC=N ~1650wm
–NH₂ . δNH sym ~1600m
R₂NH δNH ~1580w
–NH– } νNH 3500–3100m
–NH₂ }
R₃NH X(–) (R = substituent or H) } νNH 2900–2300m, bt.
CHART V
4000 3000 2000 1500 1000 900 800 700
C, H, N, O COMPOUNDS
C–NO ~ 1550s
C–ONO ~ 1650s } νN=O
C–NO₂ ~ 1540s
O–NO₂ ~ 1625s } νNO₂ antisym.
C–NO₂ ~ 1340s
O–NO₂ ~ 1270s } νNO₂ sym.
Solid state: solution values may be different
[cf. Fig. 9]
R–N=C=O
2270vs
RCONH₂
RCONHR
RCONR₂ } νC=O ~1670s Amide I
RCONH₂
RCONHR } ~1570m Amide II
CHART VI
4000 3000 2000 1500 CM⁻¹ 1000 900 800 700



= opaque regions for 0.1 mm. thicknesses of common solvents (Chart VIII)

Correct recognition of a band is dependent on an appreciation of whether its approximate intensity is reasonable for the assignment in question. Quantitative solution data are preferable, but some idea can be gained from solid state or other qualitative spectra by careful comparison of the intensity of the band with the intensities of more readily-assigned vibrations in the same spectrum.

Careful intensity measurements (ε^a or, better, B) can be of value in determining the number present of a particular molecular grouping. For example, the hydroxyl, ketone and acetate groupings have been determined in this way, the intensity values for model compounds under the same conditions being taken as standards. Identical groupings in the same molecule, when sufficiently far apart not to interact, usually behave additively.

Summary. Examine the region 3650–1500 cm^{-1} for the principal functional group absorptions. Seek confirmation of assignments in rest of spectrum and allow for confusion caused by other strong absorptions. Change state, solvent and concentration for information on association, nature of groupings, etc. Use in conjunction with other methods (ultraviolet, etc.). Confirm presence or absence of groupings by chemical or other means. Try all the structural possibilities for agreement with the spectra. Intensities should be of the right magnitude. Make direct comparisons with closely related compounds. Derivatives of the compound, and degradation and elaboration products, should show predictable changes in absorption maxima.

Factors Affecting Group Frequencies

The factors affecting the frequency and intensity of group absorptions have been widely studied. In the following section they are classified under two heads—*Physical State* i.e. factors operating from *outside* the molecule containing the group, and *Intramolecular Factors* i.e. those operating from *within.* The discussion has as its core the behaviour of three important groupings—the carbonyl group, the hydroxyl group, and the carbon–carbon double bond.

For many organic chemists the most vital single attribute of infrared spectroscopy is the ability first to detect and then to distinguish the different types of *carbonyl group* (Chart IV). The group is polar, $\overset{\delta+}{\backslash}C=\overset{\delta-}{O}$, and may be conveniently regarded in terms of relative contributions of the hypothetical canonical forms, $\backslash C=O \leftrightarrow \overset{+}{\backslash}C-\overset{-}{O}$. Thus,

any of the effects discussed below which tend to increase the contribution of the single bond form will lower the force constant of the carbon–oxygen bond and will result in a fall in frequency of the stretching vibration $\nu C{=}O$. Similar approaches can sometimes† be applied to other X=Y groupings, e.g. C=N, S=O, P=O, etc.

In the case of the *hydroxyl* group (Chart IV), we are concerned chiefly with the readily-identified O–H stretching mode, νOH. Useful correlations are also available for $\nu C{-}O$ and for δOH and γOH, though the last three modes are often coupled one with another with the result that the actual absorptions may not strictly be assigned in these simple terms. Most of the changes in νOH derive, firstly, from the ability of the slightly acidic hydrogen to form 'hydrogen bonds' with electron-rich atoms (X), outside (intermolecular) or inside (intramolecular) the molecule, and secondly, from the extent to which electrons are withdrawn from, or supplied to, the oxygen atom. Hydrogen bonding normally results in small to large frequency drops in νOH, together with proportionate broadening and intensification (p. 61 and Fig. 6).

$R-\overset{\delta-}{O}-\overset{\delta+}{H} \leftarrow :\overset{\delta-}{X}-$	$R \leftarrow O \leftarrow H$	$R-O-H \leftrightarrow R=\overset{+}{O}-\overset{-}{H}$
Intermolecular hydrogen bonding	Inductive electron withdrawal	Mesomeric electron withdrawal

The relatively non-polar *carbon–carbon double bond*, e.g. $R^1R^2C{=}CHR^3$, presents an interesting contrast to the above polar groupings. Three vibrations, νCH, $\nu C{=}C$ and γCH, are of prime importance in the detection of an olefinic bond and in the identification of its type (Chart III). The γCH mode is probably the most valuable on account of its moderate to strong intensity and the relative constancy of its frequency for a given substitution type. The appearance of the weak, but sharp, νCH peak near 3050 cm^{-1} on the high frequency side of the main aliphatic νCH absorption is a useful guide, but neither it nor $\nu C{=}C$ are as reliable as γCH for the identification of substitution type. $\nu C{=}C$ is often so weak, for example in pseudo-symmetrical systems like $R^1R^2C{=}CR^3R^4$, that it is easily missed; in fact, a fairly strong band is indicative of either the unsymmetrically substituted groupings

† The need for caution is illustrated by the behaviour of the sulphoxide group S=O. The stretching absorption, $\nu S{=}O$ ~1050vs cm^{-1}, is known to be more or less independent of ring strain (p. 63) and of substitution by C=C (p. 65); the C–S and S=O links are not coplanar as are the C–C and C=O bonds of the carbonyl group, and some effects are not transmitted to the S=O linkage.

in $R^1R^2C=CH_2$ and $R^1CH=CH_2$, or of other polar-type double bonds discussed below.

Physical State and Group Frequencies

Infrared spectra are dependent on the physical state of the sample. Specific group frequencies show a general trend to lower values from gas → liquid → solid but are sometimes much perturbed by interactions such as hydrogen bonding. Molecules are nearly always single in the *vapour* or *gas state* and the group frequencies ν^{gas} or $\nu^{vap.}$ are often quoted as the reference values for 'solvent shifts' (p. 58). In the *liquid state* each molecule is involved in frequent collision with the surrounding cage of identical molecules. The group frequencies of non-polar molecules show a 1–2% drop commonly ascribed to the very considerable increase in dielectric constant from the gas to the liquid state. Polar groups experience much larger shifts. Group frequencies for the pure liquid state are less valuable than corresponding solution data because the environment of the molecule inevitably varies with each compound.

Solid state spectra are extremely useful for qualitative work, though not satisfactory for precise correlations. Unless the sampling method has resulted in partial orientation, the spectrum is that of the randomly oriented unit cell. The group frequencies reflect the influence of the molecules on one another. Crystal forces, hydrogen bonds or dipolar interactions can have quite dramatic effects. Thus, splitting of the $-(CH_2)-$ rocking mode (Spec. 15, p. 90) can result from the interaction of the movement of one $-CH_2-$ with that of another $-CH_2-$ in a parallel chain. The 'crystallinity' of a sample of polythene can be estimated from the extent of this splitting, since the non-orientated chains give only a single peak. It is generally true that for flexible molecules (e.g. long-chain acids, esters, etc.) the solid state spectra, especially in the fingerprint region, are much sharper and more distinctive than the liquid state or solution spectra; the innumerable conformations present in the liquid state generally result in broad bands. Rigid systems (e.g. the steroids) give almost equally sharp spectra in both states. Cyclohexyl bromide, an equilibrium mixture of conformers in the liquid state, crystallises with the bulky bromine atom equatorial and the numerous bands solely due to the axial isomer then disappear. Hydroxy or amino-groups of solid compounds are almost invariably bonded either to each other or to other basic centres and the OH and NH stretching modes are broader and at lower frequencies than those of the free molecules (p. 61). Polymorphic forms of the same substance can

have very different spectra especially in the fingerprint region. Consequently, if two solids are to be identified both should have been crystallised from the same solvent.

It is possible to modify the optics of an infrared instrument so that the radiation is largely *polarised* in one plane. Spectra of single crystals or of orientated fibres may then be measured with the electric vector first parallel to a crystal plane and then at right angles to it. The differences between the two spectra reveal the approximate planes in which the absorbing groupings lie, since the dipoles can only absorb radiation when they are in the same plane as the electric vector. Some examples of this technique, and its use for the study of crystalline polypeptides etc., are given in ref. 8.

Solution data. Much useful information can be obtained by comparing group frequencies which have been measured in solution. The dilution must be sufficient to eliminate solute–solute interactions. In non-polar solvents, such as *n*–hexane and carbon tetrachloride, the changes in group frequencies from one compound to another give a measure of the various factors operating *within* the molecules (pp. 62–78). In polar solvents, such as acetonitrile (CH_3CN) and chloroform, specific associations (e.g. hydrogen bonds and dipole interactions) occur, and the group frequencies of solute molecules no longer have the relationship to one another that they had in hexane or CCl_4. Consequently care is necessary when interpreting solution data acquired in polar solvents.

The absorption behaviour of different types of groups is summarised in Table 1. The dielectric constant of the solvent appears to have only a minor effect. There is often a close correlation between the shift of infrared group frequencies from one solvent to another ('solvent shifts') and solubility differences, ultraviolet spectral shifts, and changes in optical activity and dipole moment. Actual values for certain group frequencies are given in Table 2. Solvent shifts $\Delta\nu$ are commonly expressed as fractions of the group frequency.

$$\frac{\Delta\nu}{\nu} = \frac{\nu(\text{hexane}) - \nu(\text{solvent})}{\nu(\text{hexane})}$$

There is always a small fall from hexane to carbon tetrachloride values, $\Delta\nu/\nu$ being approximately the same for all the groups in Table 2 (CS_2 and CCl_4 values are approximately the same).

TABLE 1. Effect* of solvents on group frequencies of solutes

Grouping in solute	*'Acidic' solvent e.g.* $CHCl_3$, $CHBr_3$	*'Basic' solvent e.g.* OR_2, NR_3 *and aromatics* (π *electrons*)
$\backslash^{\delta+}\ ^{\delta-}$ $X=Y$ dipole $/$ (e.g. C=O, C=N, P=O, S=O, N=O)	Weak to moderate association. $\nu X=Y$ falls and broadens.	No association. $\nu X=Y$ approx. that in CCl_4.
$\backslash^{\delta-}\ ^{\delta+}$ $X-H$ dipole $/$ (e.g. OH, NH, SH, acidic† CH)	Weak association. νXH may fall slightly (possibly due to association of solvent with the O or the N atom).	Strong association. νXH falls, broadens and intensifies.
Non-polar X=Y (e.g. C=C and N=N)	Very little shift, unless vibration νXY is coupled with that of a polar group, or involved in an interaction such as Fermi resonance.	

* Effect relative to the non-polar solvents, hexane or CCl_4. Dilute solutions; solute–solute interactions are not considered.
† e.g. R – C≡CH

TABLE 2. Values for group frequencies in different solvents

Solute	*Group frequency*	*Vapour*	*Hexane*	CCl_4	$CHCl_3$	*Ether*
$PhCO_2Me$	$\nu C=O$	*	1735	1730	1720	1730
$PhCO_2H$	$\nu C=O$†	*	1750	1744	1734	1726
$PhCOCH_3$	$\nu C=O$	1709	1697	1692	1683	1694
PhOH	νOH	3654	3622	3611	3595	3344
Pyrrole	νNH	3530	3506	3500	3486	3352
PhC≡CH	νCH‡	3337	3323	3313	3309	3250
Phyllocladene§	νCH	*	3068	3066	3067	3069
	$\nu C=C$	*	1657	1656	1654	1658
	δCH	*	873	876	876	873

Values have been determined with grating spectrometers and are probably correct to $\pm 1\ cm^{-1}$.

* not measured
† monomeric species
‡ ethynyl CH
§ see p. 92

For $CHCl_3$, the $\Delta\nu/\nu$ values are somewhat larger for $\nu C=O$ than for $\nu X-H$, the acidic hydrogen of the solvent presumably being involved in a hydrogen-bond type association $\backslash C=O\cdots H-CCl_3$; $\nu C=O$

consequently falls (p. 56). In ether the reverse holds, the X—H groupings forming quite strong hydrogen bonds with the ether molecules, $X-H\cdots OEt_2$ (i.e. $X-H \leftarrow : OEt_2$). Consequently $\nu X-H$ drops markedly for the acidic OH, NH and CH groups of phenol, pyrrole and phenylacetylene respectively. The carbonyl values for methyl ben-

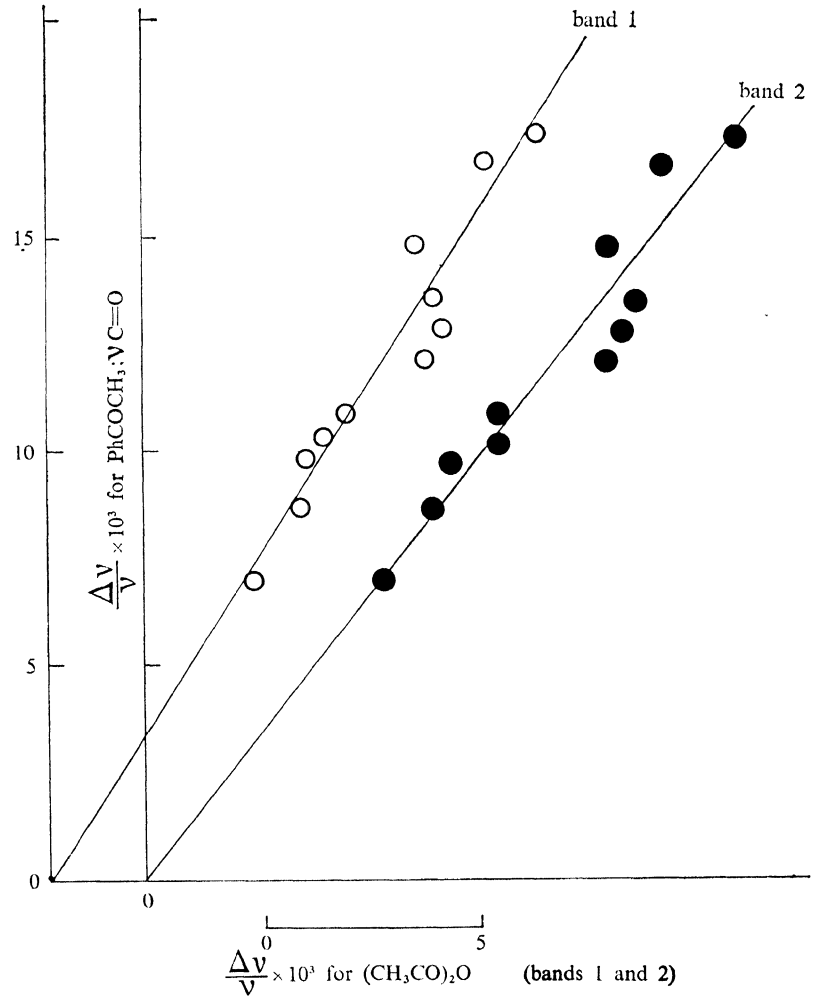


FIG. 5. 'Solvent-shift' diagram for the two bands in the carbonyl region of acetic anhydride. Each point represents the $\Delta\nu/\nu$ value for a particular solvent (from ref. 23). The reference solvent is *n*-hexane ($\Delta\nu/\nu = 0$).

zoate and acetophenone approximate to those in CCl_4 but the acidic hydroxyl of benzoic acid forms a complex $PhCOOH \cdots OR_2$ with the ether and the carbonyl value is therefore anomalous.

In contrast to the above shifts, the various modes of the comparatively non-polar olefinic double bond of phyllocladene are almost completely insensitive to solvent changes.

Solvent-shift information can be conveniently presented in graphical form, the shifts for a given band in the spectrum of one molecule being plotted against the shifts for a band in that of another, each point representing a single solvent. If a straight line results, it is a reasonable inference that both absorptions originate in groupings of similar type. Fig. 5 is such a plot for the *two* bands in the 1850–1700 cm^{-1} region of the spectrum of acetic anhydride, $(CH_3CO)_2O$. The solvent-shifts of both give good straight lines when plotted against those for the typical carbonyl group of acetophenone and it has therefore been concluded that the two bands are the result of coupling (p. 40) between the two identical carbonyl groups.

Carbonyl groups vary in their susceptibility to solvation and hence give different slopes for the $\Delta\nu/\nu$ plots. It might be thought that the more polar $\overset{\delta+}{\diagdown}\overset{\delta-}{\diagup}X=Y$ links, i.e. those with the lower frequencies, would be most solvent-sensitive, but other factors, such as steric hindrance, the presence of neighbouring electronegative and hydrogen-bonding substituents, and Fermi resonance, often complicate the situation.

Intermolecular hydrogen bonding. Molecules which bear active-hydrogen groupings, $-\overset{\delta-}{X}-\overset{\delta+}{H}$, usually self-associate readily and their

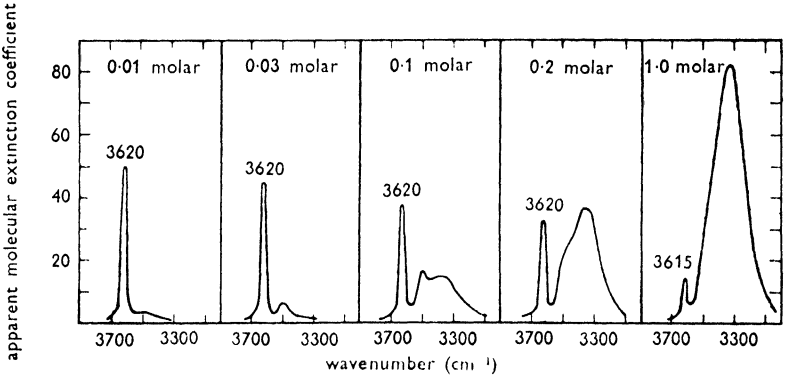


FIG. 6. The hydroxyl stretching absorptions, νOH, of cyclohexanol at various concentrations in CCl_4 (from ref. 2)

spectra are accordingly very dependent on the state of the sample. Hydroxy-compounds in the solid and liquid states normally exist as polymeric aggregates held together by hydrogen bonds. The resultant wide band at *ca.* 3300 cm^{-1} is of little use for diagnostic purposes and precise information concerning the type of hydroxyl group present is best obtained by studying the compound in dilute solution in an inert solvent (such as CCl_4), when the polymers break up, first to trimers or dimers and then to monomers (Fig. 6). The low frequency, broad, intense, 'bonded' νOH band† $\begin{bmatrix} O-H\cdots O-H\cdots O-H\cdots \\ | \quad\quad | \quad\quad | \\ R \quad\quad R \quad\quad R \end{bmatrix}$ diminishes and the high frequency, sharp, rather weak ($\varepsilon^a \sim 60$), 'free' νOH peak of the unbonded hydroxyl group increases. Other modes of the hydroxyl group, $\nu C-O$ and γOH for example, are not affected to the same extent (cf. Spec. 11, p. 86). Most hydroxy-compounds are monomeric in CCl_4 at concentrations less than 0·005M (cells of $\sim$1 cm path required) and if there is no intramolecular H-bonding (p. 66) an ordinary alcohol will absorb close to 3625 cm^{-1}.

The hydroxyl groupings will sometimes bond intermolecularly with other basic centres; when a carbonyl group is involved, as is the case with the carboxylic acids, $\nu C=O$ falls (p. 100). The tightly bonded dimers only break up in very dilute or polar solutions.

Intramolecular Factors and Group Frequencies

A large amount of information has been accumulated concerning the way in which factors operating *within* a molecule affect the characteristic group frequencies. Several factors may be operating; if they cancel one another their presence may be unsuspected.

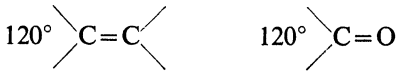
Mass changes. Isotopic substitution provides the simplest form of mass change, since the electrical nature of the atom is not altered. The exchange of an H atom for a D atom in a CH, NH or OH link has proved useful as a means of checking the nature of the grouping concerned. Thus for C–H, $\bar{m} \sim 1$ and for C–D, $\bar{m} \sim 2$ (see p. 38); hence $\nu C-D \simeq \nu C-H/\sqrt{2}$, i.e. $\sim 2300\,cm^{-1}$. Deviations from these expected values are usually explained as being due to some degree of coupling between the vibrations and those of adjacent atoms.

Mass changes of substituent groups generally have little influence on

† There is no single, unanimously-held theory to account for the unexpected breadth and intensity of most bonded νOH bands, but the shift to lower frequencies can be interpreted in terms of a lengthening and consequent weakening (lower force constant, lower frequency) of the O – H link.

functional group absorptions; for example diethyl ketone and the long-chain ketone $CH_3(CH_2)_8CO(CH_2)_{18}CH_3$ have $\nu C=O$ (in CCl_4) at 1720 and 1717 cm^{-1} respectively. In fact, it is this lack of dependence on molecular size which permits the functional group approach to the interpretation of infrared spectra.

Strain. Doubly-bonded carbon atoms in supposedly unstrained molecules have planar, trigonal bond angles.



The absorptions $\nu C=C$, νCH, δCH, γCH and $\nu C=O$ etc. of such groupings are regarded as normal. Enforced deformation of these

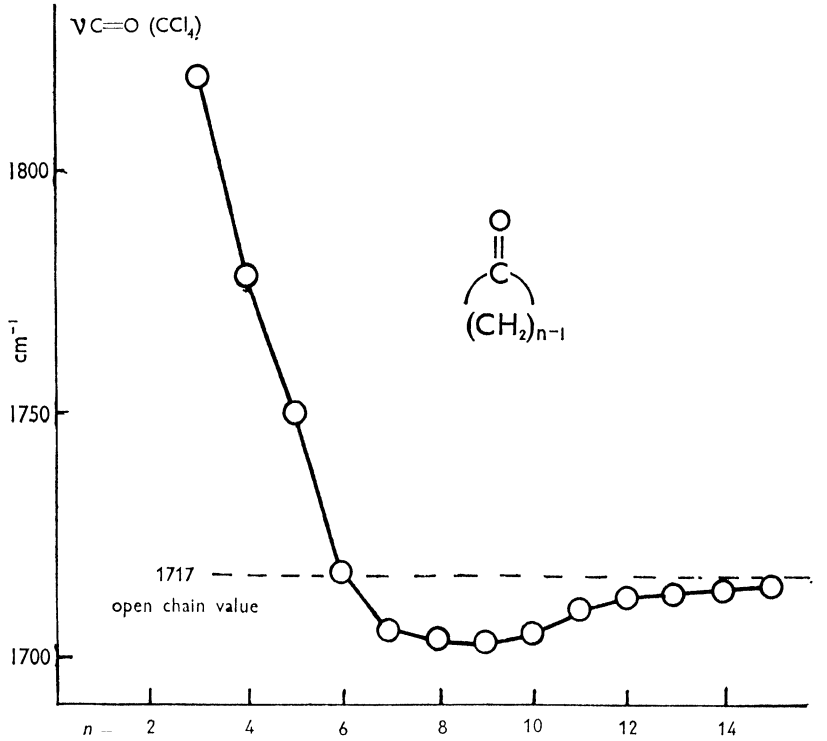


FIG. 7. Variation of $\nu C=O$ (in CCl_4) with ring size of alicyclic ketones

bond angles, within ring systems (ring strain) or as a result of bulky substituents ('steric compression', p. 72), affects these frequencies. For

example, as the angle subtended at the *carbonyl group* diminishes (6-ring, ~120°, 5-ring ~109°, 4-ring ~90° etc.), $\nu C=O$ rises. The flexibility of carbocyclic rings larger than 8-membered permits the adoption of strainless, non-planar conformations and $\nu C=O$ approximates to the value for acyclic long-chain ketones (1717 cm^{-1}; Fig. 7). The differences (summarised in Table 3) between the '5' and the '6' ring values for ketones, lactones, lactams and anhydrides have been widely used diagnostically. Bicyclic systems are more complicated to deal with, though on the whole strain is increased.

The effect of ring size on the absorptions of *carbon–carbon double bonds* is illustrated in Table 4. νCH and γCH are both slightly affected but the $\nu C=C$ values are the most interesting. The *exo*methylene group, being formally analogous to a carbonyl group, likewise shows a rise in the stretching frequency on going from 6- to 5-ring. The frequency for endocyclic double bonds conversely falls as ring size is decreased.

TABLE 3

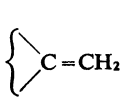
$\nu C=O$ values (grating, in CCl_4) for 5- and 6-membered rings and acyclic systems

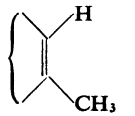
	Ketone	*Lactone* (*ester*)	*Lactam* (*amide*)	*Anhydride**
	–CO–	–CO–O–	–CONMe–	–CO–O–CO–
5 Ring	1751	1785	1698	1871, **1795**
6 Ring	1717	1750	–	1821, **1774**
Acyclic†	1719	1749	1662	1832, **1762**

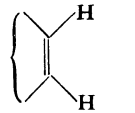
* main peak in bold type.
† refers to CH_3COCH_3, $CH_3CO_2CH_3$, CH_3CONMe_2, and $(CH_3CO)_2O$.

TABLE 4

Exocyclic and endocyclic double bonds in 5- and 6-membered rings



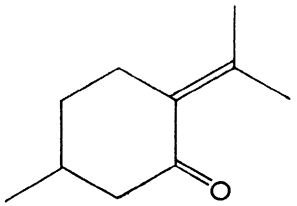




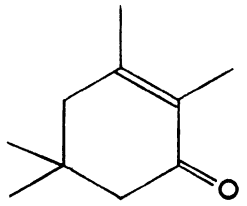
	νCH	$\nu C=C$	γCH	νCH	$\nu C=C$	γCH†	νCH†	$\nu C=C$†	γCH†
5	3073	1656	879	3053	1657	791	3045	1612	695
6	3072	1648	892	3040	1670	800	3020	1650	718

Grating values for CCl_4 solutions, except for † (CS_2).

Conjugation. When a *carbonyl group* and a *carbon–carbon double bond* are conjugated, both stretching frequencies fall, the classical viewpoint being that there is a contribution of the type $\overset{+}{C}-C=C-\overset{-}{O}$ (increased single bond character for C=O and hence lower frequency). There is usually an intensification (ε) of the C=C absorption and a broadening (increase in area, A) of the C=O frequency. The γCH modes are also affected, the frequencies rising, e.g. *trans*-disubstituted ethylene, $\gamma CH \sim 970 \rightarrow 990\ cm^{-1}$, and *cis*-disubstituted ethylene, $\gamma CH \sim 700 \rightarrow 820\ cm^{-1}$. Conjugated systems are fairly easily spotted in the infrared but identification of the exact type is more readily achieved in the ultraviolet region (p. 139). For the carbonyl group there is a drop of about 30 cm^{-1} for the first C=C in conjugation, and of approximately 15 cm^{-1} for the next one; thereafter the effect diminishes rapidly. As in the ultraviolet, the position, stereochemistry and degree



cisoid



transoid

(CCl_4 solutions)

Band	*cisoid* ν	$\Delta\nu_{\frac{1}{2}}^{a}$	ε^{a}	$10^{-2}A$	*transoid* ν	$\Delta\nu_{\frac{1}{2}}^{a}$	ε^{a}	$10^{-2}A$
C=O	1687	21	260	190	1669	19	454	295
C=C	1618	57	60	125	1639	14	82	35
	$A_{C=O}/A_{C=C} = 1{\cdot}52$				$A_{C=O}/A_{C=C} = 8{\cdot}4$			

ν, $\Delta\nu_{\frac{1}{2}}^{a}$ in cm^{-1}; ε^{a} in litre $mole^{-1}\ cm^{-1}$ and A in litre $mole^{-1}\ cm^{-2}$ (see p. 41)

of substitution of the double bond definitely affect the frequency, shape and intensity of the band maxima but no rules of the ultraviolet (Woodward) type (p. 140) are yet available. However, it has recently been shown[22] that the νC=C absorption of *cisoid* (i.e. *s–cis*) $\alpha\beta$-unsaturated ketones can be almost as strong as the νC=O absorption, whereas in *transoid* systems νC=C is relatively much the weaker. Area measurements provide the clearest differentiation (see Table above). This relative enhancement of the νC=C band in the *cisoid* form is ascribed to a larger induced dipole in the C=C bond (cf. the dipolar effect of α-halo-

gen atoms, p. 70). Another feature of these systems is that the carbonyl frequency of a *cisoid* enone is generally higher than that of the corresponding *transoid* enone by some 20 cm^{-1}.

Conjugation of heteroatoms with carbonyl groups *via* double bonds is discussed on p. 69.

Intramolecular hydrogen bonds. An acidic hydrogen, e.g. that of OH or NH, is sometimes sufficiently close to a basic centre (e.g. O<, O=C<, N< etc.) in the same molecule for the formation of intramolecular hydrogen bonds to be energetically favourable. The OH stretching frequency for an intramolecularly bonded hydroxyl is at a

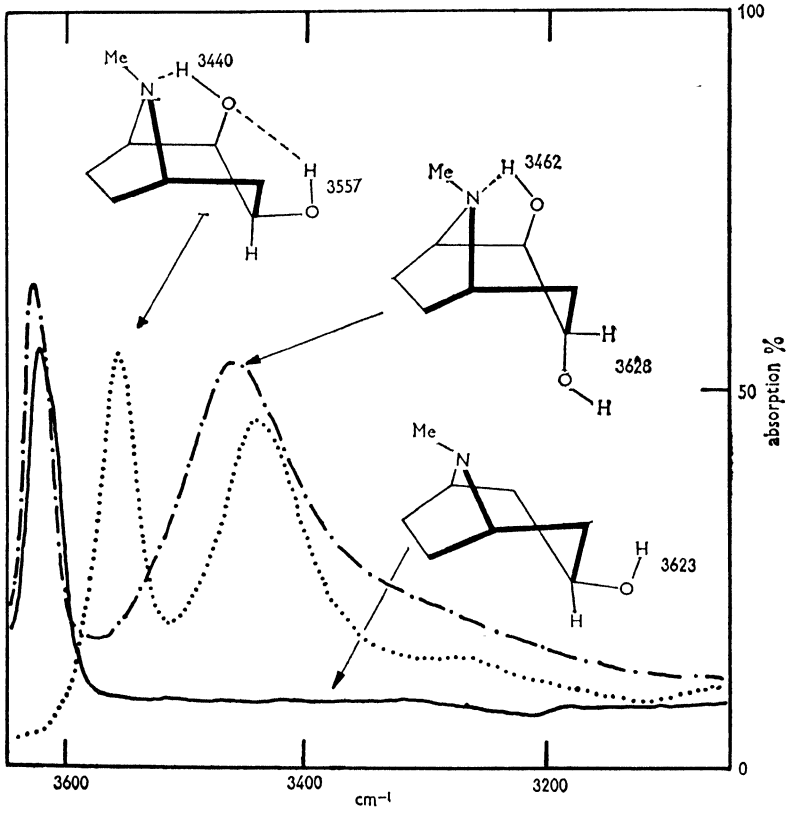
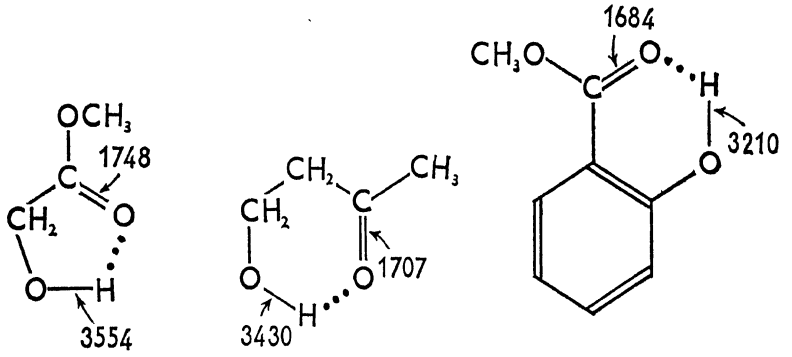


FIG. 8. νOH bands of ψ-tropine (0·00125M in 5 cm cell) and of two 2,3-dihydroxy-tropanes (0·013M, 5 mm) in CCl_4 (from *Spectrovision*, No. 9, 1960, by courtesy of Unicam Instruments Ltd.)

weak *medium* *strong*



compare: (grating values, CCl_4)

CH_3CO_2Me, $\nu C{=}O$ 1749 CH_3COCH_3, $\nu C{=}O$ 1719 $PhCO_2Me$, $\nu C{=}O$ 1730
C_2H_5OH, νOH 3625 C_2H_5OH, νOH 3625 PhOH, νOH 3611

TABLE 5. Frequency shifts for intramolecular hydrogen bonds

Hydrogen bond strength	*Approx. shifts from non-bonded values* (cm^{-1}) $\Delta\nu OH$	$\Delta\nu CO$†	*Compound type*
weak	<100	<10	1, 2- and 1, 3-diols (cf. Fig. 8); α- and β-hydroxy-ketones and -esters, etc.; α- and β-alkoxy alcohols.
medium	100–300	10–50	some hydroxy-ketones; hydroxy-amino compounds (cf. Fig. 8); hydroxy-nitro compounds.
strong	>300	50–150	conjugate chelate systems (p. 104) e.g. *ortho*-hydroxyaryl-ketones, -acids or -esters; hydroxy-quinones; tropolones; β-diketones etc.

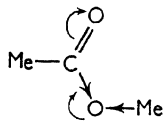
† when the carbonyl group is the basic centre.

lower value than that for a free hydroxyl and is broader. When the basic centre is a carbonyl group, $\nu C{=}O$ also falls somewhat. The crucial

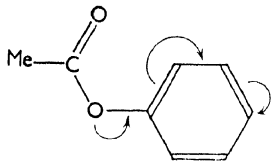
test for *intra*bonding is that these absorptions should not be affected† by dilution (intermolecular associations break up, page 61). Thus, the spectra shown in Fig. 8 for the two tropane diols are concentration independent and the assignments of configuration and conformation have been made by considering the interatomic distances between the acidic hydroxyls and the available basic centres. For an OH...O bond the maximum permissible oxygen–oxygen distance is ~3 Å. At lower values the hydrogen bond frequency shift, $\Delta\nu$ [i.e. $\nu OH^{free} - \nu OH^{intrabonded}$] has an inverse linear relationship with the O...O distance.

There are, very roughly, three types of intramolecular hydrogen bond: weak, medium and strong. The resultant ring is usually 5- or 6-membered, including the hydrogen atom. Three examples are given above and frequency shifts are summarised in Table 5.

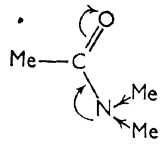
Direct substitution with electronegative atoms. Direct substitution by an electronegative atom considerably affects the *carbonyl group*. Thus, in a molecule of the type CH_3CO-X, the double bond character of the C=O group is dependent on the electronegativity of X (withdrawal of electrons along bonds, inductive process, −I) and on its power to supply electrons by the mesomeric process (+M). The double bond character is increased (νC=O rises) by electron withdrawal and de-



1749



1768



1662 cm^{-1}

νC=O (in CCl_4)

creased (νC=O falls) by electron supply. The actual carbonyl frequency depends on the balance between these two effects (see Fig. 9, p. 75). The high value for Cl is interpreted as being almost entirely due to electron withdrawal by this electronegative atom. The competing mesomeric process brings the frequency down somewhat in the −OR series

† Weak intrabonds will exist only when the molecule is isolated or surrounded by non-polar molecules, as in a dilute solution in an inert solvent; in concentrated solutions and in the liquid or solid states they may be superseded by stronger *inter*molecular bonds.

(see above). With the methyl ester this is assisted by a +I effect but in the phenyl ester it is opposed by delocalisation (−M) of the electrons into the phenyl ring. In the amide series the +M character of the nitrogen atom seems to take precedence and $\nu C=O$, the amide I frequency, is very low.†

Esters show an interesting inverse proportionality between the stretching frequency of the carbonyl group and that of the acyl C−O (see Table below). Electronic changes in the substituents R and R′ affect the electron distribution, and hence the force constants and stretching frequencies, of both the C=O and the C−O bond. This relationship can be diagnostically useful.

Esters	RCOOR′	$\nu C=O$ values in CCl_4 (cm^{-1})	$\nu C-O$‡
phenolic acetate	CH_3COOPh	1768	1213, 1198
enolic acetate	$CH_3COOCH=CH_2$	1765	1221, 1142
phenyl benzoate	PhCOOPh	1744	1264, 1198
alkyl acetate	$CH_3COOAlk$	1745	1245
alkyl benzoate	PhCOOAlk	1725	1270

‡ Band splitting probably due to coupling with other modes.

Attachment of an electronegative atom to a *carbon–carbon double bond* has similar, but less marked, effects. The increase in double-bond polarity results in a more intense $\nu C=C$ absorption and a shift of γCH to lower frequencies. For example, vinyl ethers, $CH_2=CHOR$, show $\nu CH \sim 3150w$ (several bands), $\nu C=C \sim 1630s$ (sometimes two bands) and $\gamma CH \sim 960s$ and 810s (a normal vinyl group absorbs at 3080wm, 1640m, 990s and 910s). A double bond, α,β both to a carbonyl group and a heteroatom, e.g. N−C=C−C=O and O−C=C−C=O, may act as a means of partial electron transfer from the heteroatom to the carbonyl group (+M). Such 'vinylogous' systems (e.g. Spec. 36, p. 103) often have quite low carbonyl frequencies together with unusually intense $\nu C=C$ absorptions.

† Amides are to a considerable degree subject to association effects and their spectra in the solid or liquid state, or in concentrated solutions, are often markedly different from those recorded for the same compounds in dilute solution. For example, solid state values, $\nu C=O$, are as follows: CH_3CONH_2 1684, $CH_3CONHCH_3$ 1655 and 1639, $CH_3CON(CH_3)_2$ 1634 cm^{-1}; all broad and irregular (compare Fig. 9). The exact nature and specificity of these associations have attracted much attention as an understanding of them will greatly assist the study of the structures and behaviour of proteins.

The amide II frequency (see Chart VI) is a coupled mode of δNH and $\nu C-N$ and is diagnostically useful.

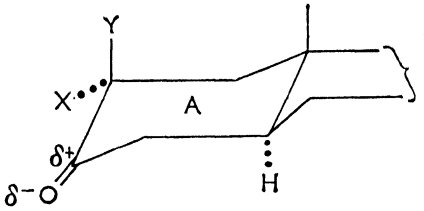
Hydroxyl groups are slightly affected by electronegative substituents thus:

	AlkOH	C_6H_5OH	$O_2NC_6H_4OH$ (*para*)	ROOH	RCOOH
νOH^{free}	3630	3610	3595	3550	3530
$\Delta\nu_{\frac{1}{2}}^{a}$	20	16	24	24	35
ε^{a}	60	200	250	60	150

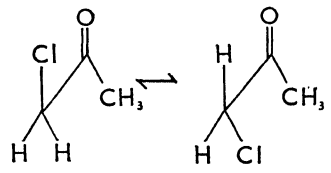
(grating values, in CCl_4)

Considerable increases in acidity of the hydroxyl, caused by electron loss from the oxygen atom, are paralleled by comparatively small drops in νOH.

Substitution by electronegative atoms on adjacent carbons. The *carbonyl frequencies* of α–haloketones are often higher than those of the parent ketones and the shift increases with increasing dipole moment of the C–X bond, F>Cl>Br>I. The effect is only operative when the C–X and C=O bonds are approximately co-planar (see below). It is believed that this effect results chiefly from an electrostatic interaction ('field effect') between the two similarly-oriented dipoles. In ring A of the steroids only the equatorial substituent, X, shows the



X	Y	νC=O (in CS_2)
H	H	1718
H	Br	1720
Br	H	1733
Br	Br	1735



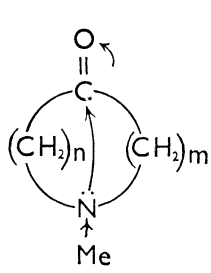
elevated νC=O 'normal' νC=O

upward shift of 15–25 cm^{-1}. This directional property would not be anticipated for a simple inductive effect. Where free rotation is possible, two or more conformers with different carbonyl frequencies may be present, e.g. in monochloroacetone (see above). The α–halogen effect extends to other carbonyl compounds, such as esters and lactones.

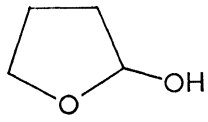
Co-planar oxygen atoms in the α–position also elevate the carbonyl

frequency though the effect is not so well documented. Both dipolar and inductive effects may be involved. α–Acetoxylketones show a marked upward shift of both the acetate and ketone carbonyls, thus $RCOCH_2OCOCH_3$ has $\nu C{=}O$ (in CCl_4) 1730 and 1756 cm^{-1} (compare Fig. 9). α–Hydroxyl groups usually hydrogen-bond (p. 66) with the adjoining carbonyl groups and the dipolar effect may not be evident. However, carbonyls bearing α–alkoxyl groups absorb at elevated frequencies, e.g. $MeCO_2Me$ and $MeOCH_2CO_2Me$ absorb at 1749 and 1761 cm^{-1}, respectively. Non-enolised α–diketones have $\nu C{=}O$ values only slightly above normal.

Rather surprisingly, a nitrogen atom substituent in the α-position has little effect though this topic has not been studied thoroughly. However, a prediction made by Robinson in 1922, that interaction effects might be expected across intramolecular space, provided that a nitrogen atom could be brought sufficiently close to a carbonyl group, has recently received support. In the type of cyclic compound shown, electron donation from the nitrogen lone pair evidently takes place for $\nu C{=}O$ is some 20 cm^{-1} lower than in cyclo-octane.



$n = m = 3$
$\nu C{=}O$ (in CCl_4) 1681 cm^{-1}



νOH 3620 cm^{-1}

Adjacent electronegative atoms have little effect on the vibrations of either *olefinic groups* or *hydroxyl groups*; νOH of the hemiacetal shown is quite normal (ν and ε).

Alkyl substitution. An increase in the chain length of *n*–alkyl groups, substituent on a *carbonyl group*, generally has little effect on $\nu C{=}O$ except that esters show a drop of *ca.* 5 cm^{-1} when methyl is replaced by ethyl. However, quite marked drops in frequency ($\sim$ 5 cm^{-1} per substituent) result on increasing the *alkyl substitution on the α-carbon atom* (e.g. ethyl to isopropyl to t-butyl). In esters and lactones, substitution appears to be equally effective whether it be on the carbon α to the carbonyl group or on that bearing the ether-type oxygen. These α–alkyl effects are sufficiently large to be of diagnostic importance,

especially in the terpenoid field, and are exemplified in Table 6. The Table shows that the above generalisations are only approximate; the inconsistencies may be explicable in terms of inductive, field and steric factors.

TABLE 6. Alkyl substitution and $\nu C=O$ (grating values, in CCl_4)

Esters	$\nu C=O$	*Ketones*	$\nu C=O$
$PhCO_2R$ ($\varepsilon\sim800$, $\Delta\nu_{\frac{1}{2}}^{a}\sim12$)		$RCOR'$ ($\varepsilon\sim300$, $\Delta\nu_{\frac{1}{2}}^{a}\sim15$)	
R = $-CH_3$	1730	CH_3COCH_3	1719
$-Et$	1724	EtCOEt	1720
$-Pr$	1724	Pr^iCOPr^i	1712
$-Pr^i$	1720	CH_3COBu^t	1711
$-Bu^t$	1717	Bu^tCOBu^t	1688
RCO_2R' ($\varepsilon\sim500$, $\Delta\nu_{\frac{1}{2}}^{a}\sim15$)		*Cyclohexanones* ($\varepsilon\sim450$, $\Delta\nu_{\frac{1}{2}}^{a}\sim12$)	
CH_3COOCH_3	1749	cyclohexanone	1717
CH_3COOEt	1744	2, 6–dimethyl cyclohexanone	1717
$CH_3COO(CH_2)_nCH_3$	1743	2, 2, 6–trimethyl cyclohexanone	1708
$CH_3(CH_2)_nCOOCH_3$	1743	2, 2, 6, 6–tetramethyl cyclohexanone	1698
$CH_3(CH_2)_nCOO(CH_2)_nCH_3$	1736		
$(CH_2)_{14}-COO$	1736		

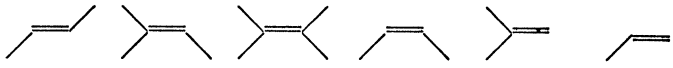
Increased alkyl substitution in *hydroxy-compounds* causes only small drops in νOH but quite large rises in $\nu C-O$ since the latter mode is coupled with the adjacent $\nu C-C$ modes (see Table below). However, $\nu C-O$ absorption is often difficult to pick out, especially in the spectra of polyfunctional compounds. Increasing α-substitution results in a decreased tendency to self-association (steric hindrance).

Carbinol	*Approx.* νOH (CCl_4) *'free'*	*Approx.* $\nu C-O$ (CCl_4)
RCH_2OH	3640	1050
R_2CHOH	3630	1100
R_3COH	3620	1150

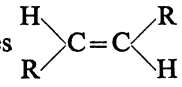
A high-resolution instrument (grating or LiF prism etc.) is necessary for meaningful measurement in the 3600 cm^{-1} region.

The degree and stereochemistry of alkyl substitution on *carbon–carbon double bonds* can often be deduced from the absorption patterns, particularly γCH modes (Chart III and the data below). Polar-substituted olefines may deviate considerably from the values given (p. 69).

Approximate positions and relative intensities for some double-bond absorptions



$\nu C=C$	1670vw	1670w	1670w	1650w	1650m	1640m
γCH	970s	820m	—	700w	890s	990s, 910s

(indicates  etc., — means 'no absorption')

Prediction of Approximate Values for Group Frequencies

In this section the effects, which have just been discussed, of factors influencing group frequencies have been summarised under each of the three groupings, C=O, OH and C=C. The empirically derived frequency shifts assigned to each factor are of necessity rather approximate as there are undoubtedly quite large and, at present, unexplained variations from one compound to another. However, provided it is realised that the frequency shifts are only approximate and that they are not strictly additive, Tables 7–9 should prove useful. A few examples illustrating their use are given later.

Table 7. The prediction of carbonyl frequencies

The 'parent' frequency is taken as that of cyclohexanone and of di-n-alkyl ketones in CCl_4 solution, i.e. ~1720 cm^{-1}.

Raise $\nu C=O$		Add (cm^{-1})
Solvent	Hydrocarbon solvents	+ 7
Ring strain	6-ring → 5-ring	+35
	Bridged system	+15
Replacement of alkyl group by an electronegative atom (other than N)	-H	+10
	-O-Alkyl (n-) (see p. 72)	+25
	-OH (monomer)	+40
	-O-C=C	+50
	-Cl	+90
Substitution on α-carbon; polar effect (co-planar; no shift if not approx. co-planar)	Halogen, -OR, -OH, -OAc, etc.	+20

LOWER $\nu C=O$		SUBTRACT (cm^{-1})
Solvent	$CHCl_3$, $CHBr_3$, CH_3CN	−15
Solid or liquid state		−10
Intermolecular hydrogen bonding	strong, e.g. RCOOH dimer	−45
	weak, e.g. single $ROH \cdots O=C$	−15
Intramolecular hydrogen bonding	weak (α– or β–hydroxyl)	−10
(cf. Table 5. Values are very	medium	−50
approximate)	strong (conjug. chelates)	−100
Conjugation	1st C=C *transoid–*	−35
	cisoid–	−20
	2nd C=C	−15
	3rd C=C	0
	Benzene ring	−20
	Vinylogous systems i.e. $-C-C=C-N-$ / $-C(=O)-C=C-O-$	−40
Replacement of alkyl group by N atom	$-NH_2$	−5
(absorptions very much lower and	−NHMe	−30
broader in solid state)	$-NMe_2$	−55
Substitution on α-carbon atom.	Each alkyl group	−5
Ring strain. (Table 3 and Fig. 7)	From 6 → medium-sized rings (7, 8, 9 etc.)	−15

Comments on Table 7

(1) Reliable correlations for intensity data are not yet available.

(2) Coupling (p. 40) and Fermi resonance sometimes disturb the carbonyl absorptions, especially when other groups are in close proximity within the molecule.

(3) Some of the above factors are, of course, interdependent e.g. the extent of intermolecular bonding is affected by the nature of the solvent. The values given are for dilute solutions. Shifts from one solvent to another are dependent on the carbonyl type (p. 61).

(4) Two carbonyl bands generally appear as separate peaks when they differ by more than 10 cm^{-1}.

(5) Fig. 9 illustrates the effect of substituting an electronegative atom or group X for one of the methyl groups of acetone.

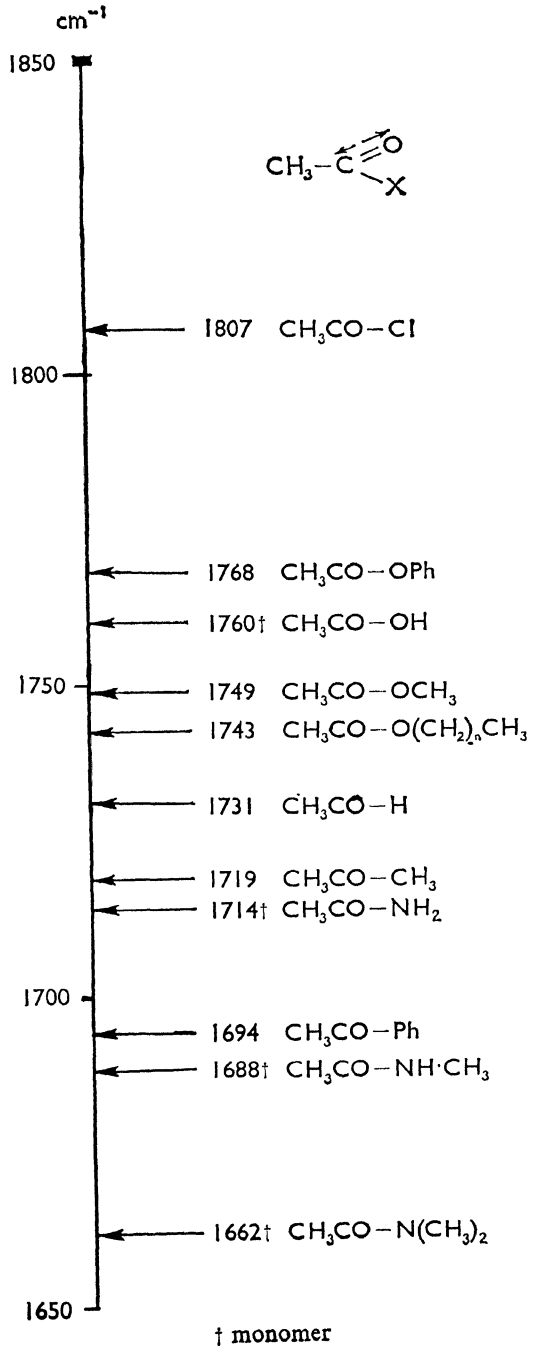


FIG. 9. $\nu C=O$ in CCl_4 for various types of carbonyl grouping, CH_3CO-X (grating values, dilute solutions, monomers only)

TABLE 8. The prediction of hydroxyl frequencies

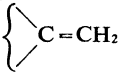
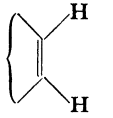
Sub-division in the manner used for the carbonyl group is not feasible as the various forms of hydrogen bonding are the major effects to be considered. 'Parent' frequencies for νOH (in CCl_4) are as follows: alcohols, 3625 ± 10 cm^{-1}; phenols, 3605 ± 10 cm^{-1}; carboxylic acids (monomer), 3530 ± 15 cm^{-1}.

RAISE		ADD (cm^{-1})
Solvents	Hydrocarbons	+ 10
LOWER		SUBTRACT (cm^{-1})
Solvents	$CHCl_3$, $CHBr_3$	− 15
	Ether	*ca.* −300
Interbonding	weak, e.g. polymeric HO···HO···	−300
	strong, e.g. RCOOH dimer	−500
Intrabonding (cf. Table 5)	weak (cyclic diols etc.)	− 20 to −100
	medium (hydroxy–amino cpds. etc.)	−100 to −300
	strong (conjugated chelates etc.)	−500 or more

With the exception of the conjugated chelates (pp. 67 and 104), the bigger the shift, the broader and more intense the bonded peak becomes.

TABLE 9. Prediction of carbon–carbon double bond frequencies

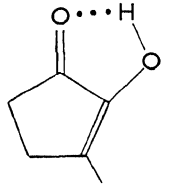
The absorption frequencies for simple olefines are summarised in Chart III, in Table 4 and on p. 73, to which reference should be made, particularly for the effects of substitution. Values for a *typical olefine* are νCH ~3040 cm^{-1} (ε 20), νC=C ~1650 (ε ~20). Physical state, solvent and concentration changes have almost no effect. The notes below can only be a rough guide, as the behaviour of olefinic modes is not readily classified.

Ring strain	6-ring → 5-ring (cf. Table 4)	*'exo'* 	νC=C rises ~10 cm^{-1} γCH falls
		'endo' 	νC=C falls ~35 cm^{-1}
Conjugation	'transoid enone'		νC=C falls ~30 cm^{-1} and intensifies (×3)
	'cisoid enone'		νC=C falls ~30 cm^{-1} and intensifies to give an ε comparable to that of νC=O γCH rises (50 cm^{-1}) in both cases
	diene		two νC=C bands, separation ~40 cm^{-1} and slight intensification
Electronegative substituent			
	Halogen, alkoxyl, etc. (acetoxyl gives only slight increase in ε)		νC=C intensifies considerably γCH frequency falls

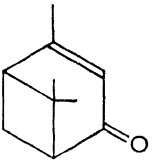
In vinylogous systems νC=C increases in intensity still further

Examples of prediction of group frequencies. The measure of agreement found below between predicted and experimental values is about average. In some cases, however, the conflicting factors result in large errors. All the values below are for CCl_4 solutions.

(*a*)



(*b*) verbenone
(Spec. 25, p. 97)



(*a*) *An enolised α–diketone*

νC=O		νOH		νC=C	
Parent	1720	Parent*	3610	Parent	~1650
6→5 ring	+35	intra H-bond	−200	Conjugation	−30
OR on α–carbon	+20			Substituent OH	?
intra H-bond (weak to medium)	−20				
Conjugation (transoid)	−35				
Predicted	~1720s		~3410m	Not predictable but ε^a will be unusually high (polar substituent)	
Found	1723s		3505m		1677s

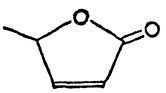
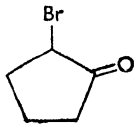
* The parent νOH value is assigned in view of the similarity with a phenolic OH.

(*b*) *A conjugated ketone*

νC=O		νC=C		γCH	
Parent	1720	Parent	1670	Parent	800
Bridged ring	+15	Bridged ring	−7	Bridged ring	−5
Conjugation (transoid)	−35	Conjugation (transoid)	−30	Conjugation	+50
α–Alkyl subst.	−5				
Predicted	~1695s		~1633wm		~845m
Found	1685s		1620wm		865wm

The 'parent' values for νC=C and γCH are from Table 4, p. 64 and the bridged ring contribution has been estimated as being approximately half that of a 6 to 5-ring contraction.

(c) *Unsaturated lactones and an α-haloketone*

$\nu C{=}O$			
Parent	1720	1720	1720
Ring strain	+35	+35	+35
Direct-OR substit.	+25	+50	—
α-Methyl (on C bearing O–CO–)	−5	−5	—
α-Bromo (neither *eq.* nor *ax.*)	—	—	+10
Conjugation	−35	—	—
Predicted	~1740	~1800	~1765
Found	**1782**† 1765	1806	1750

† Two bands are observed, that in heavy type being the stronger. This splitting has been ascribed to Fermi resonance (pp. 40 and 122). The frequency values are unexpectedly high.

PART II. INFRARED SPECTROSCOPY— EXAMPLES OF APPLICATIONS

Introductory comments. The spectra are recordings made with a Perkin-Elmer Infracord (Model 137, sodium chloride prism) which was in routine use by research students at the time. No special precautions were taken other than the polystyrene calibration checks (the sharp, strong peak at 1604 cm^{-1}) which were recorded on most of the charts immediately after the spectra themselves. A spectrum can be recorded in about 12 minutes (the latest model, 137-B, requires only 3 minutes). The horizontal scale is the normal Infracord scale (linear in wavelength, see Chart VIII), but the spectra are marked in cm^{-1} for convenience. The spectra are reproduced approximately $\frac{1}{3}$ actual size. Frequencies are best read off with a grid line (e.g. that on a plastic ruler). For the

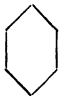
calculation of approximate intensity data (ε^a) subtract the 'base-line' optical density (p. 24) from that of the peak (the most extensive flat portion of the trace, e.g. between 1800–2500 cm^{-1} in Spec. 7, may be taken as the base-line). The instrument used is unlike most Infracords in that the vertical scale is % absorption rather than % transmission and the peaks are accordingly inverted (p. 26).

Impurity bands are indicated by 'imp.' and are regretted. Many 'pure' compounds lose their reputation on infrared examination. The KCl disc spectra often show weak to medium strength conical hydroxyl peaks at *ca.* 3500 and 1650 cm^{-1} due to adsorbed water (Spec. 43, 44 and 52). A dip or a peak at *ca.* 2350 cm^{-1} is usually due to incomplete balancing of the strong absorption due to the atmospheric CO_2 present in both beams. Similarly the 'wrinkles' at *ca.* 1600 cm^{-1} (e.g. Spec. 1) are due to incompletely balanced water-vapour vibration-rotation bands; they can be rather misleading when the weak $\nu C=C$ absorptions are being examined. In more complicated instruments the light path may be evacuated or filled with dry air. The solution spectra sometimes show negative absorption where imperfect balancing of the solvent has occurred e.g. at *ca.* 1550 cm^{-1} (CCl_4) and 1210 cm^{-1} ($CHCl_3$), cf. Chart VIII and Spec. 2, 4 and 35. This is especially true of very thin and very thick cells. Some compounds have been examined in several solvents or media in order to obtain a complete unobscured spectrum (e.g. Spec. 26, p. 97). The different portions of a particular spectrum have been 'matched' by adjusting the thickness or concentration of the sample until a common, unobscured band was of the same strength. Where frequency or intensity values are more accurately known from other work, they are quoted in preference to the data read directly from the spectra illustrated; 'G' indicates values obtained with a grating spectrometer (these are usually accurate to ± 1 cm^{-1}). For details of other abbreviations, see p. 50.

The examples will repay careful study and half an hour or more spent on a single example would not be excessive. It is suggested that each spectrum be compared first with the others in the example and then with the assignments indicated in the group-frequency charts (p. 51) and in the appropriate sections in the survey (pp. 55 to 78) of the factors affecting group frequencies. Finally, if desired, reference may be made to the major infrared texts [2–14] and to the original literature. The spectra themselves can be used as problems if they are first studied without the help of the captions which are generally on the facing page. Not all the bands capable of assignment have been assigned in the discussions.

Example 1. Solvents. The spectra of the mulling agents Nujol and Florube–3 (a chlorofluorocarbon) are given in Spec. 1. Spec. 2, 3 and 4 may be used to discern the regions which will be unobscured by solvent absorption when a solute is examined in the solvents CCl_4, CS_2 and $CHCl_3$. When a matched pair of cells filled with the same solvent is recorded (one in each beam) a *balanced-background* is obtained; in Spec. 3 the slightly thicker reference cell has resulted in slight *over-compensation* of the CS_2 absorption in the sample beam. Any absorption peaks recorded for a solute in CS_2 solution at this thickness (0·5 mm) would be valueless both between 2350 and 2100 cm^{-1} and between 1620 and 1400 cm^{-1} owing to the absorption of all the energy of the beam by the solvent.

Example 2. Functional group absorptions. (I) In simple alicyclic systems. The principal absorptions of the CH_2, C–O–H and C=O groupings emerge clearly in the series shown.

Compound	*Frequency* (cm^{-1})	*Assignment*	
Cyclohexane (Spec. 5) 	2900–2800s	νCH	CH_2; C – H stretching (two peaks)
	1455s	δCH	CH_2; C – H in-plane bend ('scissor')
	1270w	ω *or* τCH	CH_2; C – H out-of-plane bend
	1030w, 920, 870 (m)	Associated with cyclohexane ring	
Cyclohexanol† (Spec. 6) OH	3400s	νOH	OH stretch (interbonded)
	1380m	δOH	OH in-plane bend
	1080s	νC – O	C – OH stretch
	980s	Associated with COH groups	

† see p. 82 *continued on p.* 82

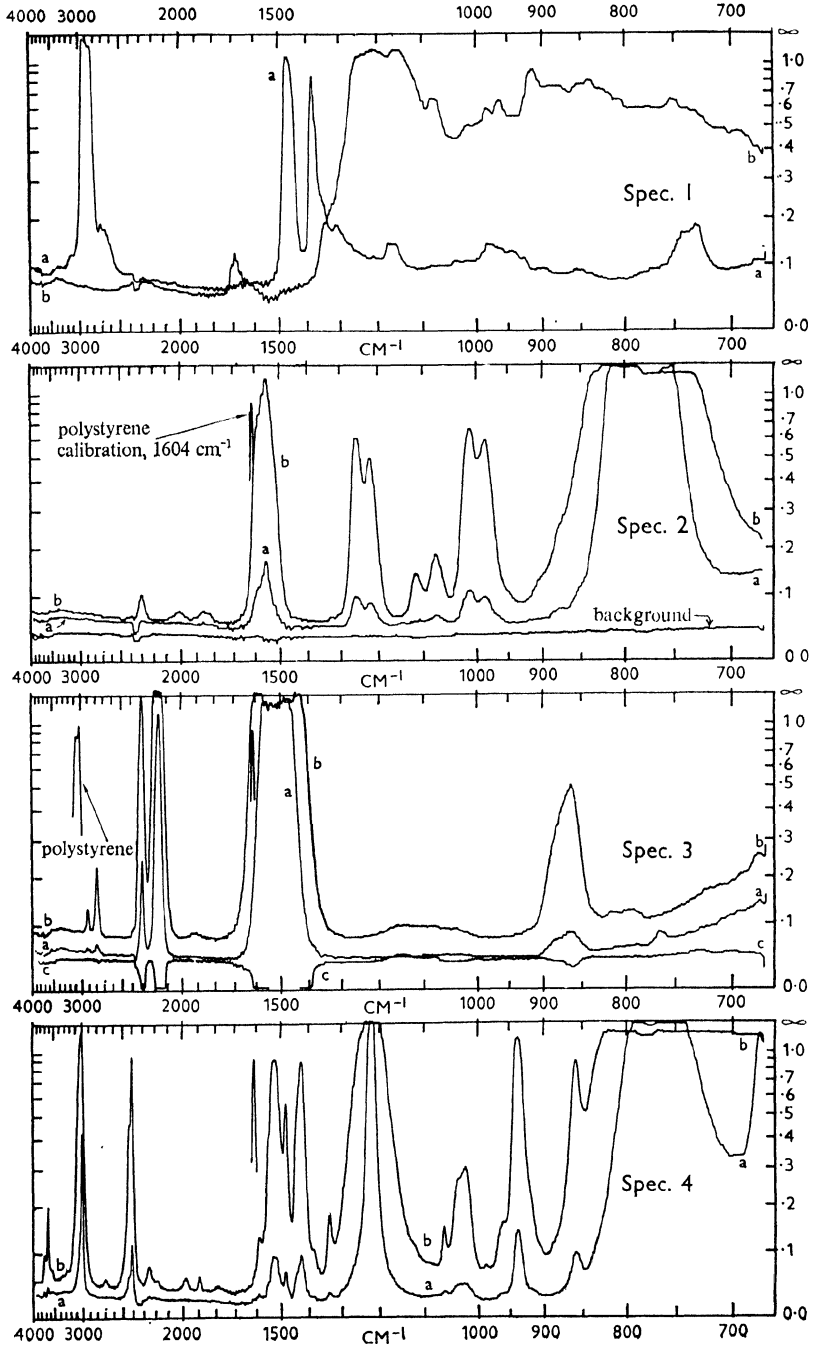
EXAMPLE 1. *Solvents.*

Spec. 1. (a) Nujol and (b) Florube–3 (both films)

„ 2. Carbon tetrachloride
„ 3. Carbon disulphide
„ 4. Chloroform (dried)

} films (a) 0·05 mm, (b) 0·5 mm and 3(c) 0·5 mm 'balanced'

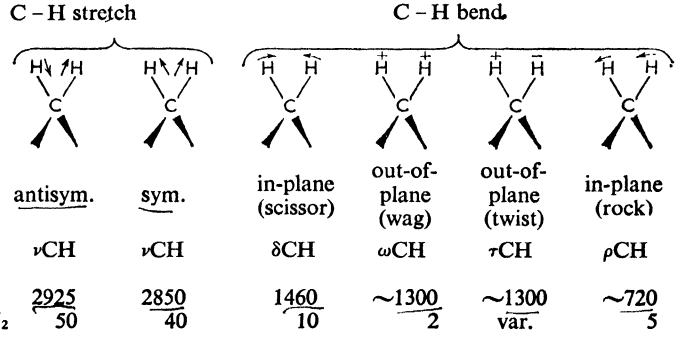
The 'background' in Spec. 2 is for the instrument alone with unobstructed beams.


4000 3000 2000 1500 1000 900 800 700
CM⁻¹
Spec. 1
Spec. 2
polystyrene calibration, 1604 cm⁻¹
background
Spec. 3
polystyrene
Spec. 4

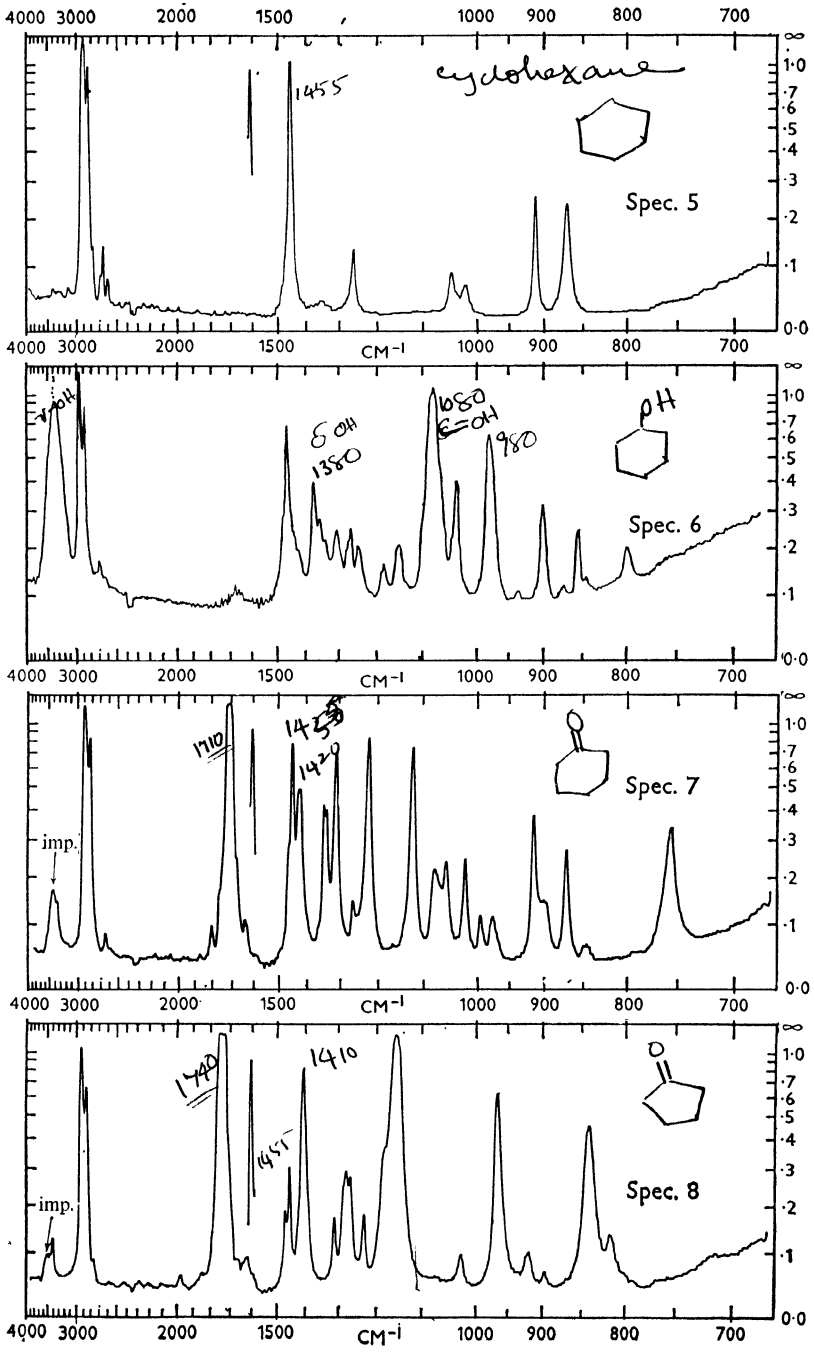
Cyclohexanone† (Spec. 7)	3400w	νOH	Impurity—cyclohexanol?
	1710s	νC=O	C=O stretch in 6-ring ketone
	1420m	δCH	CH_2 adjacent to CO, in-plane bend
Cyclopentanone† (Spec. 8)	1740s	νC=O	C=O stretch in 5-ring ketone (note overtone 2νC=O at approx. 3400)
	1410s	δCH	CH_2 adjacent to CO, in-plane bend
	1160s	Intense skeletal mode	

† The unperturbed CH_2 group absorptions are given for cyclohexane but are not listed for the other compounds as they are practically the same: a similar procedure has been adopted in later examples.

The two stretching and the four bending modes of the CH_2 group (see below) are remarkably consistent for any predominantly hydrocarbon molecule (compare the other three spectra), but intensities usually fall and frequencies shift somewhat in the neighbourhood of polar groups, e.g. $-O-$ and $-CO-$. Overtones of fundamentals below 1600 cm^{-1} may also complicate the CH stretching region (note the weak overtone bands at *ca.* 2650 cm^{-1}). The bending modes, being rather weak, are often not very obvious in the spectra of polyfunctional compounds.

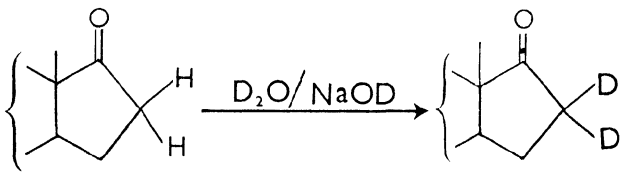


EXAMPLE 2. *Functional group absorptions* (I).
Spec. 5. Cyclohexane (film, 0·025 mm)
„ 6. Cyclohexanol (film)
„ 7. Cyclohexanone (film, 0·025 mm)
„ 8. Cyclopentanone (film, 0·025 mm)


4000 3000 2000 1500 1000 900 800 700
1455
cyclohexane
Spec. 5
CM-1
νOH
δ OH
1380
1680
C=CH
980
pH
Spec. 6
1710
1435
1420
imp.
Spec. 7
1740
1410
1455
Spec. 8

Cyclohexanol, having a more polar and less symmetrical molecule than cyclohexane, has a more complicated spectrum. The 3400 cm^{-1} band and the strong bands in the 1000 cm^{-1} region typify the absorption of a hydroxyl group; the marked changes in νOH which occur with changes in the extent of hydrogen bonding are illustrated in Fig. 6 (p. 61). The other bands are less sensitive in this respect but the bands in the 1000 cm^{-1} region are dependent on the substitution at the carbon atom carrying the OH group, tertiary alcohols absorbing the highest (see p. 72).

The carbonyl stretching absorption makes its appearance in cyclohexanone and cyclopentanone; in CCl_4 (grating spectrometer) the values of $\nu C=O$ are 1717 and 1750 cm^{-1} respectively (ε 300–500 under average resolution) reflecting the differences in strain in the 6- and 5-rings (p. 63).† The 'scissoring' vibration of the CH_2 adjacent to the carbonyl group is more intense than that of a non-adjacent CH_2 and is shifted from 1460 to ~1420 cm^{-1}. Methylene groups adjacent to certain double bonds also absorb near here (~1430 cm^{-1}). Infrared estimates of the number of CH_2 groups adjacent to a carbonyl group have been frequently used in natural product chemistry; the area of this band is measured in the unknown and compared with that in closely related model compounds. The absorption has been identified by virtue of its disappearance on deuteration of these enolisable hydrogens (see p. 62).



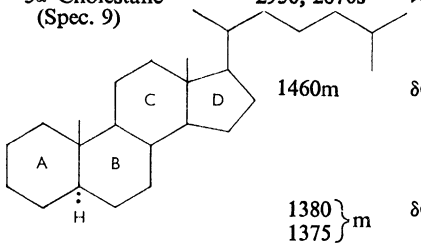
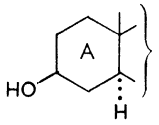
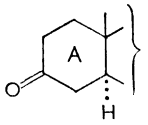
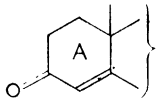
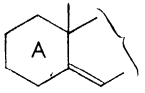
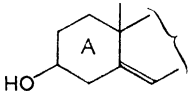
CH_2 'scissor' 1410 cm^{-1} (<1300 cm^{-1} for CD_2)

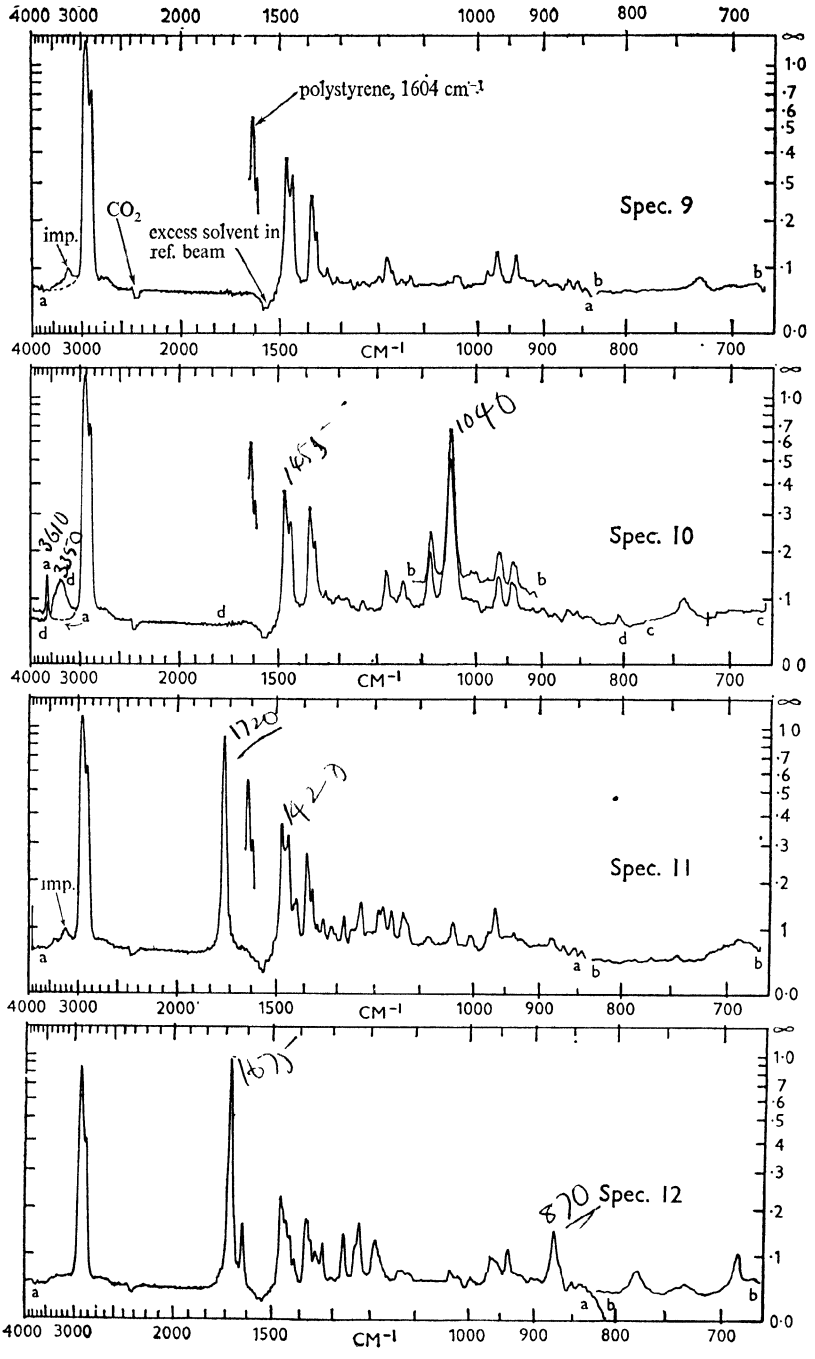
Most of the bands between 1350 and 650 cm^{-1}, some of which are quite strong, are not assignable to specific groups. However, the same general pattern can often be found in the spectra of larger molecules which contain the cyclohexanone or the cyclopentanone system.

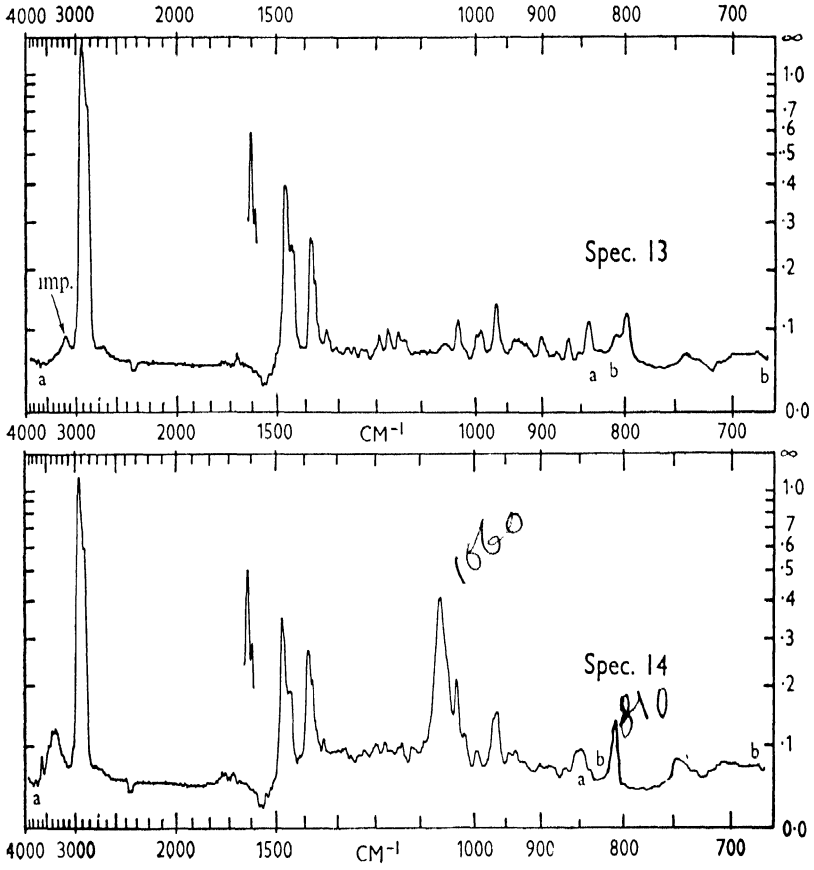
Example 3. Functional group absorptions. (II) In the 5α-cholestane skeleton.‡ This series, in paralleling the previous one, demonstrates the

† In cyclopentanone (Spec. 8) the sharp weak band adjoining the impurity band 'imp.' (ROH) is the overtone, $2\nu C=O$, of the carbonyl stretching frequency. This also applies to cyclohexanone (Spec. 7) but the situation is not so clear.

‡ See p. 225 for details of the stereochemical conventions used in steroid chemistry.

Compound	*Frequency* (cm^{-1})		*Assignment*
5α–Cholestane (Spec. 9) 	2950, 2870s	νCH	C – H stretch of CH_2 (2925, 2850 cm^{-1}) and CH_3 (2962 and 2872 cm^{-1}) which overlap one another
	1460m	δCH	C – H bend of CH_2 ('scissoring') and CH_3 (antisymmetric, see below). Band shows split but this is of little significance
	1380, 1375 m	δCH	C – H bend of CH_3 (symmetric) (cf. pp. 88 and 92)
5α–Cholestan–3β–ol (Spec. 10) 	3620w	νOH	O – H stretch, free (very dilute solution, *a*)
	3350w, br.	νOH	O – H stretch, interbonded (conc. solution, *d*) (cf. Fig. 6, p. 61)
	1040ms	νC – O	C – O stretch in C – OH (not much affected by dilution)
5α–Cholestan–3–one (Spec. 11) 	1719s	νC=O	C=O stretch in 6-ring ketone
	1420w	δCH	CH_2 adjacent to CO, in-plane bend
Cholest–4–ene–3–one (Spec. 12) 	1675s	νC=O	C=O stretch of 6-ring conjugated ketone
	1620wm	νC=C	C=C stretch of 6-ring conjugated ketone
	870wm	γCH	C – H out-of-plane bend of conjugated trisubstituted C=C (ε ~140)
Cholest–5–ene (Spec. 13) 	3040vw	νCH	C – H stretch of C=C
	835, 800 w	γCH	C – H out-of-plane bend of trisubst. C=C (ε ~65)
Cholesterol (Spec. 14) 	3620w	νOH	O – H stretch, free } cf. Fig.
	3350w, br.	νOH	O – H stretch, bonded } 6, p. 61
	1060ms	νC – O	C – OH stretch
	810w	γCH	C – H out-of-plane bend of trisubst. C=C


4000 3000 2000 1500 1000 900 800 700
polystyrene, 1604 cm⁻¹
CO2
imp.
excess solvent in ref. beam
Spec. 9
CM⁻¹
Spec. 10
1040
Spec. 11
1720
1427
imp.
Spec. 12
870



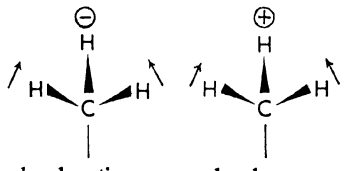
EXAMPLE 3. *Functional group absorptions* (II).

Spec. 9. 5α–Cholestane (a) 0·25M in CCl_4/0·1 mm and (b) KCl disc
„ 10. 5α–Cholestan-3β-ol (a) 0·005M in CCl_4/5 mm, (b) 0·005M in CS_2/5mm, (c) Nujol mull and (d) 0·25M in CCl_4/0·1 mm
„ 11. 5α–Cholestan-3-one
„ 12. Cholest-4-ene-3-one
„ 13. Cholest-5-ene
„ 14. Cholesterol

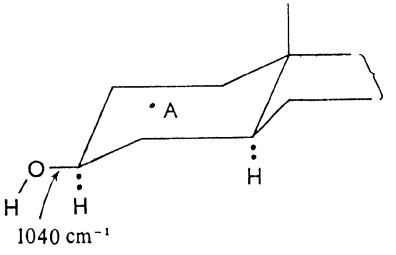
(a) 0·25M in CCl_4/0·1 mm and
(b) Nujol mull

constancy of CH_2, O–H and C=O group frequencies in spite of the increase in molecular complexity. All six compounds have the 5α-cholestane skeleton but differ as regards unsaturation and oxygen functions. The rigid skeleton of the steroids (some 4000 have been examined), and also that of the triterpenoids, have proved almost ideal for the study of group frequencies since groupings can be positioned accurately in relation to one another. Furthermore, the reverse process (structural diagnosis) with unknown steroids has been of immense value in the study of the steroidal hormones.

Cholestane, the parent hydrocarbon, has five methyl groups which absorb characteristically at *ca.* 1370 cm^{-1} (see diagram below; with high resolution it is possible to disentangle the contributions due to the various types of methyl). Cyclohexane does not absorb here but the existence of a band at *ca.* 1370 cm^{-1}, although suggestive of the presence of a methyl group, certainly *does not prove* it (cf. cyclohexanol and cyclohexanone). The 1460 cm^{-1} methyl vibration overlaps the CH_2 bending vibration and is not diagnostically useful. As the skeleton is almost constant, the CH_2 and CH_3 stretching and bending modes give a consistent pattern in the 6 compounds; the absorption due to bending of the CH_2 group adjacent to the carbonyls may be discerned in the spectra of the two ketones.



	bend, antisym.	bend, sym.
cm^{-1}	1460	1375
ε	~50	50



Spec. 12 illustrates the typical intensity relationship between the νC=O and the νC=C when the groupings are conjugated. The νC=C band of the non-polar double bond in cholest-5-ene, however, is only just detectable at about 1670 cm^{-1} and even the out-of-plane C–H bend at *ca.* 800 cm^{-1} is not very strong. The latter mode has often several component bands but the average position should be correct. With such weak bands positioned in the complex 'fingerprint' region the best course may be comparison of the spectra with those of similar olefins or of the hydrogenated compounds (absence of these bands).

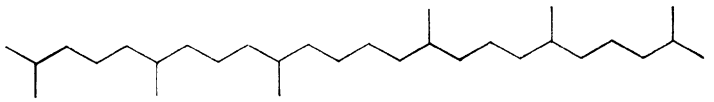
Stronger solutions and higher resolution would reveal the olefinic CH absorptions and the C=C stretches to better advantage. The exact frequency values can often define the site of the C=C link in the steroid[10] or triterpene[4] skeleton and they have been widely studied. In practice, they are useful in determining which direction the elimination of water, hydrogen halide, etc. has taken. The out-of-plane C–H deformation mode of the trisubstituted double bond is at a higher frequency in the enone as a result of conjugation.

In 5α-cholestan-3β-ol and cholesterol, the 3β-OH is equatorially orientated and the C–O stretch is near 1050 cm^{-1}. The axial isomer (3α-OH) absorbs at 1000 cm^{-1}, the difference being attributed to the greater restoring force operating in the equatorial bond. Specific correlations of this type, often useful within a group of closely related compounds, must not be used indiscriminately.

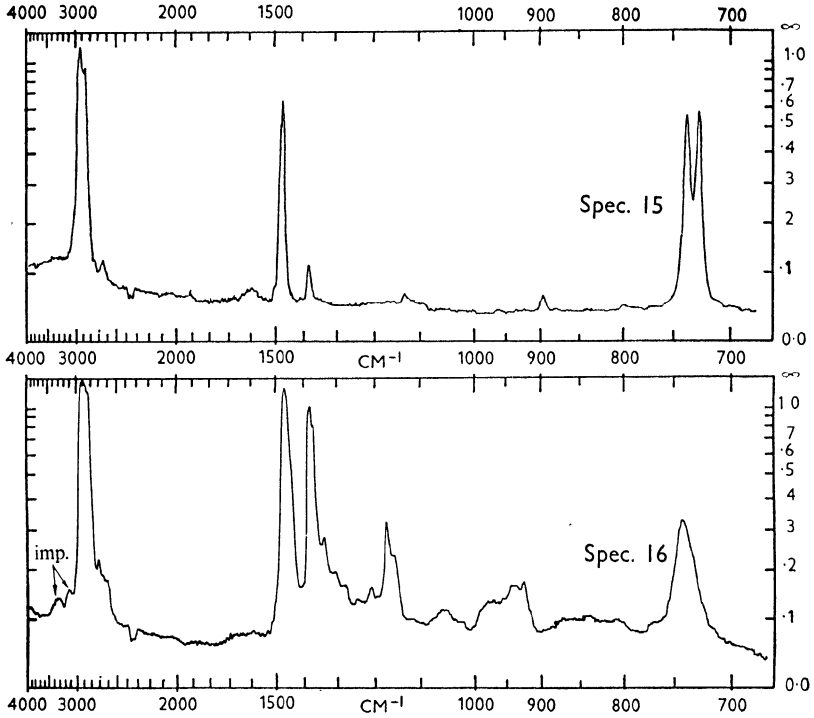
The first overtones (2ν) of the stretching vibrations of the C–H, N–H and O–H bonds fall in the near infrared region, 10,000–5000 cm^{-1} (1–2 μ). The frequency separation between overtones is approximately double that between the corresponding fundamentals, and although it is difficult to distinguish the νC–H for the CH_2 group from the νC–H for the CH_3, it is relatively easy to resolve the first overtones. There are disadvantages, however; e.g. the weakness of the overtones necessitates very large samples.

Example 4. Straight- and branched-chain hydrocarbons

Compound	*Frequency* cm^{-1}		*Assignment*
n–Tricosane	2900–2800s	νCH	C–H stretch
$CH_3(CH_2)_{28}CH_3$ ($C_{30}H_{62}$) (Spec. 15)	1470s	δCH	C–H bend in CH_2 and in CH_3 (antisymmetric)
	1375w	δCH	C–H bend of CH_3 (sym.). Note small CH_3 content
	740s, 730s	ρCH	$-(CH_2)_n-$ rocking (see p. 82). Split when chains are adjacent and parallel, i.e. in crystalline state (see p. 57)
Squalane ($C_{30}H_{62}$) (Spec. 16)	Assignments as above, but note large methyl peak at 1370 cm^{-1}		
	1170m		Isopropyl skeletal mode?



The intensity difference between the 1470 (CH_2 and CH_3 antisym. bend) and 1370 (CH_3 sym. bend) cm^{-1} peaks gives an approximate measure



EXAMPLE 4. *Straight and branched chain hydrocarbons.*
Spec. 15. *n*–Tricosane (crystalline film)
„ 16. Squalane (film)

of the methylene/methyl ratio. n–Tricosane has CH_2/CH_3 groups in the ratio 14/1 and squalane has 2/1 (squalane is the fully-hydrogenated derivative of squalene, the widely-occurring unsaturated triterpenoid hydrocarbon, $C_{30}H_{50}$). This analysis for methyl group content has been of considerable use in the study of long-chain hydrocarbon polymers, as chain branching has important effects on the properties of a polymer.

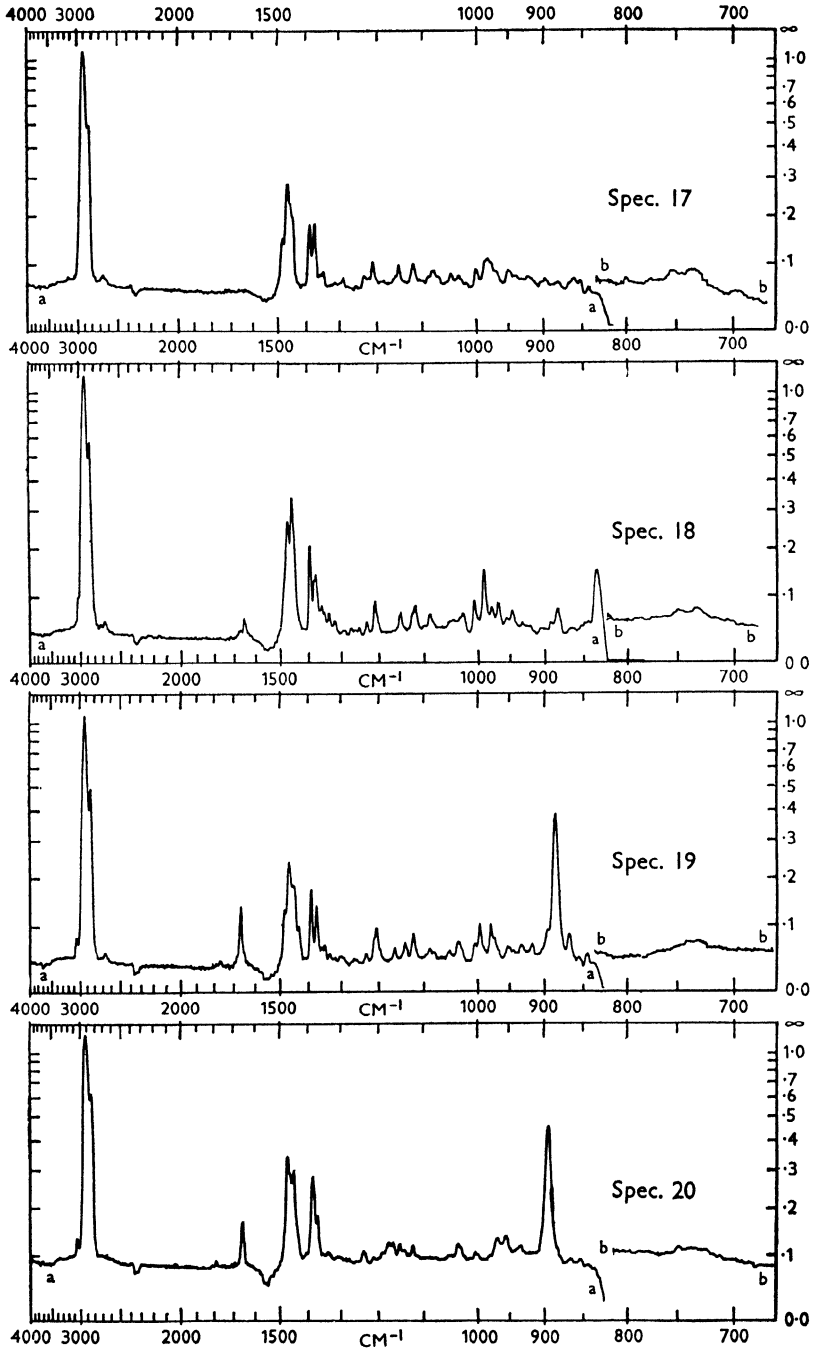
Example 5. The gem-dimethyl group and double bond absorptions

The diterpene (C_{20}) hydrocarbons, phyllocladene and isophyllo-

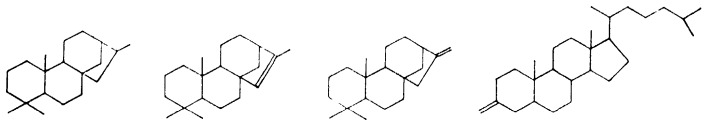
EXAMPLE 5. *The gem dimethyl group and double bond absorptions.*

Spec. 17. Phyllocladane
„ 18. Isophyllocladene
„ 19. Phyllocladene
„ 20. 3–Methylene–5α–cholestane

(a) *ca.* 0·5M in CCl_4/0·1 mm and
(b) Nujol mull


4000 3000 2000 1500 1000 900 800 700
Spec. 17
Spec. 18
Spec. 19
Spec. 20
CM⁻¹
1·0
·7
·6
·5
·4
·3
·2
·1
0·0
a
b

Phyllocladane	Isophyllocladene	Phyllocladene	3–Methylene–5α–cholestane
(Spec. 17)	(Spec. 18)	(Spec. 19)	(Spec. 20)



Double bond absorptions

νCH	—	3038 (30)	3066 (30)	3070 (35)
νC=C	—	1636 (20)	1656 (70)	1649 (80)
δCH	—	—	1428 (~40)	1434 (40)
γCH	—	827 (50)	876 (180)	887 (200)

Values are given for CCl_4 solutions measured with a grating instrument. Approx. ε^a values are in parenthesis. The δCH band is clearly resolved under these conditions.

cladene, have been isolated from numerous essential oils. Phyllocladane is their reduction product. All show a prominent split methyl peak at 1380–1370 cm^{-1}. This is characteristic of gem-dimethyl groups and is ascribed to vibrational coupling (p. 40) between the symmetrical bending modes of the methyl hydrogens. The presence or absence of this splitting has been of considerable assistance in natural product work.[24] In 3–methylene–5α–cholestane, the isopropyl group is just detectable but is rather swamped by the vibrations of the other methyl groups.

Recognition of the type of unsaturation present has also been of great importance in the natural product field. The out-of-plane CH bending modes, γCH, seen in Spec. 18–20, are well suited to this purpose, for the endo- and exo-cyclic double bonds differ markedly both in the position (827 and 876 cm^{-1} respect.) and in the intensity of their absorptions. The relative intensities of the other modes of the double bonds provide confirmation. The spectra of phyllocladene and 3–methylene–5α–cholestane are strikingly alike, though the exact positions of the double bond absorptions reflect the effect of ring size (Table 4, p. 64). The reason for their similarity rests in the fact that the spectra are dominated by the strong absorptions of the same functional group—the unsymmetrically substituted and, therefore, polar, *exo*methylene double bond. The weak but sharp νCH of this group can be seen at *ca.* 3070 cm^{-1} in Spec. 19 and 20. The CH_2 group of a cyclopropane ring sometimes absorbs in this region (due to strain) and the peak will then remain when

any double bonds in the molecule have been reduced. However, there are no completely reliable bands for the detection of cyclopropane and cyclobutane rings.

Example 6. Polynuclear aromatic hydrocarbons. The Nujol bands (*ca.* 2900, 1465 and 1380 cm^{-1}) should first be located and marked to avoid misinterpretation; it can be embarrassing to diagnose the presence of alkyl substituents, when they are not there! The crystals of polynuclear hydrocarbons are difficult to grind as the flat sheets of molecules lubricate in the manner of graphite: the large particles cause extensive light-scattering which is seen as a background falling steeply from 4000 to 2000 cm^{-1}. Hydroxylic impurities or adsorbed water account for the absorption near 3400 cm^{-1}. Contamination by carbonyl containing impurities is evident at 1690 cm^{-1} in the rather ancient pentacene sample. Some of the observed splitting, e.g. of the bands near 750 cm^{-1}, is probably due to interactions in the crystal and should not be present in solution spectra of the same compounds.

The spectra are typically those of *aromatic hydrocarbons*, though certain heterocyclic systems such as pyridine, furan, etc. give similar absorptions:

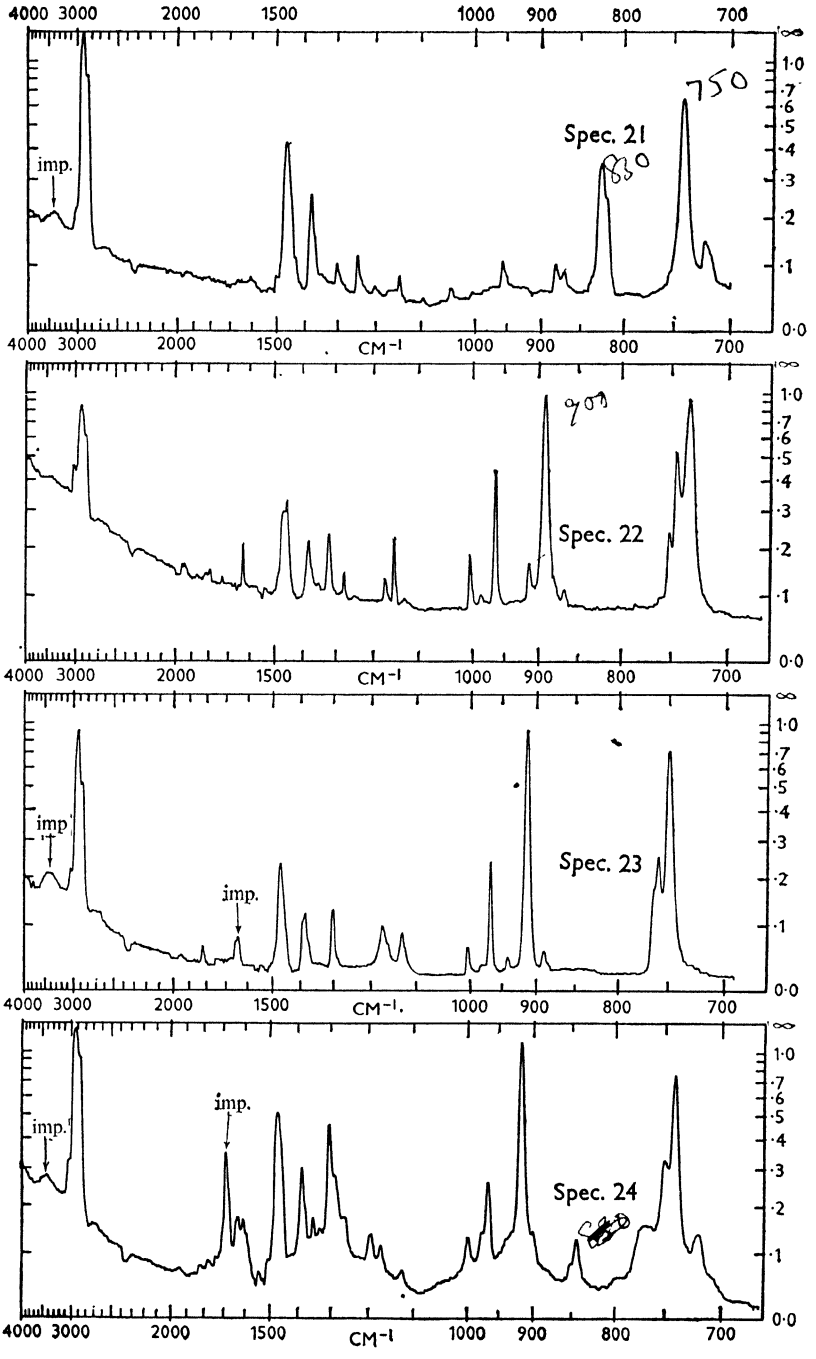
(*a*) $\sim$3030 cm^{-1}; a weak ($\varepsilon \sim 60$), but sharp νCH observable as a low shoulder on the left of the main Nujol peak.

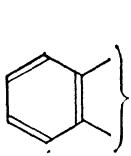
(*b*) Region 2000–1700 cm^{-1}; the pattern of weak bands ($\varepsilon \sim 10$; best seen in Spec. 22) is characteristic of the position of substitution but high concentrations are necessary to reveal it adequately. The bands are overtones and combination modes of the γCH fundamentals.

(*c*) 1600, 1580 and 1500 cm^{-1}; the ring-stretching vibrations (they are very weak in the spectra shown). They are useful for detection of aromatic rings and are intensified by conjugation and by electron-donating or electron-attracting substituents.

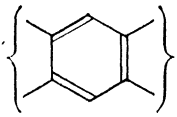
(*d*) Region 1500–1000 cm^{-1}; various vibrations (several sharp bands, wm) involving the rings; also the in-plane C–H bends. Two bands near 1450 cm^{-1} can be confused with CH_2, CH_3 and certain olefinic bending modes, and are therefore of limited diagnostic value.

(*e*) Region 1000–650 cm^{-1}; several intense bands ($\varepsilon \sim$ 200–1000) due to CH out-of-plane bends, γCH, which are very sensitive to the position of substitution, but only slightly so to the nature of the substituents. They sometimes emerge clearly even in polar, complex molecules and can be used for quantitative analysis (p. 46). The strongest bands are due to the 'umbrella' mode—all the hydrogens moving synchronously above and below the plane of the ring. In the molecules shown the substitution types (cf. Chart III, p. 52) are:

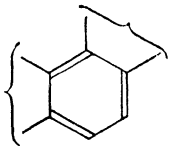

4000 3000 2000 1500 1000 900 800 700
imp.
Spec. 21
830
750
CM-1
Spec. 22
905
imp.
imp.
Spec. 23
imp.
imp.
Spec. 24
1·0 ·7 ·6 ·5 ·4 ·3 ·2 ·1 0·0



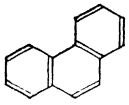
4 adj. H's
~750 cm^{-1}



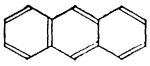
Single *p*-H's
~900 cm^{-1}



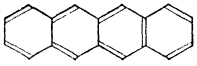
2 adj. H's
~830 cm^{-1}



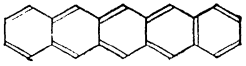
(Spec. 21)
Phenanthrene
(colourless)



(Spec. 22)
Anthracene
(colourless)



(Spec. 23)
Tetracene
(orange)



(Spec. 24)
Pentacene
(blue)

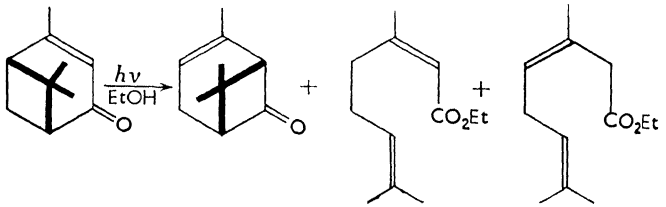
Thus, phenanthrene is alone in having a prominent 830 cm^{-1} band. The spectra of the three 'linear' *acenes* are very alike, the substitution types being the same, though the number of rings of each type does vary. Although the infrared spectra of the linear acenes differ little, the ultraviolet and visible spectra show marked differences and readily characterise the conjugated system (p. 148). The methods are complementary; alkyl substitution is difficult to detect by ultraviolet but is readily pinpointed by infrared measurements.

Example 7. A reaction study. The photochemistry of verbenone.[25] Suitably constituted α, β–unsaturated ketones are known to be susceptible to photocatalysed rearrangement and it had been anticipated that irradiation of the naturally-occurring verbenone might give the known isomer chrysanthenone (formulae on p. 96). When verbenone was irradiated in ethanol solution (silica flask, mercury arc) the ultraviolet absorption maximum characteristic of verbenone gradually disappeared and the crude product had infrared bands (in CCl_4) at 1787, 1740–1715 and 1685 cm^{-1}. Careful chromatography over alumina with light

EXAMPLE 6. *Polynuclear aromatic hydrocarbons.*

Spec. 21. Phenanthrene
„ 22. Anthracene
„ 23. Tetracene
„ 24. Pentacene
} Nujol mulls

petroleum as eluent gave successively, ethyl geranate (1715), ethyl 3, 7–dimethylocta–3, 6–dienoate (1740), chrysanthenone (1787) and unchanged verbenone (1685 cm^{-1}). Irradiation in a less reactive solvent (cyclohexane) gave a crude product with bands (in CCl_4) at 1787 and 1685 cm^{-1} (i.e. chrysanthenone and verbenone only). This work is a good example of the way ultraviolet and infrared spectroscopy can be used together. The progress of the reaction can be followed by examination of small portions of the reaction mixture. Additional data (analysis, hydrogenation, optical rotation etc.) obtained from the purified products permit their structures to be elucidated.



	Verbenone	Chry-santhenone	Ethyl geranate	Ethyl 3,7–di-methylocta–3,6–dienoate
	(Spec. 25)	(Spec. 26)	(Spec. 27)	(Spec. 28)
λ_{max} (mμ) (in EtOH)	253 (ε 6730)	290 (ε 120)	215 (ε 9800)	transparent above 210
ν_{max} (cm^{-1}) (in CCl_4)	1685*s (G) 1620wm 865wm	1787s (G) 3030vw 1660†vw 820w 800w	*1715s 1160vs 1650m 865m	1740s 1160s

* This peak incorrectly appears near 1700 in Spec. 25; the chart paper had not been positioned accurately.

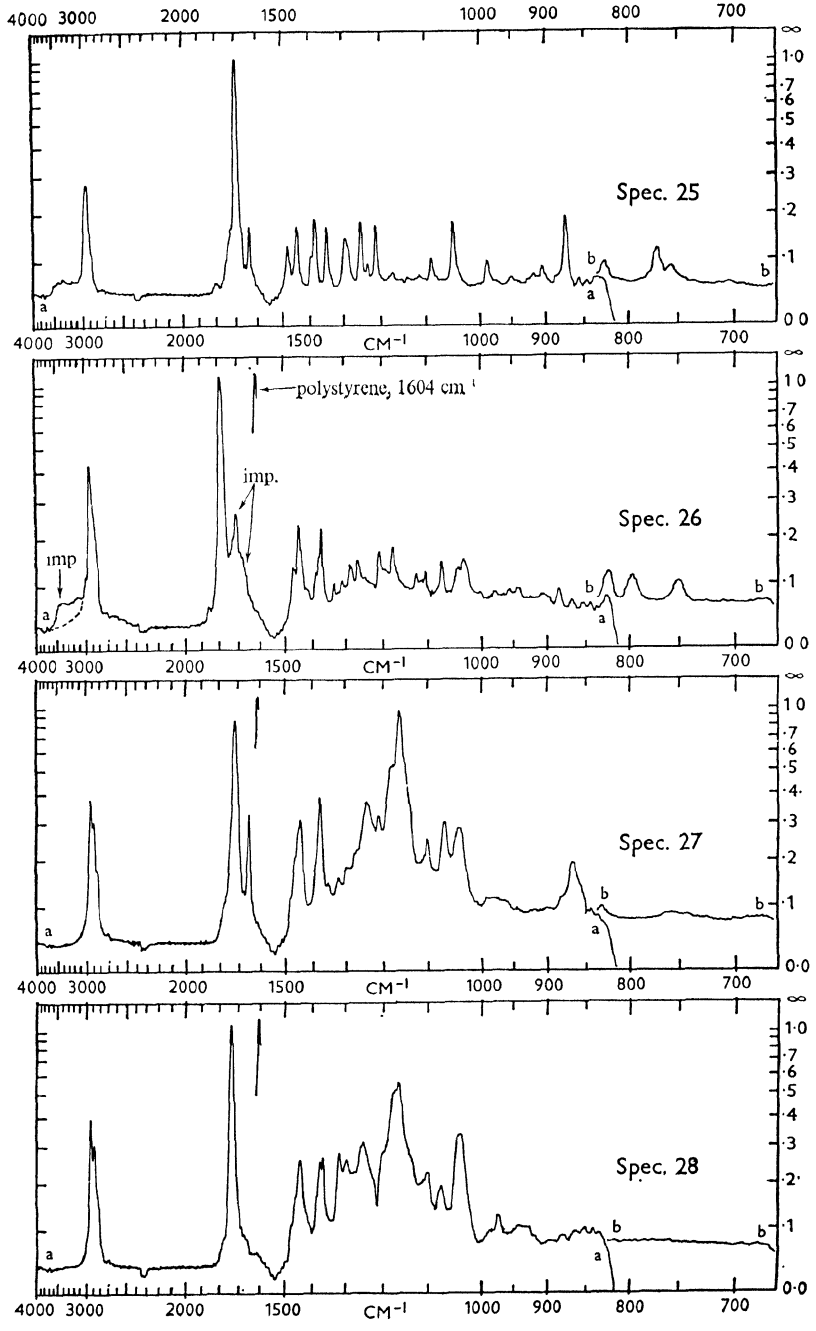
† This peak could be clearly seen in the spectrum of a much purer specimen.

Verbenone. The ultraviolet absorption, the carbonyl frequency and the fairly strong νC=C absorption are compatible with the presence of

EXAMPLE 7. *A reaction study. The photochemistry of verbenone.*

Spec. 25. Verbenone
„ 26. Crysanthenone
„ 27. Ethyl geranate
„ 28. Ethyl 3,7–dimethylocta–3,6–dienoate

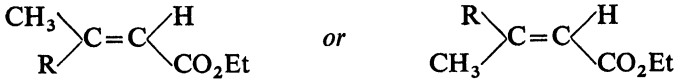
} (a) 0·2M in CCl_4/0·1 mm and (b) liquid film


4000 3000 2000 1500 1000 900 800 700
Spec. 25
Spec. 26
polystyrene, 1604 cm⁻¹
imp.
imp
Spec. 27
Spec. 28
CM⁻¹
a
b

the enone chromophore (cf. Spec. 12). The twin peaks near 1370 cm^{-1} might be assigned to the gem-dimethyl grouping but the splitting in the same region of the chrysanthenone spectrum is barely perceptible.

Chrysanthenone. The high carbonyl frequency (1787 cm^{-1}) and the low intensity ultraviolet absorption at 290 mμ are in accord with the strained cyclobutanone ring formulation (Fig. 7, p. 63). γ-Lactones and enol-acetates, which also absorb near 1780 cm^{-1}, may be ruled out on several grounds; for example, the lack of any C—O absorption 1300–1000 cm^{-1} (cf. Spec. 27 and 28) and, of course, the analytical data ($C_{10}H_{14}O$)!

Ethyl geranate. The pyramid of absorption in the C—O region at *ca.* 1170 cm^{-1} with subsidiary maxima near 1000 cm^{-1} (acyl C—O and alkyl C—O respectively) is strongly suggestive of an ester. The νC=O value, the ultraviolet absorption, the intensity of the νC=C band and the position of the γCH band are in agreement with the groupings



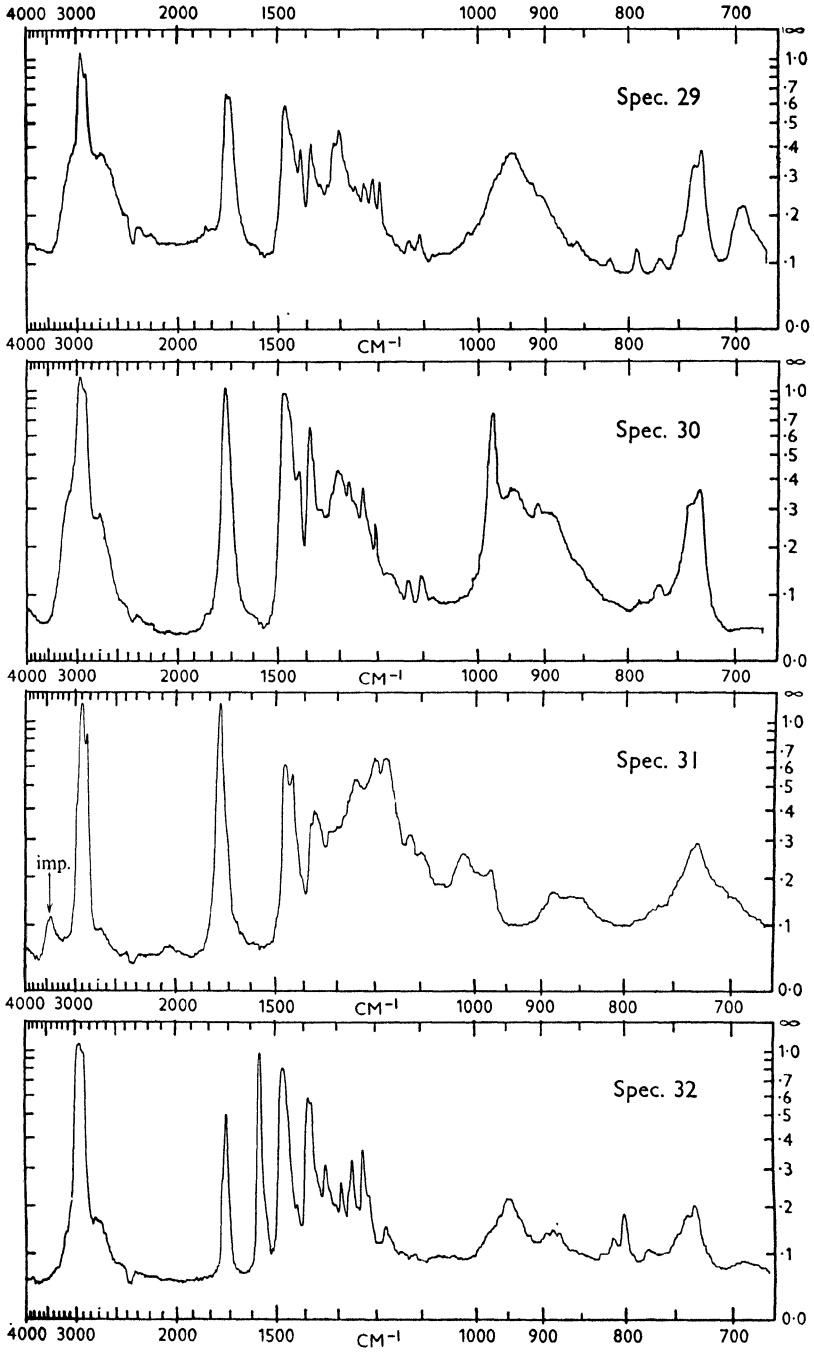
(both are present according to n.m.r. evidence). The other double bond is not in conjugation (ultraviolet), is separated by at least two methylenes from the first (not rearranged by base) and is not of the methylidine (C=CH_2) type (absence of 890 cm^{-1} band); it must therefore be in the isopropylidene position.

Ethyl 3, 7-*dimethylocta*-3, 6-*dienoate.* Both ultraviolet and infrared spectra indicate that neither double bond is conjugated with the ester grouping or the other double bond. The positions of the double bonds follow uniquely from this lack of conjugation and from the lack of methylidine ($\diagdown$C=CH_2) absorption.

Example 8. Long-chain acids and esters. Infrared spectroscopy is used extensively in the chemistry of oils and fats,[12] both in a diagnostic and in a quantitative fashion.

EXAMPLE 8. *Long-chain acids and esters.*

Spec. 29. Stearic acid (Nujol mull)
„ 30. Elaidic acid (Nujol mull)
„ 31. Methyl oleate (film)
„ 32. 6,7-*threo*-dinitro-octadecanoic acid (Nujol mull)

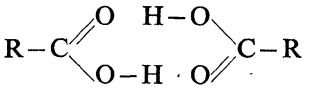

4000 3000 2000 1500 1000 900 800 700
Spec. 29
Spec. 30
Spec. 31
imp.
Spec. 32
CM⁻¹
∞ 1·0 ·7 ·6 ·5 ·4 ·3 ·2 ·1 0·0

$CH_3(CH_2)_{16}CO_2H$
Stearic acid
(Spec. 29)

trans-
$CH_3(CH_2)_7-CH=CH-(CH_2)_7CO_2H$
Elaidic acid
(Spec. 30)

cis-
$CH_3(CH_2)_7-CH=CH-(CH_2)_7CO_2Me$
Methyl oleate
(Spec. 31)

$CH_3(CH_2)_{10}-CH(NO_2)CH(NO_2)-(CH_2)_4CO_2H$
6, 7-*threo*-Dinitro-octadecanoic acid
(Spec. 32)


Carboxylic acid dimer

The hydrocarbon chains account (p. 82) for the absorption at *ca.* 2900vs cm^{-1} [the comparatively narrow band superposed on the broad νOH band], 1460s, 1370m and 730m cm^{-1}. The split in the 730 cm^{-1} band, characteristic of long $-(CH_2)_n-$ chains ($n>4$) packed parallel, is just perceptible in the three solid state spectra.

The carboxylic acid group, present as the *dimer* (see above), can best be picked out by the very broad hydroxyl νOH band at ~3400–2500 cm^{-1}. When this band is examined under higher resolution it is found to be composed of a number of subsidiary maxima which are generally held to be due to Fermi resonance of the νOH vibration with overtone and combination bands from lower frequency fundamentals, and *not* to a variety of hydroxyl groups (cf. Spec. 45, p. 111). The other principal absorptions which may be assigned to the *acid dimer group* are:

1710s cm^{-1}	νC=O
1420wm cm^{-1}	coupled C–C, C–O stretch and C–OH in-plane bending modes
1300ms cm^{-1} ($\varepsilon\sim100$)	
940m cm^{-1} ($\varepsilon\sim80$)	out-of-plane bend of OH, γOH

Long-chain acids in the solid state often have a series of weak, closely-spaced, sharp bands between 1300 and 1200 cm^{-1}—the so-called 'band

progression' (e.g. Spec. 29). They disappear in solution and probably originate in the CH_2 bending modes of the linear chains. Polymorphic forms often give very different patterns in this region.

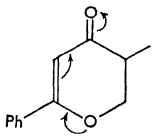
Acids are usually examined in the solid state to ensure that only the dimer is present (the monomeric forms have νOH ~ 3530m *sharp* and νC=O ~ 1760s cm^{-1} in CCl_4). Additional information can be obtained by conversion (CH_2N_2) of a few mg to the methyl ester; the disappearance of the OH absorption of the acid dimer greatly facilitates interpretation of the 3650–2500 cm^{-1} region. For example, the hydroxylic impurity in the methyl oleate of Spec. 31 is obvious. In the carbonyl region there is an upward shift of *ca.* 30 cm^{-1} on conversion of an acid to its methyl ester and the ester carbonyl generally gives a sharper and more symmetrical band.

An important application for infrared spectroscopy in the long-chain field is the determination of the type of unsaturation present (see also p. 73). The strong out-of-plane CH bend absorption of the double-bond at 975 cm^{-1} (ε ~ 130) is quite plain in elaidic acid even though there is overlap with the γOH at 950 cm^{-1}. The much weaker (ε ~ 50) band of the *cis*-isomer at 713 cm^{-1} is coincident in methyl oleate with the $(CH_2)_n$ rock common to all four compounds. Neither in elaidic acid nor in methyl oleate is the νC=C strong enough to be seen, though, when more material is placed in the infrared beam, it can be shown that the *cis*-isomers absorb more strongly than do the more symmetrical *trans* double bonds.

In the dinitro-acid, the intense antisymmetric vibration of the NO_2 group is easily spotted at 1565 cm^{-1}, but the weak symmetrical mode near 1375 cm^{-1} is obscured by the Nujol absorption. The nitroso (−NO), nitrite (−ONO) and nitrate ($-ONO_2$) groupings are also readily detected when present.

Example 9. Synthetical confirmation of a natural product structure.[26] Bullatenone, a crystalline optically-inactive unsaturated ketone, $C_{12}H_{12}O_2$, was first isolated from a New Zealand shrub in 1954. Two dihydro-γ-pyrone structures were initially considered. The spectrum of one of them is given (Spec. 36, formula p. 102). Both isomers were synthesised and neither was identical with bullatenone. The infrared spectrum of bullatenone was then found to have a single carbonyl band at 1695 cm^{-1} (in $CHCl_3$) and this was rightly considered to be too high a value for a system of the dihydropyrone type. (Release of electrons from the oxygen atom causes a fall additional to that caused by the C=C and the phenyl group. An even bigger fall occurs in the γ-pyrones: compare flavanones and flavones respectively).

2, 3–Dihydro–3–methyl–6–phenyl–4–pyrone

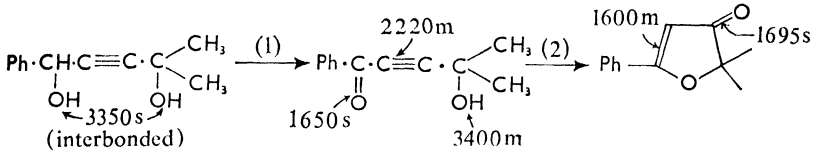


(Spec. 36)

λ_{max} (mμ) 222 (ε 8400), 242 (ε 5500),
(in EtOH) 296 (ε 1700)

νC=O (in $CHCl_3$) 1660s cm^{-1} (G)

The isomeric dihydro-oxofuran structure shown (Spec. 35) was proposed on the grounds that ring strain might bring the νC=O up to the value found. The synthesis below fully confirmed that this compound and bullatenone are identical (mixed m.p. undepressed, and superposable infrared and ultraviolet spectra). The reaction course is readily followed by the spectral changes indicated. Bullatenone gives a sharply defined spectrum, even in solution; the numerous strong bands between 1200 and 770 cm^{-1} are probably associated with coupled vibrations involving the rigid ring system.



(Spec. 33) (Spec. 34) (Spec. 35)

λ_{max} (mμ) 264 (ε 14,700) (in EtOH)

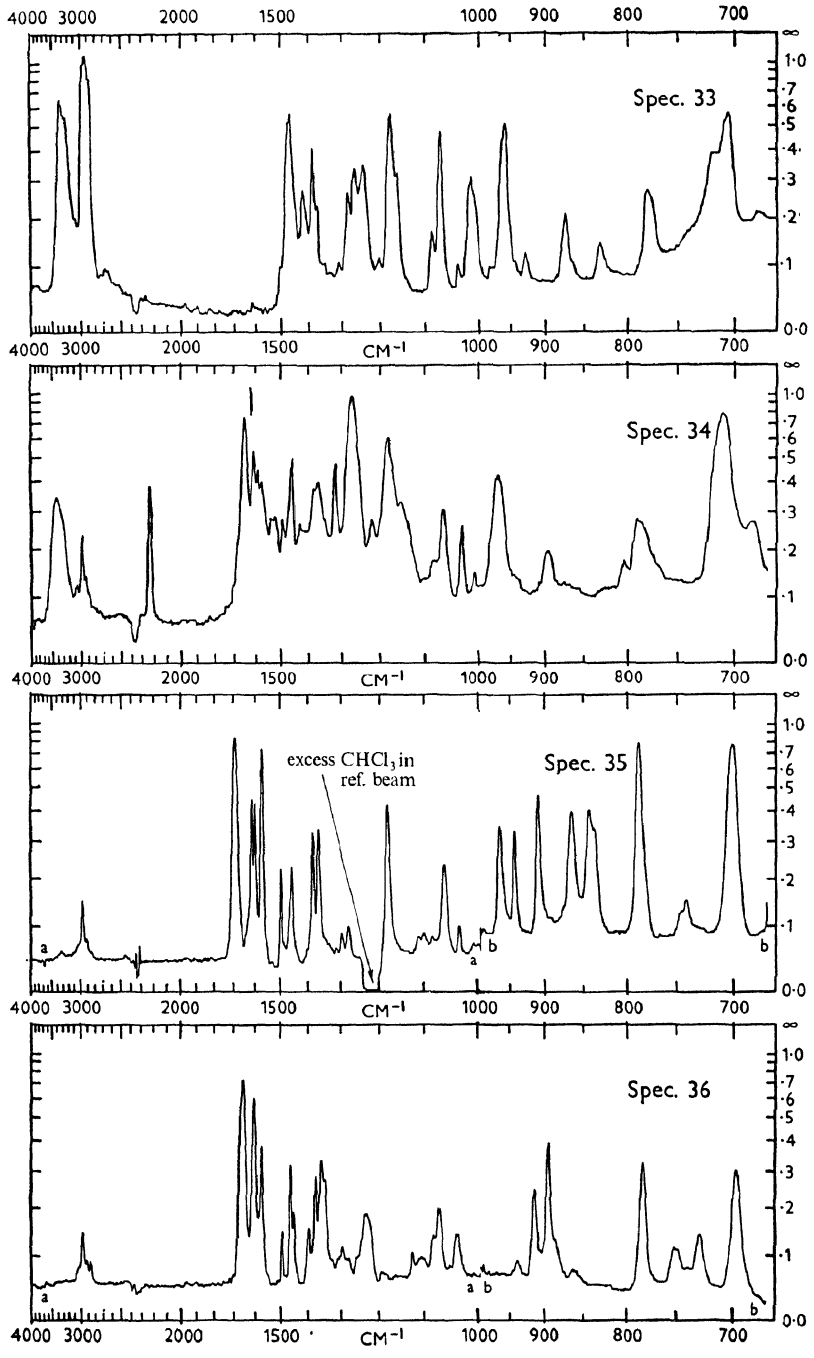
λ_{max} (mμ) 242 (ε 8000), 296 (ε 16,000), 304 (ε 16,000) (in EtOH)

Reagents: (1) MnO_2 or CrO_3/AcOH. (2) diethylamine followed by distillation (yield 60%).

EXAMPLE 9. *Synthetical confirmation of a natural product structure.*

„ 33. 4–Methyl–1–phenylpent–2–yne–1,4–diol (Nujol mull)
„ 34. 4–Hydroxy–4–methyl–1–phenylpent–2–yn–1–one (film)
„ 35. Bullatenone
„ 36. 2,3–Dihydro–3–methyl–6–phenyl–4–pyrone

(a) 0·1M in $CHCl_3$/0·1 mm and (b) Nujol mull


4000 3000 2000 1500 1000 900 800 700
Spec. 33
Spec. 34
Spec. 35
excess $CHCl_3$ in ref. beam
Spec. 36
CM⁻¹
1·0 ·7 ·6 ·5 ·4 ·3 ·2 ·1 0·0
a
b

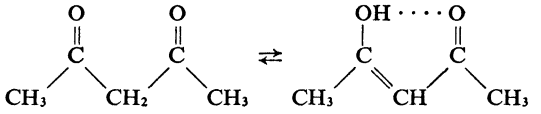
Further comments are:

Phenyl group. νCH of the ring hydrogens can be seen in Spec. 34 near 3050 cm^{-1} just to the high frequency side of the methyl group νCH ($\sim$ 2980 cm^{-1}). In the chloroform solutions used for Spec. 35 and 36 the solvent has inactivated the pen in this region by absorbing most of the radiation (cf. Spec. 4). The aromatic absorption (p. 93) between 1600 and 1500 cm^{-1} is strong in Spec. 34–36 owing to polar substitution and conjugation effects. The strong γCH absorption for the five adjacent hydrogens is at $\sim$700 cm^{-1} in all four spectra (see p. 110). The weak aromatic bands between 2000 and 1800 cm^{-1} do not show up at the concentrations used, which had been chosen for correct presentation of νC=O.

Gem–dimethyl. This region is unreliable with polar molecules. Both bullatenone and the dihydropyrone have split bands at *ca.* 1375 cm^{-1} but only the former has a gem–dimethyl group!

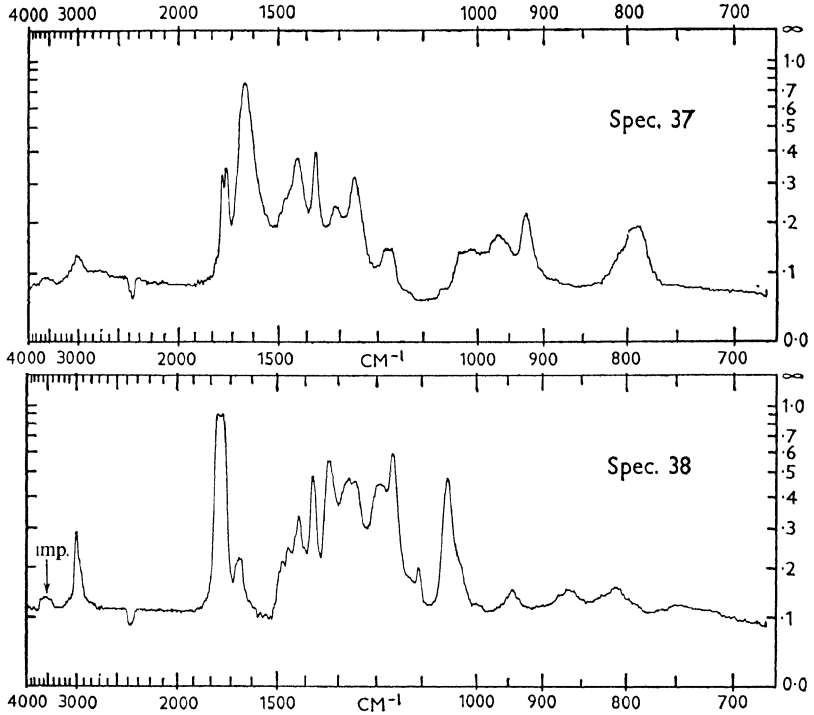
The triple bond. νC≡C for the almost symmetrically substituted C≡C of the diol is too weak to be detected. The same fundamental for the terminal ethynyl group (−C≡CH) can usually be found at *ca.* 2100 cm^{-1} as a weak but sharp band. However, the sharp and moderately intense ($\varepsilon \sim 150$) νCH absorption at *ca.* 3320 cm^{-1} is the chief diagnostic feature of this latter group (Table 2, p. 59). Conjugation of a triple bond with the carbonyl group causes a marked intensification of νC≡C, as seen in Spec. 34 (cf. the effect of conjugation in the enone system, p. 65).

Example 10. Chelate systems. β–Diketones and β–ketoesters are well known to exist as tautomeric mixtures and the individual tautomers have been isolated in some cases. As a class they have been extensively studied,[27-29] both chemically and physically (e.g. dissociation constants; ultraviolet, infrared and Raman spectra).



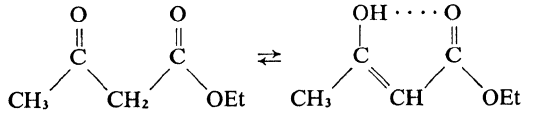
νOH	—		~ 3000vw, br.
νC=O	1724 (doublet)	}	1608s, br.
νC=C	—		

Acetylacetone (Spec. 37)



EXAMPLE 10. *Chelate systems.*

Spec. 37. Acetylacetone (film)
„ 38. Ethyl acetoacetate (film)



νOH	—	~ 3300w, br.	
νC=O	1733, 1710s	}	1645s
νC=C	—	}	

Ethyl acetoacetate (Spec. 38)

The above frequency values are for chloroform solutions.

The enol tautomer of acetylacetone predominates in the liquid state (Spec. 37). Intramolecularly hydrogen-bonded systems of this conjugate chelate type have extraordinarily broad and weak νOH bands. The peak *ca.* 3000 cm^{-1} in Spec. 37 mainly represents νCH, νOH being almost indistinguishable from the background (to check this assignment

conversion to $CH_3C(OD)=CHCOCH_3$ can be used). The intense, low-frequency $\nu C=O$ band of the enol form can be ascribed to the dual effect of hydrogen bonding and of mesomeric electron supply from the hydroxyl group.

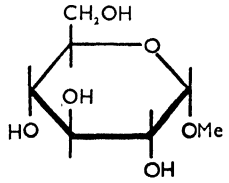
In contrast to acetylacetone, ethyl acetoacetate exists mainly in the keto form as can be seen from Spec. 38. The greater complexity in the 1250 cm^{-1} region has its origin in the C–O vibrations of the additional oxygen of the ester group.

Phenols with a carbonyl group substituent in the *ortho*-position are analogous to the enol forms discussed above, both νOH and $\nu C=O$ being at low frequencies (cf. p. 67).

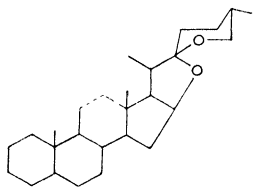
Example 11. Ethers, ketals and carbohydrates. Compounds of the type of the polyethylene glycol shown (Spec. 39) give spectra dominated by the $\nu C-O$ vibration of the ether linkages (*ca.* 1100 cm^{-1}). In the fairly short-chain polymer illustrated (M.Wt. $\sim$ 400, $n=6$) the absorption due to the terminal hydroxyl group is also prominent [in anhydrous samples it is possible to estimate hydroxyl end groups, and hence molecular weights, from the observed intensities of νOH; cf. Chap. 7]. Polymers of this type are water-soluble, and for this reason are widely used in industry. Dissolved water is responsible for some of the $\nu O-H$ peak and for the peak at *ca.* 1650 cm^{-1} (δOH of liquid water). Inadequately dried compounds often have weak absorption here due to water† (cf. Spec. 40).

$$HOCH_2CH_2-(O-CH_2CH_2-)_nOCH_2CH_2OH$$

(Spec. 39)
Polyethylene glycol

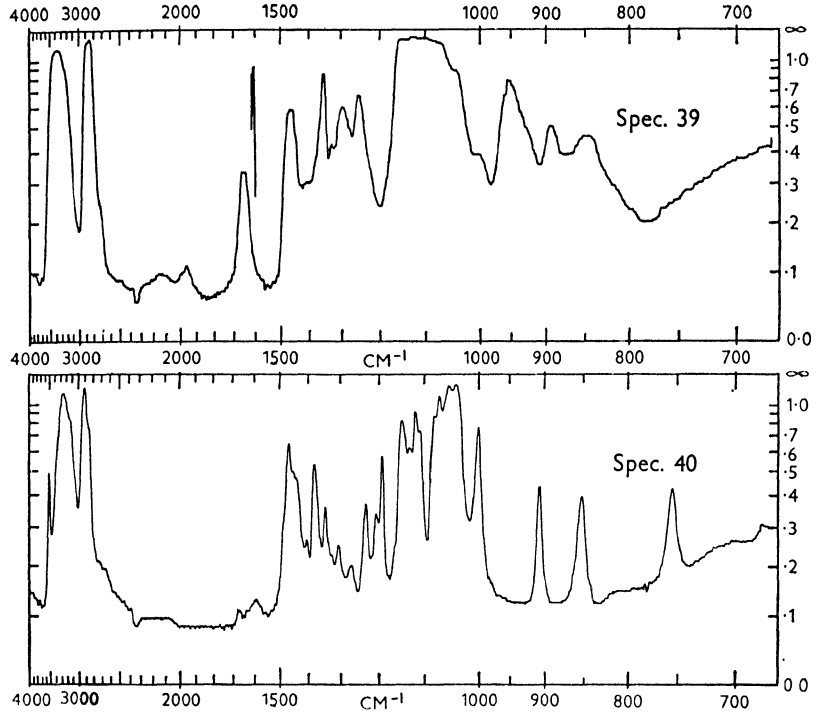


(Spec. 40)
Methyl α-D-gluco-pyranoside



(Spectrum not shown)
3-Deoxytigogenin

† Delightfully put by Barker, Bourne and Whiffen[13]: 'Thus infrared analysis may find wide application as a sedative for the conscience on those disturbing occasions when it is necessary to invoke water of crystallisation in order to explain discrepancies in the results of chemical analysis.'



EXAMPLE 11. *Ethers, ketals and carbohydrates.*
Spec. 39. Polyethyleneglycol-400 (film)
„ 40. Methyl α-D-glucopyranoside (Nujol mull)

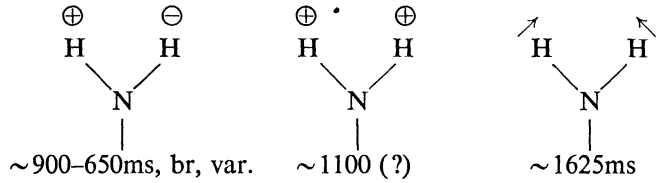
The ether $\nu C-O$ band is to be found at 1150–1000 cm^{-1} in alkyl ethers but is susceptible to coupling with $\nu C-C$ and is therefore variable in frequency. However, when carbonyl and hydroxyl groups are absent, strong bands in this region are a good indication of the presence of ether linkages. In ketals and acetals, the vibrations associated with the groupings O–C–O are often sharp, intense, and characteristically placed. The steroid, 3-deoxytigogenin has seven sharp bands between 1240 and 900 cm^{-1} of $\varepsilon \sim 150$–350, whereas the parent hydrocarbon 5α-cholestane (Spec. 9) has no band of $\varepsilon > 40$ between 1350 and 650 cm^{-1}. The bands are highly dependent on the stereochemistry at the ketal carbon.[2] Considerable effort has been devoted to the infrared characterisation of carbohydrates and some correlations have been made.[13,30] Thus it is claimed that the α- and β-anomers of the monosaccharides can be differentiated by an examination of the region 800–900 cm^{-1}, e.g. the spectrum of methyl α-D-glucopyranoside (Spec.

40) has a characteristic band near 850 cm^{-1} which is not present in that of the β–isomer. Carbohydrates are much subject to polymorphism (see p. 57). 'Crystalline' portions of long-chain carbohydrates, e.g. cellulose, can be detected by the reluctance of their hydroxyls to exchange deuterium in D_2O; the amorphous portions *do* deuterate and the νOH becomes νOD which absorbs at ~2200 cm^{-1}.

Example 12. Amines, amine salts and alkaloids. Since amines and amine salts are of biological as well as chemical interest, they have been chosen to exemplify the application of infrared to nitrogen-containing compounds. The following modes of amines are of interest:

NH stretching. The primary amine grouping has two νNH modes, antisymmetric (~3500 cm^{-1}) and symmetric (~3400 cm^{-1}) (cf. the CH_2 modes, p. 82). These two bands always appear clearly in solution (CCl_4—not CS_2, with which amines react) and sometimes in the solid or pure liquid state. Secondary amines have a single, sharp νNH in dilute solution. Tertiary amines are very hard to detect spectroscopically, except by the appearance of νNH after salt formation (Spec. 43 and 44). Primary and secondary amines can form hydrogen bonds though the shifts in νNH are generally much smaller than, and not so predictable as, the shifts of νOH. νNH bands are generally sharper than νOH bands of the same frequency.

NH bending. In primary amines these vibrations resemble the bending modes of the CH_2 group (p. 82):

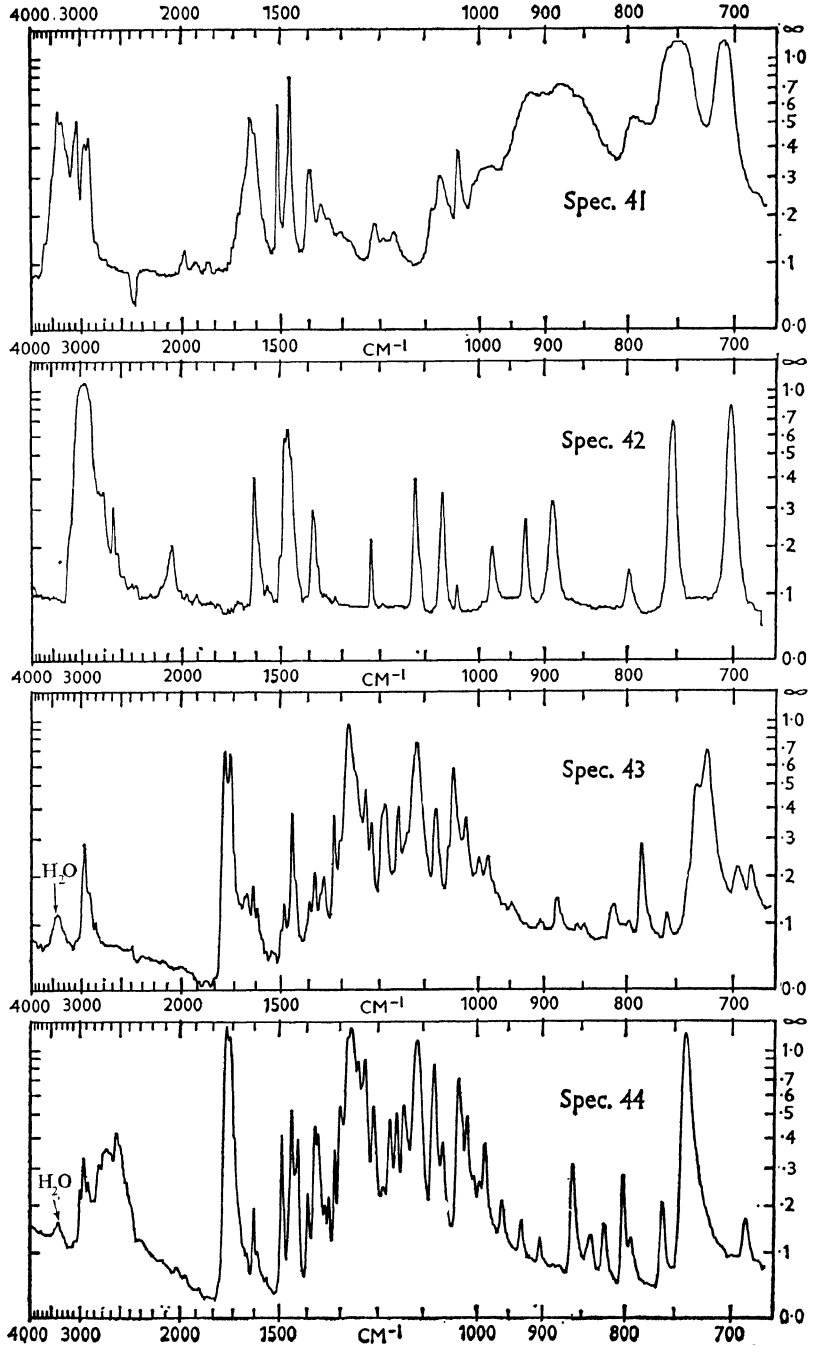


The NH bend absorption (*ca.* 1580 cm^{-1}) in secondary amines is very weak and may be confused with aromatic bands.

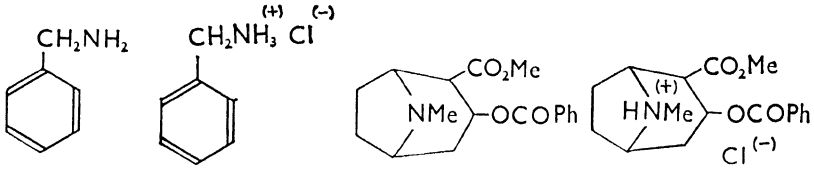
CN stretching. This is strong only in the aromatic amines, and occurs between 1360 and 1250 cm^{-1}.

EXAMPLE 12. *Amines, amine salts and an alkaloid.*

Spec. 41. Benzylamine (film)
„ 42. Benzylamine hydrochloride (Nujol mull)
„ 43. Cocaine
„ 44. Cocaine hydrochloride } KCl discs, 1·7 mg in 300 mg


4000 3000 2000 1500 1000 900 800 700
Spec. 41
Spec. 42
Spec. 43
Spec. 44
H2O
CM-1
1·0
·7
·6
·5
·4
·3
·2
·1
0·0

The spectrum of benzylamnie (Spec. 41) illustrates some of these points; in addition, the aromatic bands can be picked out at ~3100, 2000–1700, 1510, 750 and 710 cm^{-1}. [The last two frequencies are those of γCH for a mono-substituted benzene ring; Spec. 42–46 show that the positions and relative intensities of these bands may vary somewhat and that their recognition may be complicated by overlap with other bands.] The aromatic bands are also discernible in the spectrum of the alkaloid cocaine (Spec. 43). The presence of the benzoate [νC=O, 1710 cm^{-1}] and methyl ester [νC=O, 1740 cm^{-1}] groupings might be inferred from this spectrum.



Benzylamine (Spec. 41) | Benzylamine Hydrochloride (Spec. 42) | Cocaine (Spec. 43) | Cocaine Hydrochloride (Spec. 44)

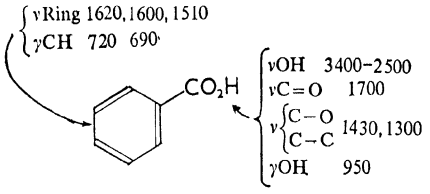
Salts of the type $[RNH_3]^+X^-$; $[R_2NH_2]^+X^-$; $[R_3NH]^+X^-$, being ionic, are normally examined as solids. The hydrogen-bonding effects can be quite significant and, although there is no universal agreement[2-8] as to the characteristic absorption positions, the following points may be useful:

NH stretching. The frequency maximum generally falls in the order $[RNH_3]^+ > [R_2NH_2]^+ > [R_3NH]^+$. Thus, in the two spectra illustrated, benzylamine hydrochloride and cocaine hydrochloride, νNH is at 3300–2500m, br. and 2800–2300m, br. cm^{-1} respectively. The peak near 2100 cm^{-1} in the former spectrum may also be a νNH mode.

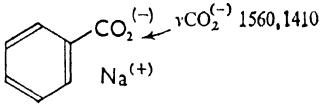
NH bending. The salts of primary and secondary amines absorb near 1600 cm^{-1} (antisymmetric deformation, for $[RNH_3]^+$). In Spec. 42, this absorption is coincident with the aromatic band. A single NH does not give a band here, and the peak near 1600 cm^{-1} in Spec. 44 must be due to the aromatic ring.

Example 13. Carboxylic acid salts and amino-acids. On conversion of a carboxylic acid to the corresponding salt, the νOH, νC=O and νC–O bands (at *ca.* 3000br, 1700 and 1300 cm^{-1} respectively) disappear and two new absorptions due to νCO_2^- appear—antisymmetric (1570 cm^{-1}) and symmetric (1400 cm^{-1}). Compare the solid state spectrum of benzoic acid (Spec. 45) with that of sodium benzoate (Spec. 46).

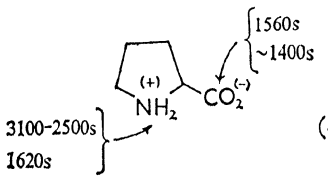
Benzoic acid shows the typical absorption of a conjugated carboxylic acid (dimer, cf. p. 100). Sodium benzoate has a remarkably simple spectrum: the 1610 band is one of the aromatic ring stretching modes (intensified by the electron-rich substitution and, possibly, by Fermi resonance with the 1560 band). The remaining bands in both spectra are mainly accountable as belonging to aromatic rings and to substituent hydrogens.



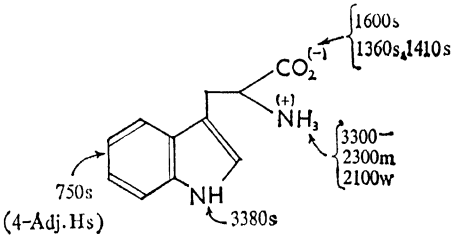
Benzoic acid
(Spec. 45)



Sodium benzoate
(Spec. 46)

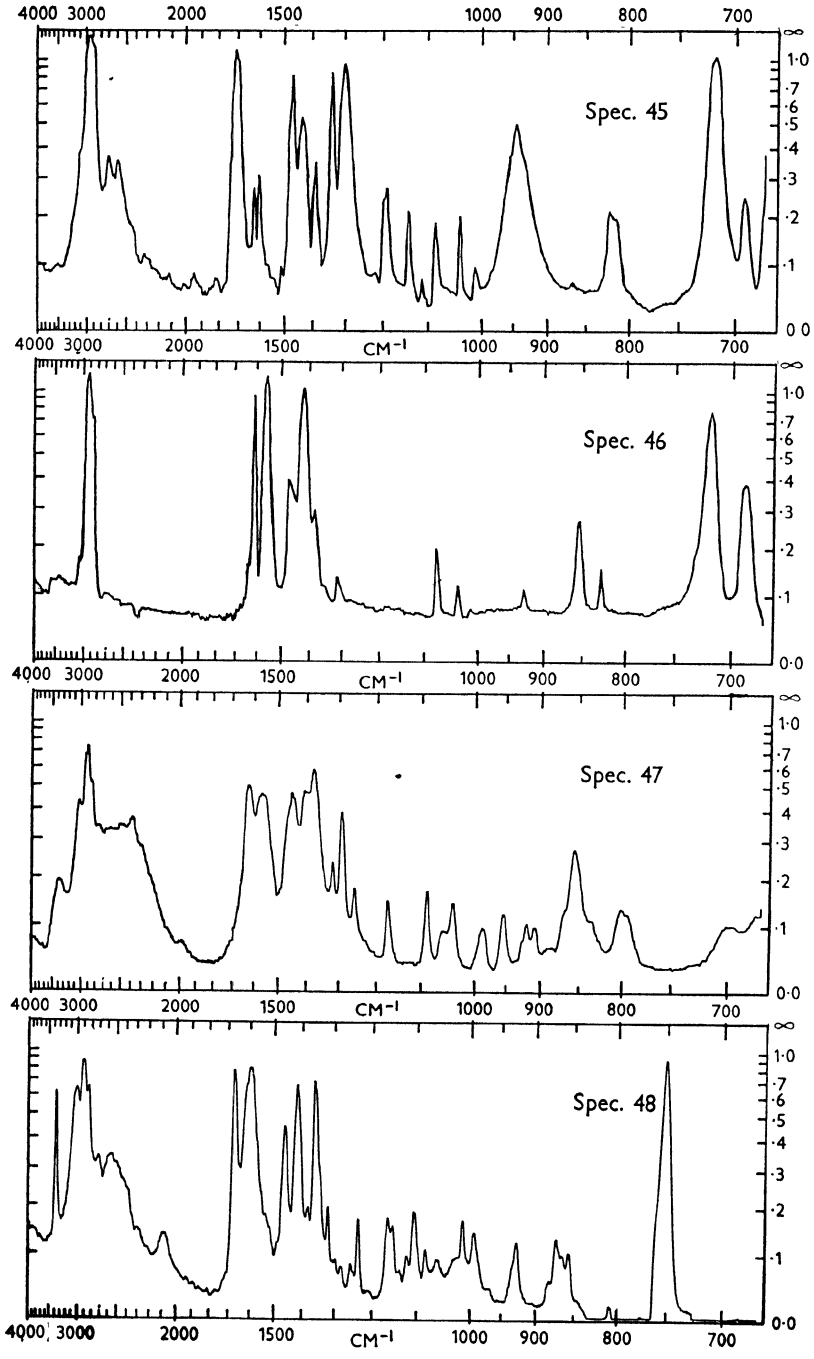


L–Proline
(Spec. 47)



L–Tryptophane
(Spec. 48)

Amino acids generally exist as zwitterions and the carboxylate portion of the amino-acid molecule absorbs in much the same way as the normal salt of a carboxylic acid. The spectrum of proline (Spec. 47) is clearly that of the zwitterion (the peak *ca.* 3400 cm^{-1} is probably due to water of crystallisation). In tryptophane (Spec. 48) the α–amino group is the basic centre of the zwitterion, as the single, acidic NH of the indole ring is quite plain at 3380 cm^{-1}. The rather high frequency of the carboxylate antisymmetric stretch may be due to the close proximity of the indole ring vibrations (including 1680 cm^{-1}?).


4000 3000 2000 1500 1000 900 800 700
Spec. 45
Spec. 46
Spec. 47
Spec. 48
CM⁻¹
∞ 1·0 ·7 ·6 ·5 ·4 ·3 ·2 ·1 0·0

In these spectra some confusion arises in the region 1300–1500 cm^{-1} from the presence of the Nujol absorptions. Care is required, but in Spec. 47, for example, it is obvious, when the relative intensities of the 1460 and 1380 cm^{-1} Nujol bands are considered, that there must be a strong absorption at *ca.* 1400 cm^{-1} due to L–proline.

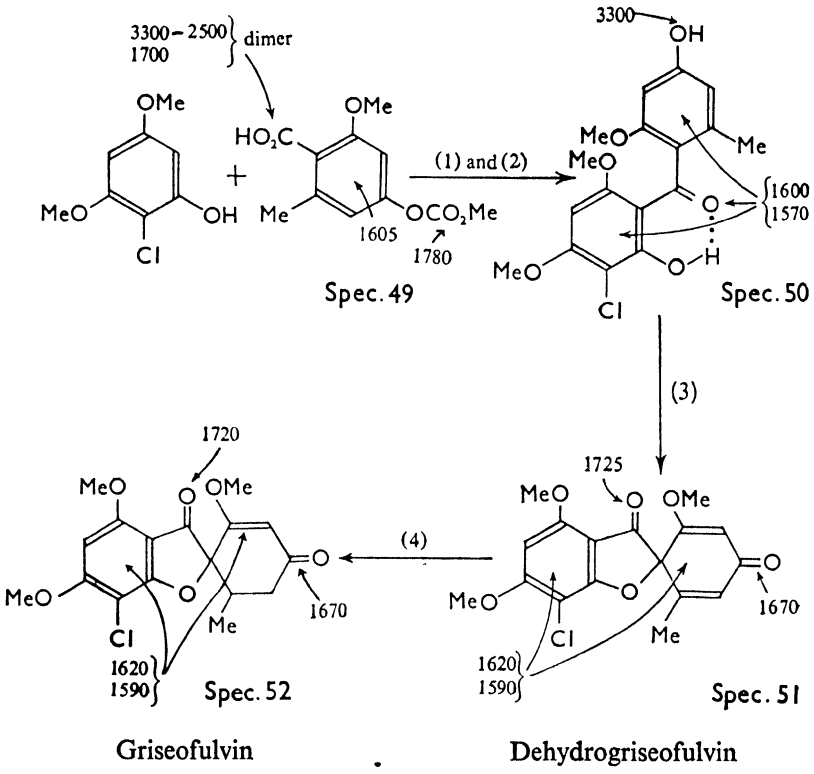
Example 14. The total synthesis of griseofulvin. Infrared measurements can still be of considerable use even when the compounds under examination are polyfunctional and of such complexity that important bands overlap. The present example is such a case.

The structure of the important oral antibiotic, griseofulvin, a metabolite of *Penicillium patulum*, has recently been confirmed by a total synthesis,[31] the last and most important part of which is outlined (p. 114). The main functional group absorptions, νOH, νC=O, νC=C were readily assigned in spite of their being measured in the solid state (the compounds are almost insoluble in chloroform) and served to establish that each step in the synthesis had gone correctly. The region 1500–1000 cm^{-1} is complex as all the compounds have several C=C–O systems (e.g. ArOMe ~ 1270, and $ArOCO_2Me$ ~ 1250 cm^{-1}); no attempt has been made to disentangle these. Likewise the polar substituents have masked the aromatic γCH vibrations and these too have been ignored. The νC–Cl vibration which normally absorbs at *ca.* 650 cm^{-1} could not be located with any certainty.

The starting acid, 2–methoxy–4–methoxycarbonyl–6–methyl–benzoic acid (Spec. 49) has the characteristic carboxylic acid dimer bands (p. 100) together with νC=O of the methoxycarbonyl grouping at 1780 cm^{-1}. [The high frequency of an arylalkyl carbonate is a consequence of the –I effect of the second electronegative oxygen attached to an electron-delocalising aromatic ring, (C=C–O←CO–OMe).] The sole carbonyl group of the *benzophenone* (Spec. 50) is chelated, doubly conjugated and flanked by methoxyls, and in fact absorbs at *ca.* 1600 cm^{-1}. It coincides with the first of the aromatic ring stretching modes (~1600, 1570 and 1490 cm^{-1}), which has itself been intensified by the substituent oxygen (compare the other spectra, especially 51 and 52). The absorp-

EXAMPLE 13. *Carboxylic acids, their salts and amino-acids.*

Spec. 45. Benzoic acid
„ 46. Sodium benzoate
„ 47. L–Proline
„ 48. L–Tryptophane
} Nujol mulls

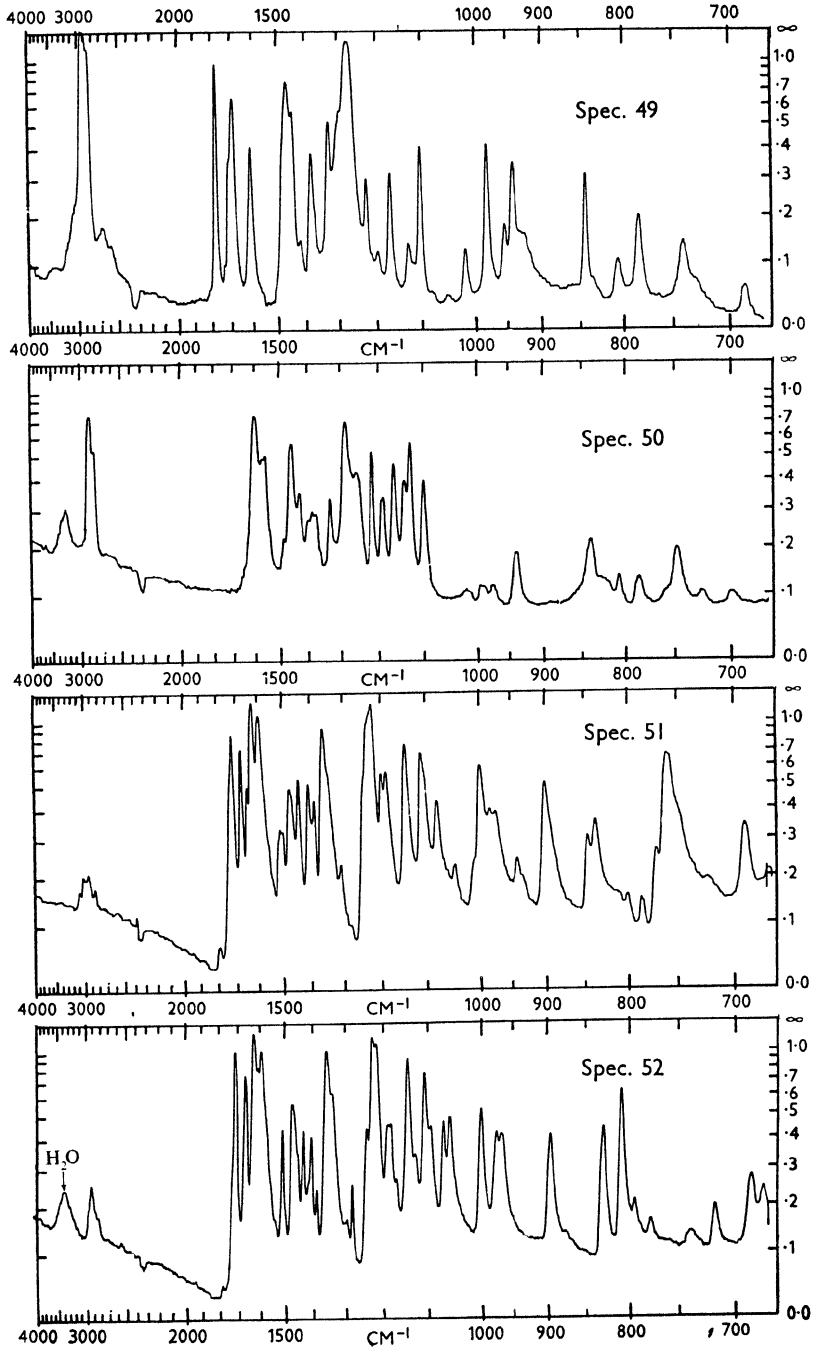


Reagents. (1) $RCO_2H \rightarrow RCOCl$, followed by addition of the phenol ($AlCl_3$/nitrobenzene/20°). (2) Hydrolysis of methoxycarbonyl grouping. (3) Alkaline $K_3Fe(CN)_6$. (4) H_2/catalyst.

tion of the chelated hydroxyl is too weak and broad to be detected but the intermolecularly hydrogen-bonded phenolic hydroxyl absorbs at 3300 cm^{-1}. The oxidative coupling, a likely step in the biogenesis, goes smoothly (60% yield) with the formation of the spiro-linked (±) *dehydro-compound* (Spec. 51). Again the ultraviolet and infrared spectra

EXAMPLE 14. *The total synthesis of griseofulvin.*

„ 49. 2–Methoxy–4–methoxycarbonyl–6–methyl benzoic acid (Nujol mull)
„ 50. The 'benzophenone derivative' (Nujol mull)
„ 51. Dehydrogriseofulvin
„ 52. Griseofulvin
} KCl discs, 1·4 mg in 300 mg


4000 3000 2000 1500 1000 900 800 700
Spec. 49
Spec. 50
Spec. 51
Spec. 52
H₂O
CM⁻¹
∞ 1·0 ·7 ·6 ·5 ·4 ·3 ·2 ·1 0·0

change dramatically and the region 1800–1500 cm^{-1} takes on a rather crowded appearance. The value of 1725 cm^{-1} for $\nu C=O$ of the five-membered coumaranone ring can be explained in an approximate fashion in terms of ring strain ($\nu C=O$ rises, 5-ring ketone), the electronegative oxygen atom in the ring (ν rises) and vinylogous conjugation (ν falls; aromatic ring with three *o* and *p* oxygen substituents). The dienone $\nu C=O$ at $\sim$1670 cm^{-1} seems high, but in any case measurements in the solid state are not strictly reliable. The conjugated double bond and the aromatic ring vibrations are all in the 1600 cm^{-1} region.

The final step gives (±) *griseofulvin* (Spec. 52). The reduction of one double bond has not changed the spectrum a great deal, though the differences (e.g. near 800 cm^{-1}) are sufficient to enable the compound to be followed during chromatographic separation from dehydro- and dihydro- griseofulvins and the ring-opened benzophenone. The inactive (±) compound and the natural, optically active (+) griseofulvin had identical infrared spectra (in the solid state this need not necessarily be the case as a racemate and a single enantiomer may crystallise in different forms, having different m.p.'s and solid-state infrared spectra). The synthesis was formally completed by resolution of the racemate *via* an optically active quinine salt of griseofulvic acid.

PART III. RAMAN SPECTROSCOPY

Raman and infrared spectra give the same kind of molecular information and either method can supplement or complement the other. In the 1930's Raman measurements were more the vogue than those in the infrared region, partly because the former could be directly recorded photographically while the latter required manual plotting of galvanometer deflections. A major change occurred about 1945 with the introduction of commercially-available, automatically-recording, infrared instruments. Raman spectra have since been little used by the organic chemist, though some return to popularity seems imminent.

Origin of Raman spectra.[1,2,32–36] The Raman effect was first observed by the Indian physicist, Raman, in 1928, though it had been predicted earlier by Smekal. Raman found that when molecules were irradiated with monochromatic light, the scattered light contained not only the original frequency (Rayleigh scattering) but also, to a very small extent, other frequencies. The frequency differences between *Raman lines* (or bands) and the original frequency turned out to be characteristic of the molecule irradiated and numerically identical with most of the infrared frequencies of that molecule.

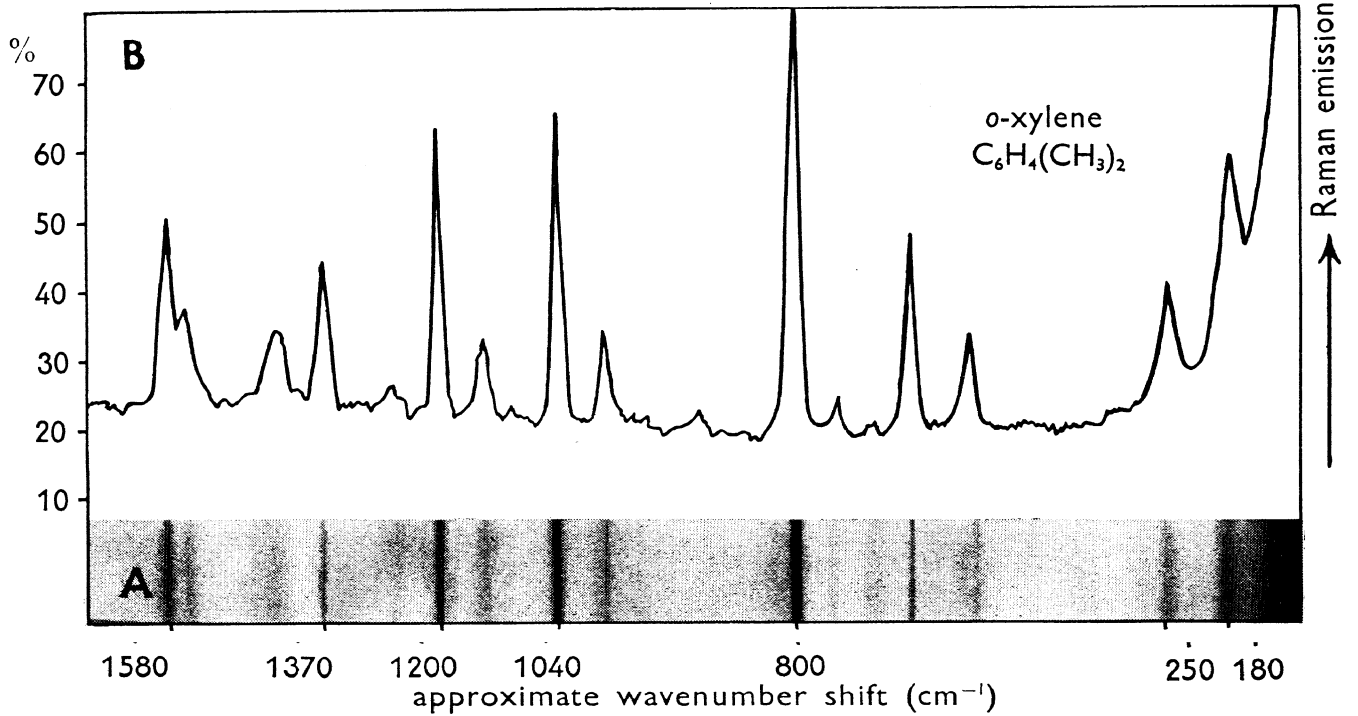


FIG. 10. Typical Raman spectrum shown (A) as the blackening on a photographic plate and (B) as it is seen either as a microdensitometer recording of the same blackening or as a direct photomultiplier recording of the scattered and dispersed radiation (by courtesy of Hilger and Watts Ltd.)

For Raman spectroscopy visible light is used; the shorter-wavelength, higher-energy radiation of the ultraviolet region is not only absorbed by many substances (Chapter 4) but also may cause molecular dissociation. The Rayleigh scattering which arises from the simple interaction of the incident light with the molecule, followed immediately by emission without permanent interchange of energy (elastic collision of the photon), accounts for some 99% of the scattered light and is registered as an intense *parent line* of unchanged frequency (Fig. 10). The *Raman scattering* (<1%) consists of two sets of lines, the Stokes and the anti-Stokes. The *Stokes lines*, the stronger set, lie to the low frequency side of the parent line and are the result of retention† by molecules of part of the energy of the incident light (inelastic collision of the photon). On irradiation, the diatomic molecule of Fig. 11 is raised from the ground

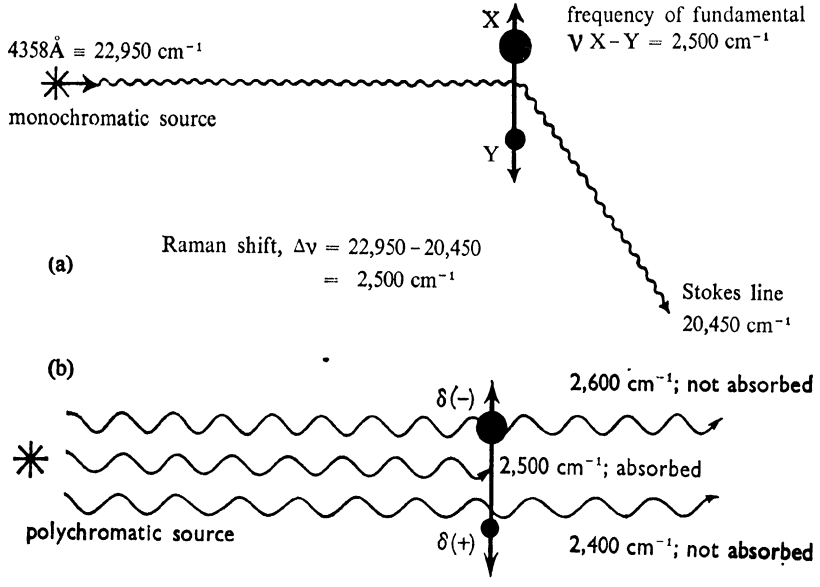


FIG. 11(a). Generation of a Stokes line by a simple diatomic molecule X - Y (substance in liquid state)
(b) Absorption and transmission of infrared radiation by the same molecule
In both cases the wavelength of the radiation greatly exceeds the molecular dimensions.

state (p. 37) to the first vibrational level ($v_0 \rightarrow v_1$) and the Stokes radiation emitted by the molecule is of correspondingly lower energy and fre-

† The light is not *absorbed* (in the conventional sense) prior to emission of the Stokes line. Absorption followed by emission results in fluorescence.

quency. In this simple case, it is a single line and the energies are related thus:

$$E_{\text{incident light}} = E_{\text{vibrational}} + E_{\text{Stokes line}}$$

For a complex molecule several Stokes lines are observed (cf. Fig. 10) owing to the excitation of the various fundamental stretching and bending modes available to a polyatomic molecule (see p. 37). The frequency separation (quoted as ν, the *Raman frequency* or $\Delta\nu$, the *Raman shift*) between the parent line and a Stokes line gives the frequency of the particular fundamental vibration concerned, and it is, of course, independent of the exciting frequency. (For a discussion of the Raman effect in relation to rotational transitions see ref. 36.)

The *anti-Stokes lines* appear as a rather faint mirror image of the Stokes lines and are generally ignored as they furnish no additional information. Each line is the result of the return to the ground state (e.g. $v_1 \rightarrow v_0$) of molecules in an upper vibrational level, the energy quanta being given up to the incident radiation.

The Raman spectrum may be suitably represented by plotting the intensities of the Stokes lines on a linear frequency-shift scale (the parent line being taken as 0 cm^{-1}). Modern Raman instruments record directly in this fashion (Fig. 13; numerous examples are to be found in a recent paper[37]). Although the result is reminiscent of the infrared spectrum of the same substance, with a close correspondence in frequencies, the intensity relationships between the bands are different. This difference derives from the fact that while the intensity of an infrared absorption band is related to the change in *dipole moment* during

TABLE 10

Comparison of infrared absorption and Raman scattering characteristics

Observation	*Inference*	*Probable origin of absorption*
weak Raman line	small polarisability change	vibration of unsymmetrical group, e.g. $\nu C=O$ and νOH *or* antisymmetrical vibration of symmetrical group e.g. $\nu C=C=C$ $\rightarrow \leftarrow \rightarrow$
strong infrared absorption	large dipole moment change	
strong Raman line	large polarisability change	vibration of symmetrical group, e.g. $\nu C=C$, $\nu C\equiv C$ *or* $\nu C=C=C$ $\leftarrow \quad \rightarrow$
weak infrared absorption	small dipole moment change	

movement of the nuclei in the bond (p. 40), the intensity of a Raman line is related to the change in *polarisability* during the same movement. For small molecules of high symmetry, e.g. CCl_4 or $HC{\equiv}CH$, selection rules based on these relationships permit the appearance of most of the fundamentals only in *either* the Raman *or* the infrared spectrum, but not in both. However, the organic molecules commonly encountered have very low symmetry and almost every line appearing in the Raman spectrum will have its counterpart in the infrared spectrum, but the weak bands in one will often be strong bands in the other (Table 10).†

Apparatus and sampling.[32,37] Samples are usually liquids or strong solutions; they should be colourless, non-fluorescent and free from dust (to prevent Tyndall scattering). Solids (other than large single crystals) and gases can be examined only with considerable difficulty. Common solvents are CCl_4, $CHCl_3$ and *water* (!), all of which exhibit only weak Raman scattering. The large quantity of substance required (0·05–10 g i.e. some hundred times greater than that required for infrared, p. 42) is a considerable deterrent to the use of Raman in academic work but not necessarily so in routine industrial analysis.

The sample, contained in a glass cell, is irradiated (see Fig. 12) with an intense beam of monochromatic light (usually the 'mercury blue' line, 4358 Å i.e. 0·4358 μ or 22,950 cm^{-1}). The scattered light emitted

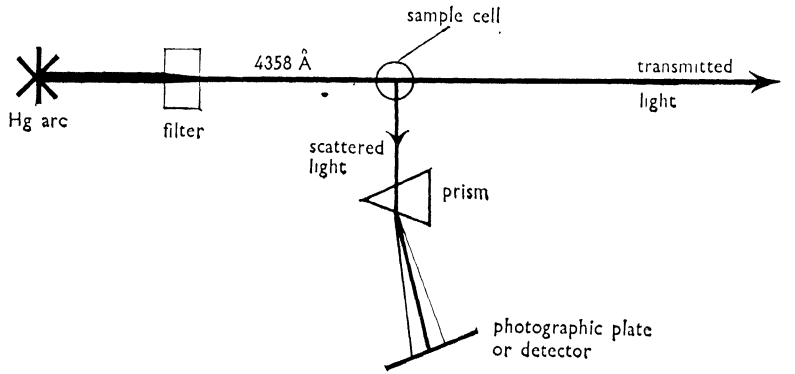


FIG. 12. Diagrammatic representation of basic Raman equipment

at right angles to the beam enters a spectrograph and the resulting spectrum is photographed using an exposure time of up to several hours (Fig. 10A). The plate can be examined visually or, better, with a micro-

† Raman lines are all polarised to varying degrees, the extent being expressed in terms of a *depolarisation factor* (ρ). ρ values are of considerable significance for symmetrical molecules but in general they are not amenable to ready interpretation.

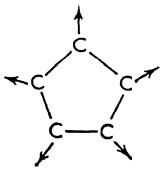
densitometer which provides a graphical record of the density of blackening on the plate at the various frequencies (Fig. 10B). The newer photoelectric instruments (e.g. Cary model 81 and the Hilger instrument) dispense with the photographic plate and record the intensity of the scattered light rapidly and directly by means of a scanning mechanism, photomultiplier detector, amplifier and strip-chart recorder (cf. Fig. 4, p. 30). The *intensity* of a line is *linearly* proportional to the quantity of substance in the sample cell (not logarithmically as with absorption spectroscopy, Chap. 2, p. 24) and is defined in terms of a *scattering coefficient*—the ratio of the intensity of the line to that of a line generated by a known thickness of a standard liquid, e.g. CCl_4.

Applications.[2,32,34,35] As yet, few organic chemists have ready access to modern Raman instruments, but with some problems it may be worth seeking one out. Some of the advantages can be listed as follows: (1) aqueous solutions can be examined; (2) a feature which should facilitate biochemical studies; groupings (e.g. $C\equiv C$, $C=C$, $C-C$, $S-H$) which generally absorb weakly or not at all in the infrared, scatter strongly in the Raman and should therefore be amenable to study and to analytical control; (3) the low-frequency region below 650 cm^{-1} (see p. 22), which necessitates special far-infrared equipment, is easily studied with Raman techniques; (4) Raman spectra are frequently less complicated than their infrared counterparts† and hence are more amenable to interpretation—though it must be admitted the field is less well documented; in addition, (5) frequencies can be determined with considerable accuracy. Sampling restrictions, such as the necessity for large liquid samples which are free from dust, colour and fluorescence and which are stable to the intense radiation, are probably the main drawback.

Raman spectroscopy can be used in much the same way as has already been discussed for infrared spectroscopy (pp. 44–55); thus a spectrum can serve as a fingerprint of a substance, as a criterion for analytical control and as a basis for structural interpretation. The Raman shifts for a particular group will generally coincide with the absorption frequencies given for that group in the infrared charts (I–VIII, pp. 51–54). Saturated and unsaturated hydrocarbons have received considerable attention, particularly as to the effect of alkyl substitution, and one analytical method is claimed to detect 0·001% benzene in a mixture. Raman spectroscopy is clearly of special value when the information to be derived from it is not readily obtained by infrared spectroscopy. Data concerning $\nu C=C$ and $\nu C\equiv C$ are good examples

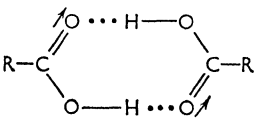
† Due to the comparative weakness of overtones and combination tones (cf. p. 39).

—as are the symmetrical ring-stretching modes of cyclic hydrocarbons (see below), the CH in-plane bending modes at 1450–1200 cm^{-1} of hydrocarbons (δCH; vs in Raman, w in infrared) and the $\nu C{=}O$ sym. stretch of carboxylic acid dimers (cf. p. 100; see below). The latter



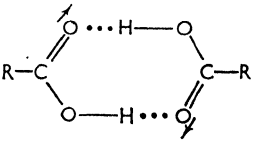
~890 cm^{-1}

Raman s
infrared vw



antisym. $\nu C{=}O$
~1710 cm^{-1}

Raman w
infrared vs



sym. $\nu C{=}O$
~1660 cm^{-1}

Raman vs
infrared vw (normally not observed)

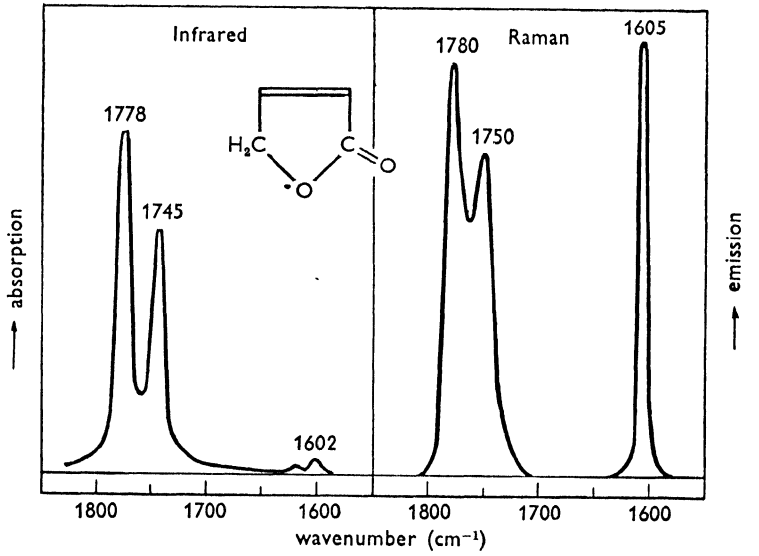


FIG. 13. Infrared and Raman spectra of $\Delta\alpha\beta$-butenolide illustrating intensity differences of the C=C stretching band at 1602–1605 cm^{-1}. The doubling of the C=O stretching band is ascribed to Fermi resonance (solutions in CS_2; from R. N. Jones, *Instituto Superiore di Sanita*, 1959, Vol. II, Part III).

example illustrates how an apparent non-coincidence of the Raman shift and the infrared absorption may be explicable in terms of two types of vibrations. In Fig. 13, the behaviour of the $\nu C=C$ mode at $\sim 1603\ cm^{-1}$ and of the carbonyl $\nu C=O$ Fermi doublet at ~ 1748 and $1780\ cm^{-1}$ typifies the differing intensity relationships in the Raman and infrared regions. More studies of this type, in which complex molecules are studied by both methods, are certain to be made in the near future.

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Chapter 4

P. Bladon

ULTRAVIOLET AND VISIBLE SPECTROSCOPY

Theoretical Aspects

As mentioned earlier (see Chapter 2, p. 23 and Chapter 1), the absorption of ultraviolet or visible light by organic molecules is accompanied by the excitation of valency electrons to higher energy levels, the frequency (ν) of the light being related to the difference (ΔE) between the energy levels by the equation $\Delta E = h\nu$. Theoretical calculation of these energy differences is not easy and to account for the spectrum of even a simple compound, such as benzene, is a relatively complex matter. Fortunately, to do so is usually unnecessary for the application of the method to organic chemistry, since the arguments used are based mainly on analogies with related compounds of known structure (cf. infrared spectroscopy, Chapter 3). Nevertheless the present chapter begins with a brief theoretical survey of the most important classes of electronic transitions, since a knowledge of these is an aid in the classification of the empirical data and in the appreciation of the types of compound to which the method can be applied. This survey is followed by a discussion of practical aspects, and the remainder of the chapter consists of a presentation of the absorptions of some common types of compounds together with examples of typical applications. Detailed theoretical considerations have been excluded for the sake of brevity and simplicity (the reader is referred to a number of excellent introductions to this aspect[1–5]). Interest in the theoretical interpretation of the spectra of complex molecules has however deepened considerably in the last few years and even a qualitative understanding of the major theories is often of value, e.g. in assessing the validity of particular analogies or in predicting the spectra of compounds for which analogies are not available.

Types of transitions. The wavelength of the absorption associated

with an electronic transition depends on the energy both of the orbital originally occupied by the electron and of the higher energy orbital (generally antibonding) to which it is promoted. Saturated hydrocarbons contain only strongly bound σ-electrons and the excitation of these to antibonding σ^*-orbitals requires very large energies, corresponding to absorption in the far-ultraviolet region. The transitions which give rise to absorption in the readily accessible ultraviolet (> 185 mμ) and visible regions are mainly of three types†:

(i) $\pi \rightarrow \pi^*$ *transitions*, i.e. transitions in which a π-electron is excited to an antibonding π-orbital. These occur with compounds which contain conventional double or triple bonds or aromatic rings. Although ethylene itself absorbs strongly only below 185 mμ, conjugated π-electron systems are more readily excited and butadiene, for example, absorbs in the accessible ultraviolet. In fact, the most important application of ultraviolet spectroscopy is the elucidation of the presence, extent and nature of conjugation. Increasing conjugation generally moves the absorption to longer wavelengths and finally into the visible region; this is well illustrated by Table 3, p. 134.

(ii) $n \rightarrow \pi^*$ *transitions*, i.e. transitions in which an electron in a nonbonding atomic orbital (lone pair) is excited to an antibonding π-orbital. These occur with compounds which contain double bonds involving heteroatoms (e.g. C=O, C=S, N=O, etc.), the most common example being the weak absorption at *ca.* 300 mμ associated with an isolated keto-group.

(iii) $n \rightarrow \sigma^*$ *transitions*, i.e. excitation from a nonbonding orbital to an antibonding σ-orbital. These are of less importance than the first two classes, the most common example being the absorptions (generally in the region around 200 mμ) of saturated compounds containing heteroatoms such as sulphur, nitrogen, bromine and iodine (the absorptions of oxygen and chlorine compounds lie at shorter wavelengths).

Groupings which give rise to absorptions of the above types are known as *chromophores* (Greek, colour bearer). This term is derived

† Numerous other classifications may be found in the literature. Thus the designation N → V is used to describe transitions from a bonding orbital to an antibonding orbital ($\sigma \rightarrow \sigma^*$ and $\pi \rightarrow \pi^*$ transitions) and the term N → Q designates transitions from a nonbonding atomic orbital to a higher energy molecular orbital ($n \rightarrow \sigma^*$ and $n \rightarrow \pi^*$ transitions). Two further types, N → R transitions (where the excited state corresponds to incipient ionisation as in Rydberg atomic spectra) and N → T transitions (where the excited state is a triplet), are of minor significance to organic chemists and are not discussed further in this chapter.

$\pi \rightarrow \pi^*$ absorptions have also been called K-bands (from the German 'Konjugation') and $n \rightarrow \pi^*$ absorptions have been termed R-bands (from an earlier view that the excited state is a radical). Numerous terms have also been devised for the classification of ground and excited states on the basis of symmetry etc.[1–6]; these cannot be considered here.

from an early theory of colour and it was originally restricted to groupings of the type (i). The term *auxochrome* (Greek, increase colour) was applied to groupings containing unshared *p*-electrons (–OH, $-NH_2$, –SH, Hal, etc.); when conjugated to π-electron chromophores these frequently move the absorption to longer wavelengths. Such a movement is often described as a *bathochromic* shift; the term *hypsochromic* denotes a shift to shorter wavelengths.

Fine structure. Transitions between electronic levels are usually accompanied by changes in vibrational level and the spectra of fairly

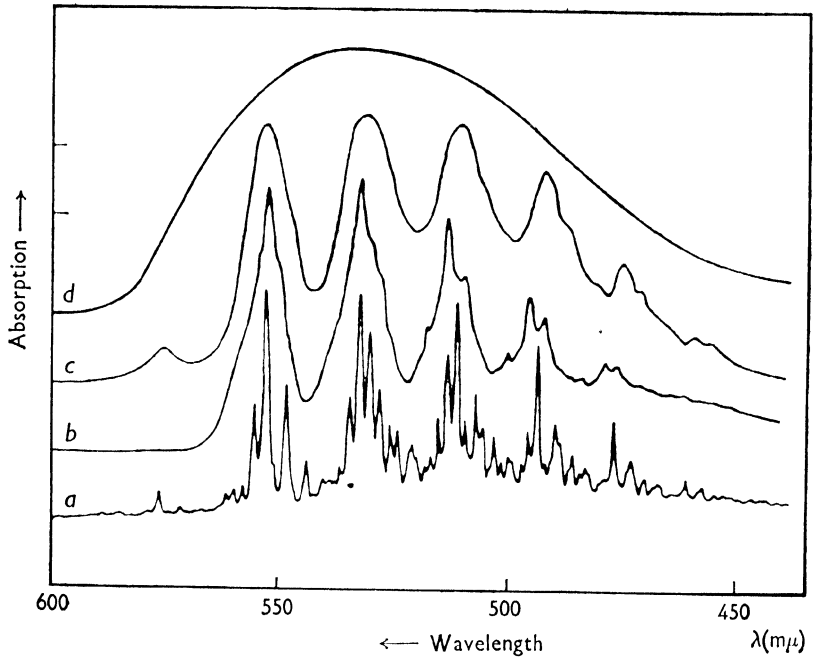


FIG. 1. Absorption of *sym*-tetrazine in the visible region in

(*a*) vapour state at room temperature
(*b*) 5:1 isopentane/methylcyclohexane glass at 77°K
(*c*) cyclohexane at room temperature
(*d*) water at room temperature.

The wavelength scale refers to spectrum (*a*). Spectra (*b*), (*c*) and (*d*) have been displaced by *ca.* −4, −6 and +20 mμ respectively. The spectra have also been displaced vertically to avoid overlap. The absorption is due to an $n \rightarrow \pi^*$ transition (cf. p. 152). Adapted from Mason.[7]

simple and rigid organic molecules often show well developed *fine structure* (see Fig. 1 and Fig. 2, p. 137), the individual peaks representing transitions to different vibrational levels of the upper electronic state. The fine structure is clearest in the vapour phase; in solution much of the detail is lost, especially in polar solvents (greater solute-solvent interaction). Fig. 1 provides a striking example. In more complex molecules the number of vibrational levels is very large and for this and other reasons the fine structure is generally entirely obliterated (cf. Fig. 3, p. 149). The spectra of most organic molecules therefore consist of broad bands. Although most of the applications of electronic spectroscopy in organic chemistry depend on empirical generalisations based on the wavelengths of the maxima of such bands, these maxima are in fact of limited theoretical significance, since they seldom correspond to the energy difference between the lowest vibrational levels of the ground and excited states (see ref. 1, p. 134 for details).

Intensities. Absorption bands differ not only in their wavelength but also in intensity. The measure of intensity usually used by organic chemists is the molar extinction coefficient at the wavelength of maximum absorption (ε_{max}, see p. 25); for theoretical purposes the band area is of greater significance. The magnitude of ε_{max} is of considerable value in identifying the nature of the chromophore responsible for the absorption, since the values of ε_{max} vary widely (Table 2, p. 32). Theoretically, the intensity of a band is related to its *transition moment* (a measure of the mean displacement of the promoted electron during the transition) and this depends mainly on the relative symmetries of the orbitals of the ground and excited states.[1,2] If the required symmetry conditions are fulfilled, the transition is said to be *allowed* ($\varepsilon > 5{,}000 - 10{,}000$); if they are not, the transition is termed *forbidden* and the absorption has much lower intensity. Departures from the typical intensities expected for a particular chromophore are often of considerable structural interest. For example, the absorption intensity of conjugated π-electron systems is decreased by departures from coplanarity (p. 159) and the intensities of forbidden transitions (e.g. the weak $n \rightarrow \pi^*$ absorption of the keto-group) may be enhanced in certain molecular environments (p. 153).

Experimental Technique

The examination of organic substances by ultraviolet and visible spectroscopic methods has become a routine operation in recent years, and in many laboratories it is provided as a service. This arrangement, while having the advantages of speed and convenience, may limit the

chemist's appreciation of the potentialities and limitations of the method. The technique involved with modern instruments (even the recording types) is simple enough to be mastered by the average chemist in an hour, and this effort is well worth while.

The basic features of spectrophotometers have already been discussed in Chapter 2. Details of instrument operation differ from instrument to instrument, and are given fully in manufacturers' instructions; repetition here would be of little value. There is, however, a large part of the experimental procedure which is common to all spectrometric work in this region, and this is briefly dealt with below (for further details see refs. 8 and 9).

Solvents. Solvents for ultraviolet and visible spectrometry must fulfil the requirements of dissolving the substance under test and of being transparent in the region of the spectrum to be examined. Compared with the infrared region the choice is fairly wide—solvent absorption bands do not usually interfere.

Ethyl alcohol is the solvent most commonly employed; it transmits ultraviolet light to below 200 mμ and is a good enough solvent for many organic compounds. Good quality absolute alcohol is necessary; this should have been dried by quicklime or calcium and not by azeotroping with benzene. If absolute alcohol of this quality is not available, 96% alcohol (rectified spirit), purified as described in ref. 10, is a good substitute, and may indeed be preferable in some cases (e.g. glycosides—for which it is a better solvent than absolute alcohol). Methyl alcohol and isopropyl alcohol can often be used in place of ethyl alcohol.

Hydrocarbon solvents (*n*-hexane, light petroleum, cyclohexane, isooctane [2, 2, 4–trimethylpentane]) are useful solvents for non-polar substances. They must be carefully purified (to remove all traces of aromatic and olefinic substances) by treatment with oleum and/or passage through silica gel.[11] When treated in this way they will transmit light to well below 200 mμ. Being less polar than alcohols, these solvents have less effect on any vibrational fine structure in spectra.

If the substance being tested is soluble in distilled water, or aqueous acid or alkali, then these solvents are excellent choices since their ultraviolet transmission characteristics are very good. Many acidic or basic substances undergo a change in spectrum on forming a salt, and if the spectra of such salts are examined in solution in water, the spectra of both the salt and the free acid (or base) formed by hydrolysis will be superimposed. In such cases it is best to examine the spectra in aqueous solutions of extreme pH (e.g. 0·1N HCl and 0·1N NaOH). Amphoteric compounds (e.g. pteridines) should be treated in the same way. Spectra

in buffer solutions of intermediate pH can be used to determine ionisation constants (see p. 161).

Dioxan, tetrahydrofuran and ether (very volatile!) are useful solvents for substances which are insoluble in alcohol. Chloroform is an excellent solvent for many substances but suffers from the disadvantage of being opaque below *ca.* 245 mμ. 2, 4–Dinitrophenylhydrazones are conveniently examined in this solvent or in dimethylformamide.

In choosing a solvent for an extended investigation, it is necessary to consider the solubility properties of all the compounds, and to choose a solvent suitable for all of them. This is important, since comparisons of spectra obtained in different solvents can be misleading. Corrections can sometimes be applied however (see p. 140).

Table 1 lists the transmission characteristics of the solvents commonly used in ultraviolet and visible spectroscopy; most of these are commercially available specially purified for this purpose (for details of purification procedures see refs. 8, 9 and 11).

TABLE 1.† Solvents for ultraviolet and visible spectrometry

Solvent	% *Transmission* at wavelengths (mμ) given at the top of each column (in 1 cm cell against water blank)								
	200	*210*	*215*	*220*	*225*	*230*	*250*	*255*	*300*
ethyl alcohol	15	34	46	56	66	73	92	95	99
methyl alcohol	21	30	44	56	66	75	94	96	99
isopropyl alcohol	—	38	53	63	73	79	95	96	99
n-hexane	28	56	73	83	90	93	99	100	100
cyclohexane	23	22	36	52	68	82	99	100	100
iso-octane	—	20	29	68	83	91	98	99	99
chloroform	—	—	—	—	—	—	20	—	97
ethyl acetate	—	—	—	—	—	—	—	25	91
acetic acid	—	—	—	—	—	—	10	—	—
carbon tetrachloride	—	—	—	—	—	—	(25 at 265 mμ)		97

† The figures quoted are taken from the current catalogues of Hopkin and Williams Ltd. and The British Drug Houses Ltd.

Cells. The cells used in ultraviolet work are generally made of fused silica. Glass cells can be used in the visible region.

Cells can be purchased as matched pairs but this is not necessary and indeed it is preferable to determine and correct for any inequalities in absorption. It is convenient to have a range of path lengths, 1 mm, 2 mm, 5 mm, 1 cm (most frequently used), 2 cm, 4 cm, and by use of several pairs in turn it is possible to cover a whole spectrum with the same solution. Variable path length cells can also be purchased. For accurate work the same cell of a pair should be used throughout for the solution and each cell should be placed in the instrument so that the same window faces the light source every time.

Every care should be taken to keep the cells clean. The windows should not be touched with fingers. To remove any traces of solution on the outside of a cell, tissues (paper handkerchiefs) moistened with solvent are useful. Air bubbles released from the solution during the recording of a spectrum can lead to errors, especially with aqueous solutions. This should be guarded against.

Preparation of solutions. If high accuracy is required, successive dilutions should be avoided in the preparation of the solutions.

In the case of non-recording spectrophotometers fitted with photoelectric cells, the greatest accuracy is obtained, in theory, when the optical density is 0·43. In practice, therefore, it is best to arrange the concentration and cell dimensions so that readings of optical density at important points are between *ca.* 0·3 and 0·8, outside which range the accuracy decreases. With recording instruments using photomultiplier tubes, it is advantageous to work at somewhat higher optical densities (*ca.* 0·9), provided that the proportion of 'stray light' (light of other wavelengths reaching the detector) is small.

The Absorptions of Some Common Structural Types

To appreciate the potentialities of ultraviolet and visible light absorption measurements in structural and analytical work one requires some knowledge of the absorptions of various types of compounds, and the following survey is intended to provide an introduction to this. As in the case of infrared spectroscopy (p. 48), a vast body of empirical data has been accumulated and the usual procedure in structural work is to compare the spectrum of the unknown with those of compounds analogous to the various possible structures (cf. Examples 1 and 2, p. 156). For some systems, the empirical data can be generalised into rules (e.g. p. 135), but these are not without exceptions and should be used with care. For certain types of compounds (e.g. polyenes and aromatic systems) generalisations based on molecular orbital theory can also be used as a basis for predictions (details may be found in refs. 1–5).

A great number of potential applications will become immediately obvious from the following survey; actual examples of some typical applications are also given (see especially p. 155). As mentioned earlier, the main value of the method is in the detection of conjugation and in the investigation of the extent and nature of conjugated systems. In general (but not always) increasing conjugation moves the absorption to longer wavelengths, often increasing the intensity of absorption. Two types of conjugation must be considered: (i) π-π conjugation

between multiple bonds and/or aromatic rings, and (ii) *p*-π conjugation, involving overlap between a lone pair orbital on a heteroatom (O, S, N and the halogens) and a π-electron system. The effect of the latter type of conjugation is most clearly seen in the aromatic series (p. 150). Alkyl groups attached to conjugated systems also tend to exert a small bathochromic effect (p. 134); this can be explained in terms of 'hyperconjugation', although this view is not without difficulties.[12]

In general, unsaturated systems which are not conjugated with each other tend to absorb independently and the absorption of the compound then approximates to the sum of the absorptions characteristic of the individual systems (cf. Example 3, p. 157). In some cases, however, interactions occur even between groups which are not formally conjugated and greatly modified absorption behaviour may then be observed (p. 153).

Absorption due to carbon-carbon multiple bonds

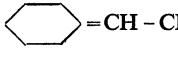
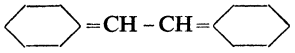
Isolated double bonds. Ethylene has a broad $\pi \rightarrow \pi^*$ absorption band at *ca.* 160 mμ which is outside the range of all instruments except vacuum spectrographs. Substitution of the hydrogen atoms by alkyl groups has the effect of raising the wavelength of the maximum, so that in tri- and tetra-substituted ethylenes it appears at ~185–200 mμ ($\varepsilon \sim 10{,}000$). Measurements in this region are just possible with conventional spectrometers fitted with fused silica prisms or with gratings, but special precautions are required to avoid false maxima and the interpretation of the results is not always unambiguous. The reader is referred to the literature for details of the data and the experimental technique.[10,13] One use of the method is in the detection of tetra-substituted ethylenic bonds; these do not give any characteristic absorption in the infrared region.

Conjugation with a heteroatom (auxochrome, cf. p. 128) moves the $\pi \rightarrow \pi^*$ absorption of ethylene to longer wavelengths, e.g. $CH_2{=}CH{-}OCH_3$ and $CH_2{=}CH{-}N(CH_3)_2$ have λ_{max} *ca.* 190 and 230 mμ respectively ($\varepsilon \sim 10{,}000$). The effect of such *p*-π conjugation is further illustrated by the aromatic compounds discussed later.

Conjugated dienes. The $\pi \rightarrow \pi^*$ absorptions of conjugated dienes lie in the accessible region and such dienes can therefore be distinguished readily from non-conjugated isomers. 1, 3-Butadiene, the first member of the series, has a maximum at 217 mμ (ε 21,000) (*unless otherwise stated all data refer to ethanol solutions*; in the particular case of olefins there is little or no change in the position of maxima on varying the solvent). Substitution of any one of the hydrogen atoms in buta-

diene by an alkyl group raises the position of the maximum by 3–6 mμ. As is seen from Table 2, this effect is additive. Included are data for some cyclohexylidene derivatives; in these, pairs of hydrogens in butadiene are replaced by a ring residue, which has a bathochromic effect slightly greater than two alkyl groups.

TABLE 2. Absorptions of aliphatic dienes

	λ_{max} (mμ)	ε
$CH_2=CH-CH=CH_2$	217	21,000
$RCH=CH-CH=CH_2$	223	24,000
$CH_2=CR-CH=CH_2$	220	22,000
$RCH=CH-CH=CHR$	227	23,000
$CH_2=CR-CR=CH_2$	226	21,000
	237	7,700
	247	18,000

Conjugated trienes and higher polyenes. Extension of the chromophore of butadiene by a third conjugated double bond (hexatriene) shifts the absorption maximum to 268 mμ (ε 56,000). Bathochromic shifts again occur on replacing hydrogens by alkyl groups.

The absorption maxima of some more extended polyenes are given in Table 3 which is adapted from refs. 14 and 15. Two series are considered, the unsubstituted polyenes and the dimethylpolyenes.

TABLE 3. Absorption of conjugated polyenes (in hexane or iso-octane)

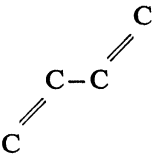
	trans $H-(CH=CH)_n-H$		*trans* $CH_3-(CH=CH)_n-CH_3$	
n	λ_{max} (mμ)	10^{-3} ε	λ_{max} (mμ)	10^{-3} ε
3	268	56	275	30
4	304	—	310	77
5	334	118	341	122
6	364	—	—	—
7	390	—	—	—
8	410	—	—	—

The Table illustrates the important (and theoretically explicable)[1,2] fact that the increase in wavelength for an additional double bond becomes smaller as the higher members of the series are reached. The absorptions show vibrational fine structure (cf. Fig. 5b, p. 159) and only the peaks of longest wavelength are listed above.

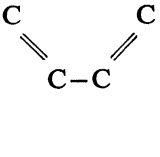
The Vitamins A and the carotenoids are important classes of polyolefinic compounds, the spectra of which have been studied closely (cf. p. 160). For reviews on these specialised fields see refs. 16 and 17.

Conjugated dienes and trienes in cyclic systems. When the diene chromophore is in a ring system striking changes are observed and the

absorptions are at much higher wavelengths than predicted by the above simple considerations. Cyclohexa-1,3-diene has a maximum at 257 mμ and the intensity (ε 8,000) is considerably lower than that of the acyclic dienes. A possible reason for this is that the diene system here has the *cisoid* conformation (i.e. *cis* disposition of double bonds about a single bond) in contrast to the *transoid* conformation of most acyclic dienes.



transoid (*s-trans*)
(most acyclic dienes)



cisoid (*s-cis*)
(cyclic dienes)

With increasing ring size the wavelength of the maximum decreases and *cis-cis*-cyclodeca-1, 3-diene does not show absorption above 210 mμ, presumably because the chromophore is forced out of coplanarity by the ring system (cf. p. 159).

Diene and polyene chromophores are often encountered in systems of condensed rings, particularly in the terpenoid and steroid fields. The great importance of ultraviolet spectroscopy in these fields cannot be overemphasised and the data on the steroids, collected in a review by Dorfman,[59] can often be applied to other types of compounds.

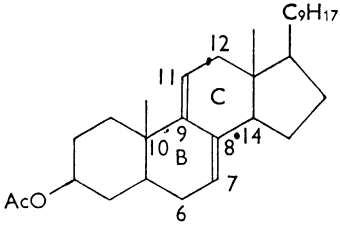
The positions of the light absorption maxima of dienes were systematised by Woodward,[18] and the rules that he devised were extended by Fieser and Fieser.[19] The method of the calculation is indicated in Table 4 and by the examples which follow.

TABLE 4. Woodward and Fieser's rules for the absorption of conjugated polyenes[18,19]

	mμ
Parent heteroannular diene	214
Parent homoannular diene	253
Increment for double bond extending conjugation	30
Increment for alkyl group or ring residue	5
Increment for each exocyclic double bond	5
Increment for oxygen functions (*O*-acyl or *O*-alkyl)	0
Solvent correction (compare Table 6)	0
	———
	λ_{max} (in EtOH) = Total

Examples of calculations

(*a*)



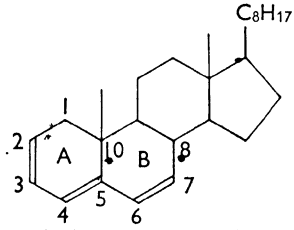
ergosta–7, 9 (11), 22–trienyl acetate

(1)

	mμ
Parent heteroannular diene	214
Four ring residues (4 × 5)	20
Two exocyclic double bonds (2 × 5)	10
λ_{max} =	244

The chromophore in this compound is of the heteroannular type (i.e. the double bonds are in different rings), therefore the parent will be of the simple butadiene type (214 mμ). The parent system is substituted on all the carbon atoms, $C_{(7)}$ by $C_{(6)}$, $C_{(8)}$ by $C_{(14)}$, $C_{(9)}$ by $C_{(10)}$, and $C_{(11)}$ by $C_{(12)}$. Each one of these counts as an alkyl group increasing the absorption wavelength by 5 mμ. In addition both double bonds are exocyclic to a ring, the double bond between $C_{(7)}$ and $C_{(8)}$ being exocyclic to ring C, that between $C_{(9)}$ and $C_{(11)}$ being exocyclic to ring B. Each of these eventualities merits a further 5 mμ shift. The calculated maximum (244 mμ) is in good agreement with the principal maximum actually observed (243 mμ). The two subsidiary maxima (236 and 251 mμ) are probably the remnants of vibrational fine structure.

(*b*)



cholesta–2, 4, 6–triene

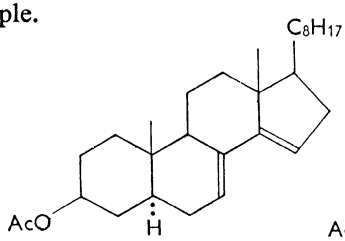
(2)

	mμ
Parent homoannular diene	253
Increment for extra double bond	30
Three ring residues (3 × 5)	15
Exocyclic double bond	5
λ_{max} =	303

The chromophore here has three double bonds and could be considered either as a homo- or a hetero-annular diene with an additional double bond. If a homoannular diene system is present (ring A in this case) the calculation must be based upon it. There are three ring-residue substituents, $C_{(1)}$ (on $C_{(2)}$), $C_{(10)}$ (on $C_{(5)}$) and $C_{(8)}$ (on $C_{(7)}$); in addition the Δ^4–double bond is exocyclic to ring B. The calculated maximum is in good agreement with the observed principal maximum (306 mμ), which has two subsidiary peaks (at 296 and 320 mμ).

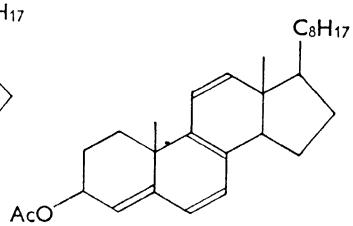
Two further examples, 5α–cholesta–7, 14–dien–3β–yl acetate (3) and cholesta–4, 6, 8 (9), 11–tetraen–3β–yl acetate (4) are given. In these the reader can check the calculated values for himself. Note that in the former example the basis of the calculation is the heteroannular diene type, notwithstanding the *cisoid* double bond arrangement.

The rules are fairly reliable for dienes and trienes, giving calculated values usually within ±5 mμ of the experimental figure, but with more extended polyenes the accuracy decreases, as illustrated by the last example.



(3)

λ_{max} 242 mμ (ε 10,000)
Calc. 244 mμ



(4)

λ_{max} 355 mμ (ε 13,500)
Calc. 343 mμ

Acetylenes and conjugated acetylenes. Simple acetylenes have an intense absorption band in the inaccessible region (~175 mμ, ε~6,000)

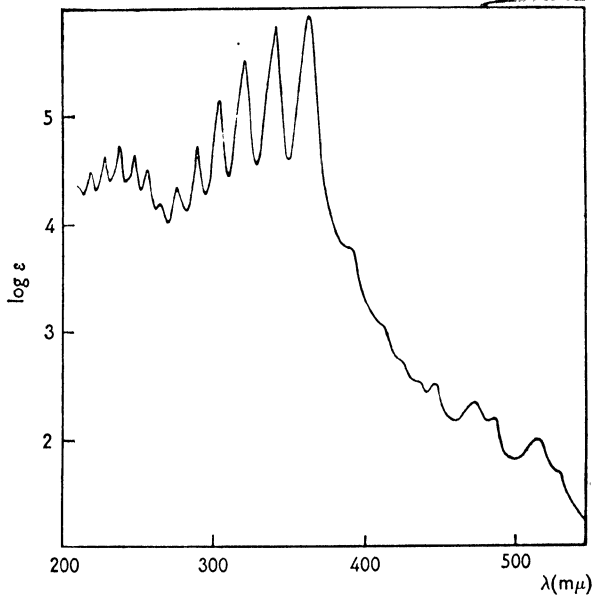


FIG. 2. Absorption of di–t–butyldeca–acetylene (from Jones, Lee and Whiting[20])

and several weaker peaks between 200 and 240 mμ. Conjugation of the acetylenic and ethylenic bonds as in vinylacetylene gives a band at 219 mμ (ε 6,400) having a shoulder on the long wavelength side, cf. butadiene (λ_{max} 217 mμ, ε 21,000). Further conjugation with ethylenic linkages gives absorption similar to that of the polyenes.

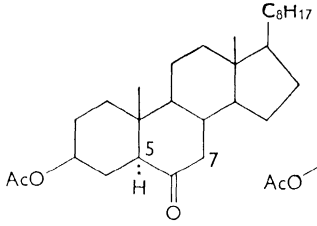
The conjugation of two or more acetylenic bonds results in compounds having very characteristic spectra, consisting of two bands both of which move to higher wavelengths with increasing conjugation and both of which show fine structure. The band at longer wavelengths is fairly weak (ε~250) but the second band is remarkably intense and shows unusually pronounced fine structure. Full details may be found in ref. 20. Fig. 2 shows the spectrum of the deca-acetylene $(CH_3)_3C-(C\equiv C)_{10}-C(CH_3)_3$ in which an additional group of bands appears at shorter wavelengths. This compound has ε_{max} 850,000! The spacings (in cm^{-1}) of the fine structure peaks are constant in each band and give the C≡C stretching frequencies in the excited states (cf. the stretching frequencies in the ground state obtained by IR; p. 39).

Absorption due to carbonyl groups

Isolated carbonyl groups. Simple ketones and aldehydes have weak light absorption in the region 270–300 mμ (ε 10–50) due to an $n \rightarrow \pi^*$ transition, the aldehydes absorbing at slightly longer wavelengths than the corresponding ketones. These bands are easily obscured by higher intensity absorption and are difficult to observe since fairly high concentrations are required. In the steroid series, the position of the maximum is not greatly affected by the position of the carbonyl group. However, these bands are useful in allowing a distinction to be made between aldehydes and ketones on the one hand, and esters and lactones on the other, since these latter compounds do not absorb in this region. This distinction cannot always be made on the basis of infrared spectroscopy.

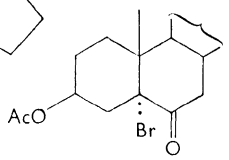
Cookson[21] has shown that the position of the low-intensity band in α-halogenated polycyclic ketones is a sensitive indication of the conformational orientation of the halogen atom. With axial bromine atoms the maximum is shifted towards longer wavelengths by 20–30 mμ and the intensity (ε) is increased from ~50 to ~150. In contrast equatorial bromine atoms cause only a slight hypsochromic shift (−5 mμ, measured from the maximum of the parent ketone), together with a slight increase in intensity (however, equatorial α-haloketones show characteristic differences in the infrared; see p. 70). Making use of these generalisations, Cookson showed that the products obtained by

brominating the steroid ketone (5) in positions 5 and 7 must both have axial halogens and hence the configurations shown in (6) and (7) (see p. 225 for details of the stereochemical conventions and nomenclature used in the steroid series).



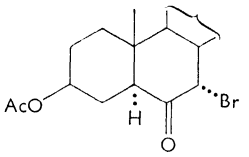
(5)

λ_{max} 280 mμ (ε 40)



(6)

λ_{max} 308 mμ (ε 125)



(7)

λ_{max} 310 mμ (ε 160)

The low intensity band of ketones is that which, in the case of optically active ketones, is responsible for the Cotton effect in the rotatory dispersion curves (Chapter 6). In fact, rotatory dispersion measurements can be used to detect keto-groups in such compounds and to observe the wavelength shifts obtained on halogenation (see also p. 229). The amount of material required for this is generally smaller than for absorption measurements.

Absorption of unsaturated aldehydes and ketones. The $\pi \rightarrow \pi^*$ absorption of an isolated carbonyl group lies in the far-ultraviolet, but conjugation with a double bond moves the absorption into the region 210–260 mμ. Table 5 gives the characteristics of typical aldehydes and ketones. It will be noted that the weak $n \rightarrow \pi^*$ band is shifted above 310 mμ in most cases. The effect on the short-wavelength absorption of introducing substituents on the carbon atoms is not constant and varies from *ca.* +5 to +15 mμ for each alkyl substituent. An additional double bond raises λ_{max} by about 30 mμ.

TABLE 5. Absorptions of unsaturated aldehydes and ketones

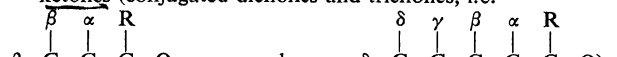
	$\pi \rightarrow \pi^*$ band		$n \rightarrow \pi^*$ band	
	λ_{max}	ε	λ_{max}	ε
$CH_2=CH-CHO$	208	~10,000	328	13
$CH_3-CH=CH-CHO$	220	15,000	322	28
$CH_3-(CH=CH)_2-CHO$	271	25,000	—	—
$CH_2=CH-COCH_3$	212	6,500	324	24
$CH_3-CH=CH-COCH_3$	224	9,750	315	38
$(CH_3)_2C=CH-COCH_3$	235	14,000	314	60
$CH_2=C(CH_3)-COCH_3$	218	8,300	319	27

Unsaturated ketones in cyclic systems. When unsaturated ketones occur in condensed cyclic systems (e.g. steroids and terpenes) prediction

of the wavelength of maximal absorption for the short-wavelength band can be made from rules devised by Woodward[22] and amplified by Fieser and Fieser.[19] These are similar to those used for polyenes but are rather less reliable. They are outlined in Table 6 and examples of the calculations are appended.

It will be noted that a solvent correction is included in the Table. The positions of the absorption maxima of ketones, in contrast to those of polyenes, are sensitive to solvent changes, a consequence of the greater polarity of carbonyl compounds compared with olefinic compounds, which results in a greater degree of solvent-solute interaction.†

TABLE 6. Woodward and Fieser's rules for the absorption of unsaturated ketones (conjugated dienones and trienones, i.e.



and

	mμ
Parent α, β–unsaturated system	215
Increment for double bond extending the conjugation	30
Increment for a homoannular diene component	39
Alkyl group, ring residue, OR, OAc — α	10
Alkyl group, ring residue, OR, OAc — β	12
Alkyl group, ring residue, OR, OAc — γ and higher	18
Increment for α–OH	35
Increment for α–Cl	15
Increment for α–Br	23
Increment for each exocyclic double bond	5
Solvent correction	see below
λ_{max} (in EtOH) =	Total

Solvent corrections for unsaturated ketones examined in solvents other than ethanol.‡

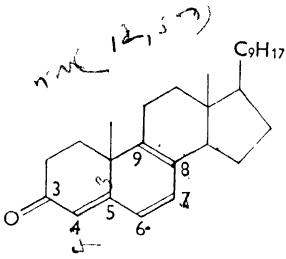
Solvent	*To convert observed* λ_{max} *to* λ_{max} *in* EtOH *add*
hexane	+11 mμ
ether	+ 7
dioxan	+ 5
chloroform	+ 1
methanol, ethanol	0
water	− 8

† The increase in λ_{max} with increase in the polarity of the solvent means that the energy necessary for the change *ground state* → *excited state* is less in the more polar solvent. There is good reason to believe that this is due to stabilisation of the excited state by interaction with the solvent. This is easily understood since the electron displacement during excitation results in an excited state which approaches the polar structure $C^+ - C = C - O^-$. In theory, this intramolecular charge transfer is analogous to the intermolecular charge transfer responsible for the absorptions discussed on p. 163.

In contrast, the $n \rightarrow \pi^*$ absorption of ketones undergoes a small hypsochromic shift on increasing the polarity of the solvent, since interaction with the solvent stabilises the lone pair electrons in the ground state.

‡ The corrections should be used for purposes of comparison only. It would be incorrect to *record* light absorption results obtained in (say) hexane as λ_{max} (in EtOH) after applying a correction.

Example of calculation

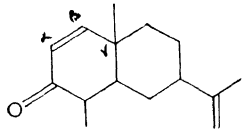


ergosta–4, 6, 8 (9), 22–tetraene–3–one (8)

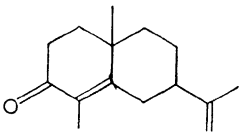
		mμ
Parent α, β–unsaturated ketone		215
Increment for two extra double bonds		60
Increment for homoannular diene component		39
Substituents	one β (on $C_{(5)}$)	12
	one ε (on $C_{(8)}$)	18
	two ξ (on $C_{(9)}$)	36
Exocyclic double bond		5
	λ_{max} (in EtOH) =	385

In fact, this compound shows a peak at 388 mμ (ε 12,300) with additional peaks at 288 (ε 2,190) and 244 mμ (ε 17,800). These two additional peaks are too far from the 388 peak to be due to vibrational fine structure but are probably absorptions due to parts of the chromophoric system only. For comparison, steroid Δ^4–3–ketones have a peak at 241 mμ (calc. 244 mμ) and cholesta–6, 8 (9)– diene has a peak at 275 mμ (calc. 273 mμ).

An illustration of the early use of ultraviolet spectra in structure determination is provided by α–cyperone. This has λ_{max} 252 mμ (ε 19,000) which is inconsistent with the structure (9) for which the calculated λ_{max} is 227 mμ. The alternative structure (10) is the correct one (calculated λ_{max} 254 mμ).



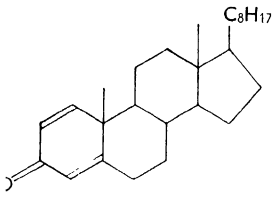
(9)



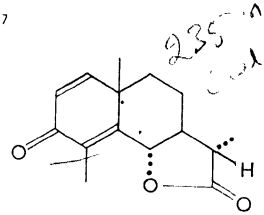
(10)

In cross-conjugated ketones in which the carbonyl group is conjugated separately to two mutually unconjugated olefin systems, maximal light absorption occurs, in general, at the higher of the two wavelengths calculated from the Woodward-Fieser rules, although agreement is not always good. The intensity is usually lowered. For example cholesta–1, 4–dien–3–one (11) has λ_{max} 244 mμ (ε 15,000). The two calculated values are 227 mμ (Δ^1–3–one) and 244 mμ (Δ^4–3–one). Examples of anomalous compounds are santonin (12),

λ_{max} 235 mμ (ε 11,000) [calc. λ_{max} 227 or 254 mμ] and cholesta–1, 4, 6–trien–3–one (13), λ_{max} 223, 256 and 298 mμ (ε 13,500, 11,900 and 15,300) [calc. λ_{max} 227 or 280 mμ].



(11)



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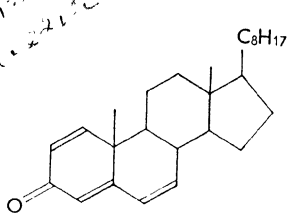
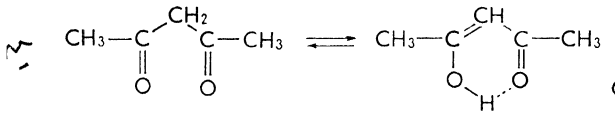


(13)

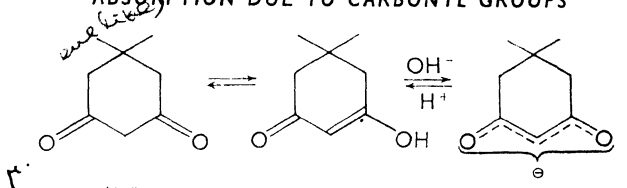
Vicinal diketones. α–Diketones show the weak $n \rightarrow \pi^*$ carbonyl band in the 290 mμ region. In addition some α–diketones and α–keto–aldehydes absorb weakly ($\varepsilon \sim 20$) at about 420 to 350 mμ in the visible region. This absorption is responsible for the pale yellow colour of glyoxal and diacetyl, two of the simplest coloured organic compounds. α–Diketones in cyclic systems frequently show evidence for the existence of an enol form. For example, cyclohexane–1, 2–dione has λ_{max} 266 mμ (ε 2,600) shifted to 294 mμ in alkaline solution. Additional double bonds serve to increase the wavelength of the maximum, the results being predictable by the Woodward-Fieser rules.

β–Diketones. β–Diketones which can enolise invariably show $\pi \rightarrow \pi^*$ absorption characteristic of the enol form, the keto form having only weak $n \rightarrow \pi^*$ absorption, and the investigation of such tautomeric equilibria is an important application of ultraviolet spectroscopy. Acetylacetone (14) is 84% enolised in ethanol and consequently shows ε_{max} 10,000 at 273 mμ. The unusually high value of λ_{max} is probably a consequence of the chelation in the enol form (15). The enol form (17)



(14) (15)

of dimedone (16) which is *transoid* and in which chelation is impossible has λ_{max} 255 mμ (ε 17,000) in acid solution, which is more in line with the value predicted from the Woodward-Fieser rules.



(16) (17) (18)

β–Diketones are acidic (being the vinylogues of carboxylic acids) and in alkaline solution the absorption maximum undergoes a bathochromic shift. For example, the anion (18) of dimedone has λ_{max} 282 mμ (ε 28,700).

Ene-diones. Ene-diones of the type of hex–3–ene–2, 5–dione have light absorption similar to that of the parent α, β–unsaturated ketone, the additional carbonyl group extending the conjugation having little effect. Compare, for example:

$CH_3COCH=CHCOCH_3$	$CH_3COCH=CHCH_3$
λ_{max} 226 mμ (ε 14,500) in hexane.	λ_{max} 224 mμ (ε 9,750).

Carbonyl derivatives. The spectra of semicarbazones, thiosemicarbazones and particularly of 2, 4–dinitrophenylhydrazones have proved useful in distinguishing aldehydes and ketones having different degrees of unsaturation and alkyl substitution.[9,23,24]

Carboxylic acids. In carboxylic acids and their simple derivatives (amides, esters, etc.) the low intensity $n \rightarrow \pi^*$ absorption of the carbonyl group (p. 138) is shifted to *ca.* 200 mμ and is often discernible

TABLE 7. Absorptions of unsaturated acids and esters

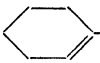
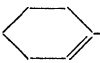
	R = H		R = Alkyl	
	λ_{max} (mμ)	ε	λ_{max} (mμ)	ε
$CH_2=CH-COOR$	<210	—	<210	—
$CH_3-CH=CH-COOR$	205	15,000	205	16,500
$(CH_3)_2C=CH-COOR$	216	12,000	217	14,000
$(CH_3)_2C=C(CH_3)COOR$	221	9,700	—	—
$CH_3-(CH=CH)_2-COOR$	254	25,000	—	—
$CH_3-(CH=CH)_3-COOR$	294	37,000	—	—
$CH_3-(CH=CH)_4-COOR$	327	49,000	—	—
ROOC – CH=CH – COOR				
cis (maleic acid)	210 (in water)	14,000	205	8,000
trans (fumaric acid)	—	—	211	16,000

only as a shoulder on a more intense band at shorter wavelengths. These absorptions are of little value for diagnostic purposes. However, in thiolesters the carbonyl group and the sulphur atom interact to give

a band at somewhat higher wavelengths (e.g. butyl thiolacetate has λ_{max} 231, ε 4,300); this absorption is useful in biochemical work.

Conjugation of a carboxyl or alkoxycarbonyl (ester) group with a double bond results in a high intensity $\pi \rightarrow \pi^*$ absorption in the region 200 to 230 mμ. Table 7 illustrates the bathochromic shifts caused by alkyl substituents on the double bond[25] and the shifts (50, 40 and 30 mμ) for successive additional double bonds.

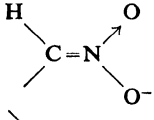
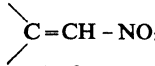
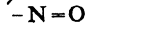
Compounds containing multiple-bonded nitrogen or sulphur. The absorption maxima of compounds containing unconjugated C=N or C≡N bonds generally lie below 200 mμ. Conjugation moves the absorption to higher wavelengths but the resulting compounds tend to absorb at somewhat shorter wavelengths than their carbon analogues, e.g. compare

	λ_{max}	ε
$CH_3CH=CH-CH=NC_4H_9$	220	23,000
$CH_3CH=CH-CH=CHCH_3$	227	23,000
 —C≡N	211	11,200
 —C≡CH	223	12,500

Many chromophores containing nitrogen give low intensity absorption bands due to forbidden $n \rightarrow \pi^*$ transitions. Examples are given in Table 8. Some of the groups can conjugate with olefinic double bonds giving intense $\pi \rightarrow \pi^*$ absorption (compare the first and third entries in the Table).

The weak $n \rightarrow \pi^*$ absorptions of isolated C=S groups lie at much longer wavelengths than those of C=O groups. The absorption is moved to shorter wavelengths in thion-esters (cf. ordinary esters) and is then accompanied by an intense $\pi \rightarrow \pi^*$ band.[26] The last three entries in Table 8 illustrate these points.

TABLE 8. Absorptions of some nitrogen and sulphur compounds containing multiple bonds

Functional group	*Example*	λ_{max}	ε
$-NO_2$	nitromethane	~270	15
	nitromethane in alkali	237	9,500
	1-nitropropene	230	10,000
	nitroso-*t*-butane	300	100
	(in ether)	665	20

Functional group	*Example*	λ_{max}	ε
$-O-NO_2$	octyl nitrate	~270 (shoulder)	15
$-O-N=O$	butyl nitrite	220 ~370 (several peaks)	2,000 50
$-N_3$	ethyl azide	220 287	150 20
$\diagdown CN_2$	diazomethane	~400	~5
$-N=N-$	azomethane	340	16
$-N=N-$ $\downarrow$ O	azoxymethane	217 274 (shoulder)	7,250 40
$\diagdown N-N=O$	diethylnitrosamine	233 350	7,400 90
$-S-C\equiv N$	ethyl thiocyanate	248	50
$-N=C=S$	ethyl isothiocyanate	245	1,000
$-C-$ $\parallel$ S	cyclohexanethione	495	weak
$-O-C-CH_3$ $\parallel$ S	ethyl thionoacetate	241 369	8,100 18
$-O-C-S-$ $\parallel$ S	*OS*-diethylxanthate	223 280 354	7,100 11,000 52

Light absorption of saturated compounds containing heteroatoms. In compounds of nitrogen, oxygen, sulphur or the halogens, absorption can arise from $n \rightarrow \sigma^*$ excitation of an electron in the lone pairs of the heteroatom. The absorption maxima of most saturated oxygen and chlorine compounds lie well below 200 mμ (it is therefore possible to use water, alcohol and ethers as solvents for ultraviolet spectroscopy). Other compounds containing heteroatoms absorb at longer wavelengths and examples are given in Table 9. One feature of particular interest is that the absorption is shifted to longer wavelengths in compounds containing two heteroatoms having overlapping lone-pair orbitals. This is illustrated in the Table by the polyhalides, the mercaptals and the disulphides. The last show striking variations due to differences in stereochemistry.

In contrast to the divalent sulphur compounds, hexavalent sulphur compounds, e.g. sulphones, sulphonic acids and esters and sulphonamides, are essentially transparent in the ultraviolet region if the remainder of the molecule is saturated.

TABLE 9. Absorptions due to heteroatoms in saturated molecules

Functional group	*Example*	λ_{max}	ε
-OH	CH_3OH†	184	150
-Cl	CH_3Cl†	173	weak
$-NH_2$	CH_3NH_2†	215	600
-Br	CH_3Br†	204	200
-I	CH_3I‡	258	365
	CH_2I_2‡	292	1,320
	CHI_3‡	349	230
-SH	C_2H_5SH	193	1,500
	(in water)	~220	200
-S-	$C_2H_5SC_2H_5$	210	1,800
		~230 (shoulder)	140
-S-C-S-	$CH_3SCH_2SCH_3$	235	540
-S-S-			
acyclic	$C_2H_5SSC_2H_5$	202	2,100
		252	420
in 6-ring	tetramethylene disulphide	295	300
in 5-ring	trimethylene disulphide	334	160

† in vapour. ‡ in saturated hydrocarbon solvent.

Absorption due to aromatic chromophores

Benzenoid aromatic systems. The ultraviolet absorption spectra of most aromatic compounds consist of three absorption bands, all due to $\pi \rightarrow \pi^*$ transitions. In the simplest case—benzene—there are two strong bands at 184 (ε 50,000) and 204 mμ (ε 7,400), the former being difficult to observe. The third band shows extensive fine structure in the vapour state and to a less degree in solution (Fig. 3); it is much weaker (forbidden transition), the 255 mμ peak having $\varepsilon \sim 200$. These three bands have been variously labelled (cf. ref. 1, p. 299) but here they will be called Bands I, II and III respectively.

In substituted derivatives of benzene and in aromatic compounds containing condensed nuclei these bands are modified, in general being moved to higher wavelengths. Fig. 3 and Tables 10 and 11 illustrate these points. It will be noted that some substituents, e.g. alkyl groups and halogen atoms (Nos. 2 to 8), have a small bathochromic effect upon both bands II and III, but have little effect upon the intensity of either band. Other substituents, such as the hydroxyl, alkoxyl, sulphonamide and amino groups (Nos. 9 to 12 and 16) which are themselves transparent in the near-ultraviolet region, have more pronounced bathochromic effects and also raise the intensity of Band III.

TABLE 10. Principal absorption maxima of benzene derivatives†

		Band II		*Band III*		*Additional bands*		*Bathochromic shift relative to benzene*	
		λ_{max} (mμ)	ε	λ_{max} (mμ)	ε	λ_{max} (mμ)	ε	II (mμ)	III (mμ)
1	benzene	204	7,400	254	200			0	0
2	toluene	207	7,000	261	225			3	7
3	ethylbenzene†	208	7,800	260	220			4	6
4	*o*–xylene†	210	8,300	263	300			6	9
5	*m*–xylene	212	7,200	265	300			8	11
6	*p*–xylene	216	7,500	268	500			12	14
7	chlorobenzene	210	7,400	264	190			6	10
8	bromobenzene	210	7,900	261	192			6	7
9	phenol	211	6,200	270	1,450			7	16
10	phenoxide ion	235	9,400	287	2,600			31	33
11	anisole	217	6,400	269	1,480			13	15
12	benzenesulphonamide	218	9,700	265	740			14	11
13	benzonitrile	224	13,000	271	1,000			20	17
14	benzoic acid	230	11,600	273	970			26	19
15	benzoate anion	224	8,700	268	560			20	14
16	aniline	230	8,600	280	1,430			26	26
17	anilinium cation	203	7,500	254	160			−1	0
18	acetophenone	246	9,800	280†	1,100	~320†	50	42	26
19	benzaldehyde	250	11,400	280†	1,100	~320†	50	46	26
20	nitrobenzene	269	7,800	obscured		~330†	~200	65	—
21	styrene	247	10,000	281	540			43	27
22	phenylacetylene†	235	10,000	~280	300			31	26
23	cinnamic acid†	273	20,000	obscured				69	—
24	cinnamaldehyde†	285	25,000	obscured				81	—
25	benzil†	259	20,000	obscured		370	80	55	—
26	benzophenone†	252	18,000	obscured		~330	180	48	—
27	diphenyl†	248	17,000	obscured				44	—
28	stilbene (*trans*)†	295	29,000	obscured				91	—
29	azobenzene (*trans*)†	320	21,300	obscured		443	510	116	—

† Data marked with a dagger are for ethanol solutions. All other data in this Table are for solutions in water containing a little methanol. The values of λ_{max} in this solvent are generally close to those in ethanol, especially for non-polar compounds.

TABLE 11. Principal absorption maxima (in ethanol) of some aromatic compounds with fused rings

		Band I		*Band II*		*Band III*		*Bathochromic shift relative to benzene*		
		λ_{max} (mμ)	ε	λ_{max} (mμ)	ε	λ_{max} (mμ)	ε	I (mμ)	II (mμ)	III (mμ)
30	naphthalene	220	100,000	275	5,700	312	250	36	71	58
31	α–naphthol	233	30,000	298	5,000	322	2,000	49	94	68
32	β–naphthol	225	70,000	285	3,000	330	2,000	41	81	76
33	α–naphthylamine	238	22,000	324	5,000	obscured		54	120	—
34	β–naphthylamine	237	60,000	280	6,300	337	2,000	53	76	83
35	β–naphthylammonium cation	222	100,000	275	5,600	311	400	38	71	57
36	phenanthrene	252	50,000	293	16,000	330	250	68	89	76
37	anthracene	253	200,000	375	8,000	obscured		69	171	—
38	tetracene	278	200,000	474	13,000	obscured		94	270	—
39	pentacene	310	270,000	580	15,000	obscured		126	376	—

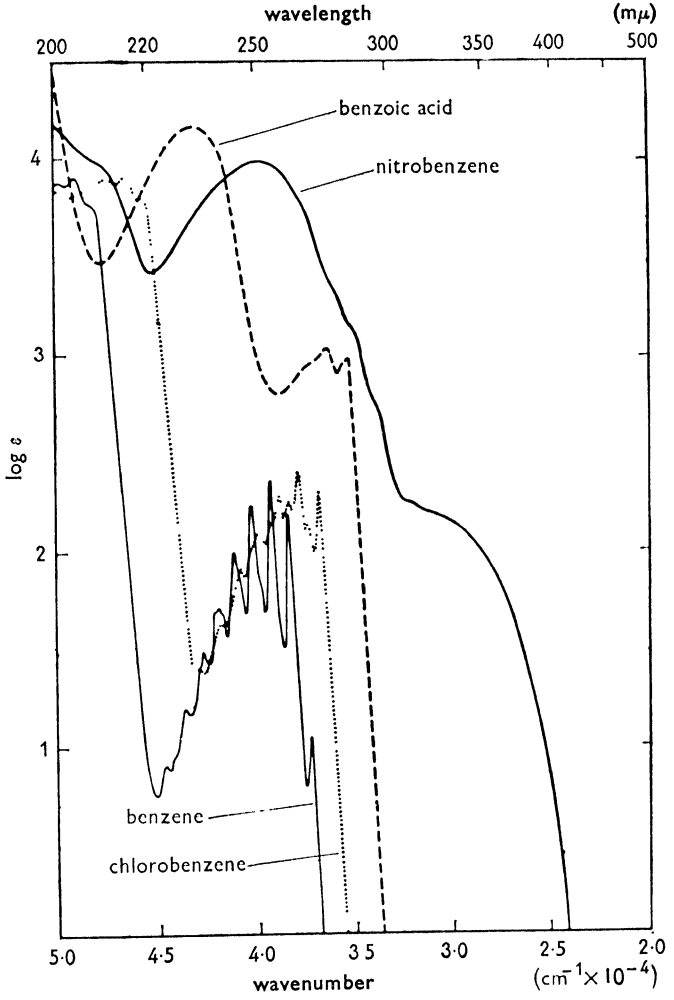
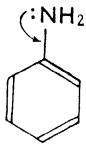


FIG. 3. Absorptions of benzene, chlorobenzene, benzoic acid and nitrobenzene in light petroleum (from Beaven, Johnson, Willis and Miller[8])

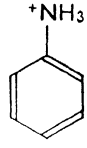
Substituents which are unsaturated (ethylenic and acetylenic, nitrile, aldehyde, carboxylic acid and nitro groupings) also have much the same effect, often to a greater degree (Nos. 13 to 15, 18 to 22). Two such chromophores (or an additional phenyl group) conjugated to the benzene ring have a strong bathochromic and intensifying effect on Band II, which moves out sufficiently to obscure Band III (Nos. 23, 24 and 27 to 29). In several cases weak bands appear at longer

wavelengths. For example, in nitrobenzene and azobenzene there are bands at 330 and 443 mμ respectively (which are responsible for the colour of these compounds). These bands, together with the bands around 320 mμ in the carbonyl compounds, are due to $n \rightarrow \pi^*$ transitions in the particular functional group (nitro, azo or carbonyl), modified by the remainder of the molecule.

The interpretation of the effect of substituents on the spectrum of benzene has been the subject of much theoretical work.[1-3,27] Details cannot be discussed here, but the data given above illustrate clearly that π-π and p-π conjugation play an important part. As an example of the latter, it is interesting to observe the difference in the spectra of aniline in neutral and acid solutions (Nos. 16 and 17).



(19)



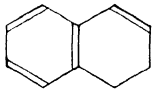
(20)

In aniline there is p-π conjugation between the lone pair of electrons on the nitrogen atom and the π-electrons of the benzene ring or, to put it in another way, there is a +M effect operating (19). In the cation (20) there is no longer a free pair of electrons, so that p-π conjugation is absent (no +M effect). Consequently the aniline cation has almost the same spectrum as benzene (except that the fine structure is absent). A similar effect is noted with β-naphthylamine and its cation (Nos. 34 and 35.)

It may be noted that charge transfer both *to* and *from* the ring (exemplified by NH_2 and NO_2 substituents) is effective in causing a bathochromic shift and intensification of the absorption. In disubstituted benzenes, the combined effect of the groups depends on their electronic type and relative orientation; p-orientated substituents of opposite type often give unexpectedly large shifts, e.g. p-nitroaniline has λ_{max} 375 mμ (ε 16,000).

In the aromatic compounds containing condensed nuclei (Table 11), Band I appears above 200 mμ; its wavelength increases with the number of rings. Band II also moves to higher wavelengths and in many cases obscures Band III. Linear hydrocarbons (e.g. anthracene) absorb at longer wavelengths than their angular counterparts (e.g. phenanthrene); this is diagnostically useful and can readily be inter-

preted theoretically.[1-3] The high intensity of the Band I absorption in the linear condensed hydrocarbons (anthracene, tetracene, pentacene) should be noted. The effect of substituents on the spectra of the polynuclear series can be roughly predicted on the basis of the effects noted in the benzene series.



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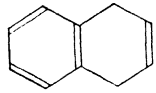
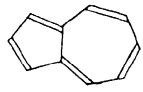


(22)

An early example of the use of the spectra of aromatic compounds was to distinguish the two dihydronaphthalenes formed by reduction of naphthalene with sodium. The two compounds had (*a*) λ_{max} 262 (ε 10,000), 296 (ε 500) and (*b*) λ_{max} 270 mμ (ε 800). Clearly the former is the 1, 2–dihydroisomer (21), having a spectrum similar to that of styrene but with the maxima at rather longer wavelengths (see Table 10). The spectrum of the latter resembles that of *o*-xylene, in agreement with the structure (22).

Non-benzenoid aromatic compounds. The spectra of non-benzenoid carbocyclic aromatic compounds often have a resemblance to those of their benzenoid analogues. Bands formally related to the Bands I, II and III of the benzene series can sometimes be identified. Often there is a considerable shift to longer wavelengths. Thus the tropylium

(23)



(24)

ion (cycloheptatrienylium cation, 23) has λ_{max} 275 (ε 4,500) (Band II?) and azulene (24) has bands with considerable fine structure at *ca.* 270 ($\varepsilon \sim 50{,}000$), 340 ($\varepsilon \sim 4{,}000$) and 600 m$\mu$ ($\varepsilon \sim 300$) corresponding to Bands I, II and III of naphthalene.[28] The weak long-wavelength absorption of azulene is responsible for its blue colour and the wavelengths of its peaks can be used for elucidation of the degree and position of substitution. Whereas with benzenoid hydrocarbons alkyl substituents always cause a bathochromic shift, alkyl substitution in azulene and certain other non-benzenoid aromatics may shift the absorption in either direction, depending on the position of substitution.[1,2,28] The absorptions of these compounds are of considerable interest from a theoretical point of view.

Heterocyclic aromatic compounds. Heterocyclic nitrogen compounds with six-membered rings have spectra which closely resemble those of their benzenoid counterparts, except that the intensity of the forbidden long-wavelength transition (Band III) is considerably higher. This may be seen by comparison of the spectra of pyridine, quinoline and isoquinoline (Table 12) with those of benzene and naphthalene (Tables 10 and 11). However, there is additional absorption at higher wavelengths due to $n \rightarrow \pi^*$ transitions involving the lone pair on the nitrogen; this appears as a shoulder at 270 mμ in the case of pyridine and as a separate band for compounds which contain more than one nitrogen atom, e.g. pyridazine (25), pyrimidine (26) and *sym*-tetrazine (Fig. 1).

(25)

(26)

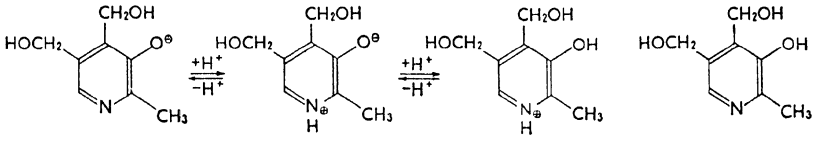
TABLE 12. The absorptions of some heterocyclic compounds

	λ_{max} (mμ)	ε	λ_{max} (mμ)	ε	λ_{max} (mμ)	ε
pyridine†	195	7,500	250	2,000	—	—
quinoline†	275	4,500	311	6,300	—	—
isoquinoline†	262	3,700	317	3,500	—	—
pyridazine†	—	—	246	1,300	340	315
pyrimidine†	—	—	243	2,030	298	326
pyridoxine in						
0·1N HCl (27c)	232	2,100	291	8,600	—	—
H_2O, pH 6·8 (27b)	254	3,900	324	7,200	—	—
0·1N NaOH (27a)	245	6,300	310	6,800	—	—
alcohol (27d)	—	—	286	5,700	—	—
N-methyl pyridoxine (pH 6·5)	256	4,300	330	6,100	—	—
O-methyl pyridoxine (pH 2–10)	—	—	280	—	—	—
2-hydroxypyridine (pH 6)	224	7,230	293	5,890	—	—
N-methyl-2-pyridone (pH 5)	226	6,100	297	5,700	—	—
2-methoxypyridine (pH 7)	—	—	269	3,230	—	—
furan†	200	10,000	250	1	—	—
thiophen†	—	—	235	5,000	—	—
pyrrole†	210	15,000	240	300	—	—

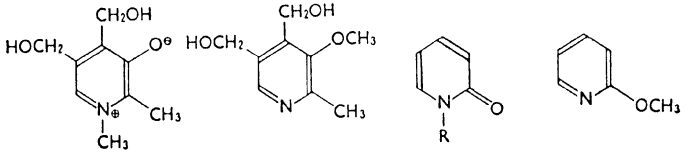
† in hydrocarbon solvent.

Ultraviolet absorption has been of great value in the characterisation and estimation of the countless heterocyclic derivatives which occur in nature. Many of these are amphoteric and the changes in absorption caused by acid and alkali may be of considerable diagnostic value, especially since the ionisation constants can often be determined spectrophotometrically (p. 161). Such changes may be illustrated by the behaviour of pyridoxine (27), a member of the B_6 group of vitamins

(see Table 12).[29] An additional feature of this compound is that the spectrum in alcohol is greatly different from that in water (pH 6·8). The latter spectrum resembles that of *N*-methyl pyridoxine (28) suggesting that pyridoxine exists in water mainly as the dipolar ion (27b) but that the uncharged species (27d) predominates in alcohol; the spectrum of pyridoxine in alcohol is in fact similar to that of *O*-methyl pyridoxine (29).



(27a) (27b) (27c) (27d)



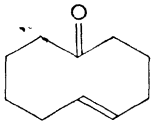
(28) (29) (30) (31)

The effect of pH on the spectrum of 2-hydroxypyridine shows that this compound is much weaker, both as an acid and as a base, than the 3-hydroxy compound. This, together with the fact that the spectrum (Table 12) resembles that of *N*-methyl-2-pyridone (30, R=CH_3) rather than that of 2-methoxypyridine (31), suggests that '2-hydroxypyridine' in fact exists as the tautomer (30, R=H). Similar arguments have been applied in related systems and to other tautomeric types.[30]

The absorption spectra of heterocycles containing five-membered rings differ greatly from those of benzene and its derivatives, a consequence of the profound difference in structure (Table 12).[1]

Non-conjugated electronic interaction.[1,2,31] In the preceding sections electronic spectra arising from π-electron unsaturated systems and from lone pair *p*-electrons have been considered and it has been assumed that interaction between two such systems can occur only if they are conjugated. In fact, evidence is accumulating to show that this is not always so, and compounds are known in which the absorption spectrum is not that expected for the two non-conjugated chromophores present. In most of these cases the chromophores are held in close proximity by the geometry of the molecule. The resulting interactions between the chromophores are sensitive to the orientation of the overlap of the

orbitals and may lead to the intensification of existing bands or even to the appearance of bands at unexpected wavelengths. The former behaviour is illustrated by phenylacetone which has ε_{max} 145 at 288 mμ (cf. $\varepsilon \sim 20$ at λ_{max} 270 for acetone), the latter by the unsaturated ketone (32) which has λ_{max} 214·5 (ε 2,300) in tetrafluoropropanol[32] and by barrelene (33) (λ_{max} 239 with ε 300).[33] The paracyclophanes (34) show shifts to longer wavelengths and a decrease in intensity as n decreases from 5 to 2, but this behaviour is due in part to distortion of the aromatic rings.[34]



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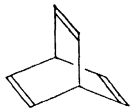

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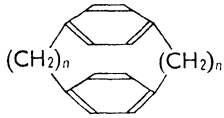
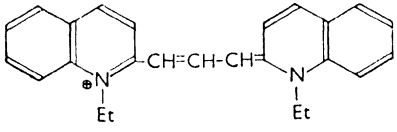


(34)

Dyes and colouring matters. No attempt will be made here to discuss even a small selection of the many complex conjugated systems which are responsible for the colour of synthetic dyes and of natural pigments such as chlorophyll. However, one structural feature common to a significant number of synthetic dyes is of special interest, namely, that many are cationic resonance hybrids of canonical forms in which the formal charge can occupy a number of positions. For example, in the cyanine dye (35) the charge may reside on either of the two nitrogens and a similar situation holds for the triphenylmethane dye, crystal violet (36). The spectra of such molecules have been called *charge resonance spectra* and their theory is well developed.[2,3,35,36]



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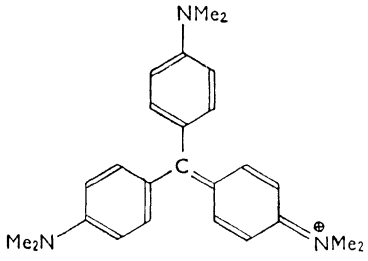


(36)

Absorption due to inorganic ions. A knowledge of the ultraviolet absorptions of inorganic ions is useful, since they are met with as salts

of organic bases (and acids) and are used in making buffer solutions. Table 13 gives the absorptions of common ions (see also refs. 2 and 37).

TABLE 13. Absorptions of inorganic ions in water

	λ_{max} (mμ)	ε
H^+	transparent	—
Na^+ K^+	transparent	—
NH_4^+	<200	—
OH^-	~187	4,000
Cl^-	183	10,000 (ϵ ~30 at 200 mμ)
Br^-	~195	12,000 (ϵ ~30 at 225 mμ)
I^-	230	12,000
	195	12,000
SO_4^{2-}	<190	~100 at 190 mμ
SH^-	230	~5,000
SO_3^{2-}	<220	1,000 at 220 mμ
NO_3^-	205	10,000
	300	7
CO_3^{2-}	~215 (shoulder)	~100

APPLICATIONS OF ULTRAVIOLET AND VISIBLE SPECTRA

A number of examples of the application of electronic spectroscopy to structural work have already been given above and also (in connection with infrared spectroscopy) in Chapter 3. In the present section, some general considerations are presented, together with further examples and a brief discussion of the many ways in which the analytical potentialities of the method can be exploited.

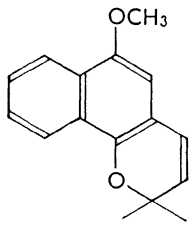
Structure determination. From the ultraviolet spectrum of an unknown compound information about the absorbing chromophore(s) can be obtained. Since these frequently involve several bonds, it is rare to be able to delineate functional groups as in infrared spectroscopy. Electronic absorption spectra are best considered in conjunction with the infrared and nuclear magnetic resonance spectra, and any other physical, chemical and analytical information which is available. They are often used to best effect in deciding between possible structures after the main functional groups and skeleton have been found by other means.

Some generalisations are possible, however. If a compound is transparent (ε<10) in the region from 200 to 800 mμ, it certainly contains no conjugated olefinic or benzenoid unsaturation, aldehyde and ketone groups are absent, and there is a high probability that no isolated double bonds are present. Strong absorption (ε~10,000) in the region 220–250 mμ is indicative of the presence of a 2-unit conjugated system (e.g. a diene or α, β–unsaturated ketone group). Similar absorption at ~260, 300 or 330 mμ is probably due to 3, 4 or 5 unit conjugated systems. A

medium intensity band ($\varepsilon \sim 200$–$1{,}000$) at about 260–300 mμ often indicates the presence of a benzene ring, while low intensity absorption ($\varepsilon \sim 20$–100) near 290 mμ is commonly due to a ketone or aldehyde group. Coloured compounds nearly always owe their absorption to a conjugated system of at least 4 units. Exceptionally, the colour may be due to one of the low intensity bands associated with certain functional groups (see Table 8).

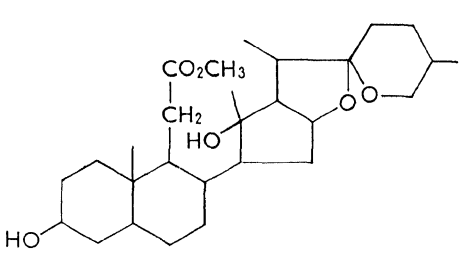
To illustrate the use of electronic absorption spectra, several examples, in which they have played an important role, follow.

Example 1.[38] Lapachenole, $C_{16}H_{16}O_2$, is a natural product present in Brazilian white peroba wood (*Paratecoma alba*). Chemical evidence showed that it is a naphthalene derivative containing a single methoxyl group, the other oxygen also being present in an ether linkage. The ultraviolet spectrum of lapachenole itself (λ_{max} 275, 362 mμ; ε 25,000, 6,000) indicated that an extra double bond must be conjugated with the naphthalene chromophore. Indeed, dihydrolapachenole has a spectrum (λ_{max} 250, 320, 338 mμ; ε 25,000, 6,000, 6,000) very similar to that of 1, 4–dimethoxynaphthalene (λ_{max} 245, 315, 330 mμ; ε 25,000, 6,000, 6,000). This similarity, coupled with degradative evidence, led to the formula (37) for lapachenole; this was confirmed by synthesis.



(37)

Example 2.[39] Treatment of methyl hecolate (38) with an excess of phenyl magnesium bromide (the first step of a Barbier-Wieland degradation) was expected to yield a tertiary alcohol (39) with simple benzenoid light absorption. Instead the product had λ_{max} 242 mμ (ε 9,700) which is very similar to that of propiophenone (λ_{max} 242 mμ, ε 13,500). Clearly the product is the ketone (40) formed by reaction with only one mole of Grignard reagent. On treatment with acetic acid, this ketone gave the cyclic enol ether (41) which has λ_{max} 263 mμ (ε 13,000). This is similar to the light absorption of β–methylstyrene (λ_{max} 251, ε 17,000), the ethereal oxygen atom having a slight bathochromic effect.



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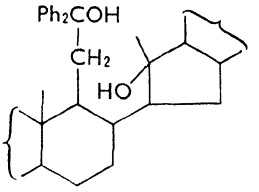

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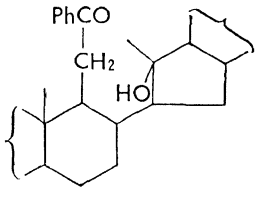

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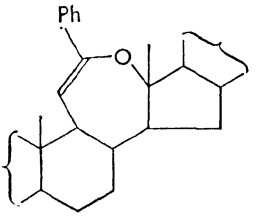


(41)

Example 3.[40] *The use of sum and difference spectra.* If two chromophores in a single molecule are sufficiently separated, no interaction occurs and the spectrum of the substance is the sum (on a molar basis) of the spectra of the chromophores. For example, the alkaloid reserpine $C_{33}H_{40}N_2O_9$ gives on hydrolysis the easily recognised 3, 4, 5-trimethoxybenzoic acid, reserpic acid $C_{22}H_{28}N_2O_5$ and methanol. Reduction of the carboxyl group in reserpic acid with lithium aluminium hydride gives reserpyl alcohol $C_{22}H_{30}N_2O_4$. The ultraviolet spectrum of reserpyl alcohol (Fig. 4, curve *c*) is characteristic of a substituted indole. If the spectrum of methyl 3, 4, 5-trimethoxybenzoate (curve *b*) is added to it, the summation spectrum (dashed line below curve *a*) is very similar to that of reserpine itself (curve *a*). Further, comparison of the spectrum of reserpyl alcohol with those of various substituted indoles revealed the closest similarity to that of 2, 3-dimethyl-6-methoxyindole (curve *d*). Neuss and his co-workers deduced from this the presence in reserpine of two distinct chromophores, namely this indole and a trimethoxybenzoate ester grouping. Similarities between reserpine and other yohimbine alkaloids, together with chemical degradative evidence, led to the formula (42) for the alkaloid.

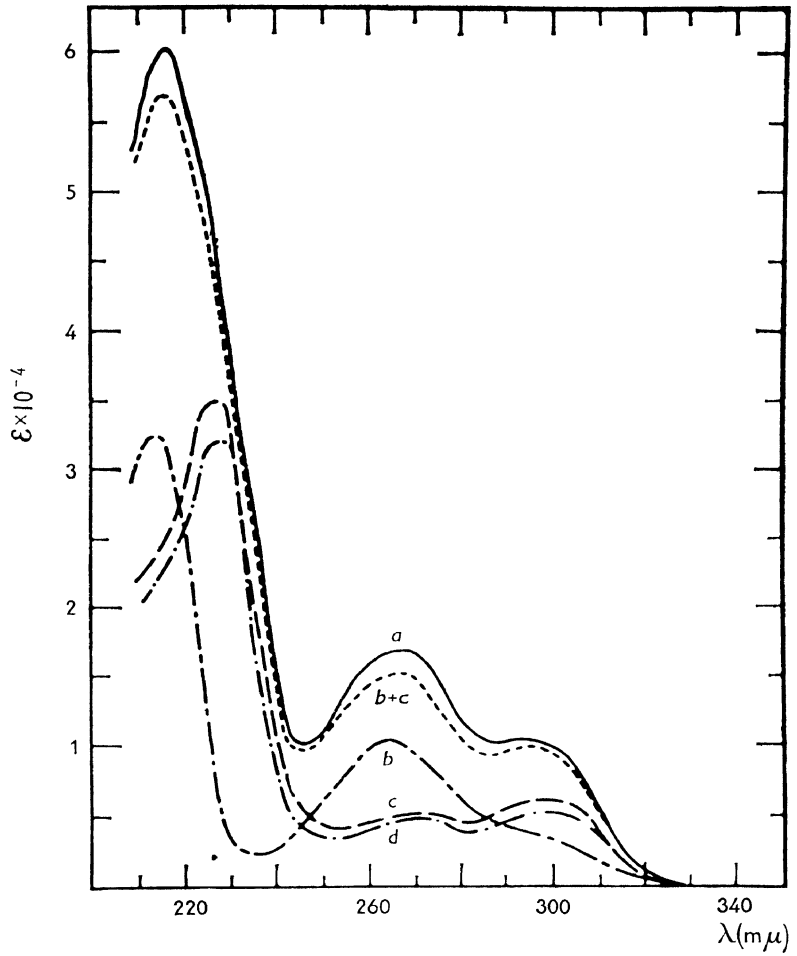
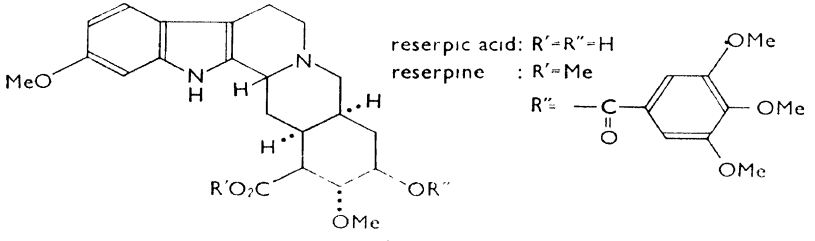


FIG. 4. Absorptions (in methanol) of (*a*) reserpine, (*b*) methyl 3,4,5–trimethoxybenzoate, (*c*) reserpyl alcohol and (*d*) 2,3–dimethyl–6–methoxyindole. The dashed curve below curve (*a*) represents the sum of curves (*b*) and (*c*) (from Neuss, Boaz and Forbes[40])



(42)

Steric effects. The overlap of adjacent π or p orbitals clearly depends on their relative orientation, and electronic spectra therefore provide a delicate tool for studying stereochemical detail in certain systems. Some applications of this type have already been discussed (pp. 138 and 153). The effects are quite complicated and only brief discussion is possible here; the reader is referred to recent reviews[1,2,74] for details and key references.

One of the commonest applications is the detection of steric repulsions which oppose the coplanarity of conjugated π-electron systems. For example, diphenyl gives an intense band at 248 mμ (see Fig. 5*a*)

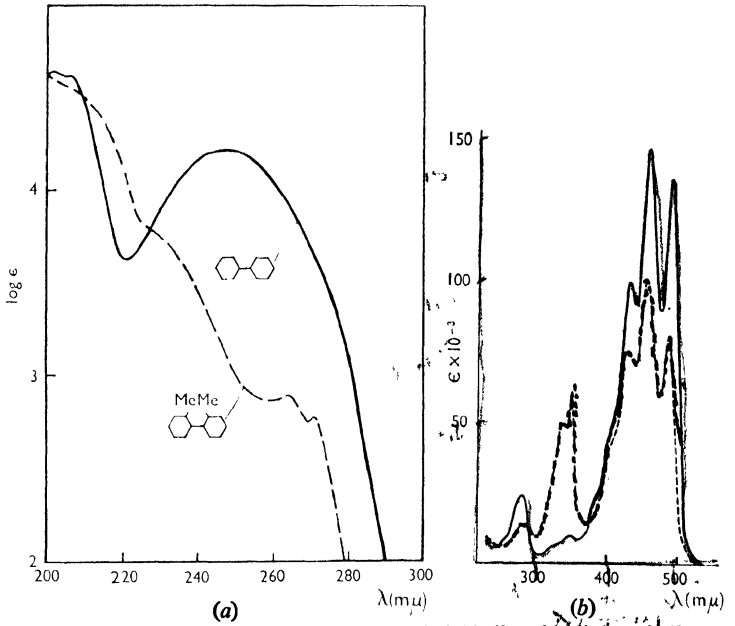


FIG. 5. (*a*) Absorption of diphenyl and 2,2′–dimethyldiphenyl (from Beaven[74])

(*b*) Absorption of all–*trans* methyl bixin (full line) and its central–*cis* isomer (dashed line) (from Inhoffen and Raspé[75])

which depends on conjugation between the two benzene rings. In 2, 2′–dimethyldiphenyl the effect of steric hindrance to coplanarity displaces this band to lower wavelengths (shoulder at ~230 mμ) and greatly decreases its intensity, revealing a band at 260 mμ probably due to transitions in the isolated benzene rings. The reduction of the intensity of allowed transitions by such steric twisting about a single bond is a fairly general phenomenon but the effect on the wavelength

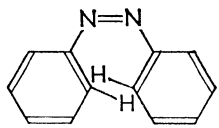
varies; for example, 2–methylacetophenone absorbs at approximately the same wavelength as acetophenone, although the intensity is decreased.

In long-chain conjugated polyenes steric hindrance to coplanarity can arise when *cis*–bonds are present. Fig. 5*b* shows the spectra of the carotenoid 'all–*trans*' methyl bixin (p. 201) and the isomer with a central *cis*–double bond; in the latter the long wavelength band is weakened and a diagnostically useful '*cis*–band', probably due to a partial chromophore, appears at shorter wavelengths.

Although absorptions due to allowed transitions are generally weakened by departures from coplanarity, the intensities of forbidden transitions may be increased. This is illustrated below by the behaviour of *trans*–azobenzene and the sterically restricted *cis*–isomer (43). Such differences between *cis* and *trans* isomers are often of analytical value.

Light absorption of azobenzene (in ethanol).

	$\pi \rightarrow \pi^*$ transition		$n \rightarrow \pi^*$ transition	
	λ_{max} (mμ)	ε	λ_{max} (mμ)	ε
trans-isomer	320	21,300	443	510
cis-isomer	281	5,260	433	1,518



(43)

Analytical applications. Since ultraviolet spectra are usually intense, small quantities of an absorbing substance can be easily detected. For example, the detection of polyacetylenes in plants and in fungus cultures[41] has been facilitated by their very intense and characteristic spectra (p. 138), and the important and common steroid diene ergosterol can be detected in the same way. In such cases, ultraviolet spectra can be used to monitor subsequent purification processes. The presence of traces of benzene in cyclohexane produced by hydrogenation or in commercial alcohol can also be revealed spectroscopically.

The application of electronic spectroscopy in *quantitative* analysis depends simply on the application of the equation discussed on p. 24 (Lambert-Beer Law); thus the concentration of a single absorbing substance can readily be calculated from the measured optical density if ε or $E^{1\%}_{1cm}$ is known. The analysis of multicomponent systems is more difficult than in the case of infrared spectroscopy (p. 46) since the absorptions of the components generally overlap, but no major problem

arises provided that the absorption curves are appreciably different (refs. 1 and 8 give details of the methods). A typical example is the estimation of mixtures of aromatic sulphonamides in biological fluids. Compared with infrared spectroscopy, electronic spectroscopy has several advantages in analytical work—(i) simple visible and ultraviolet spectrometers are cheaper and more accurate than their infrared counterparts, (ii) the method is more sensitive (larger extinction coefficients) and (iii) aqueous systems can be examined without difficulty.

In addition to the more obvious analytical uses of electronic spectroscopy, the method provides a powerful tool for the investigation of the rates and equilibria of the many reactions which involve a change in an absorbing group. A classical example of a reaction whose kinetics can be determined spectrophotometrically is the acid-catalysed transformation of allyl alcohols of the type $C_6H_5CH(OH)CH=CH_2$ to the conjugated isomer $C_6H_5CH=CHCH_2OH$. The latter has an absorption maximum with ε 19,500 at 251 mμ, while the former has $\varepsilon < 1{,}000$ at this wavelength[42]; the extent of reaction at any time can therefore be readily determined from the intensity of absorption. Typical equilibria for which the relative concentrations of the components have been deduced from the spectra are the hydration of aldehydes ($RCHO + H_2O \rightleftharpoons RCH(OH)_2$; the carbonyl band at $\sim$280 mμ is absent in the hydrate), the formation of metal complexes, and tautomeric changes (pp. 142 and 153). Two types of equilibria of special interest—ionisation and the formation of charge transfer complexes—are considered individually below.

Determination of ionisation constants.[1,43] If in an organic molecule there is a basic or acidic function which is part of a chromophore, then the spectrum of the substance will vary with the pH of the solution. At low pH the spectrum will be that of the protonated form, at high pH, that of the unprotonated form will be observed.

	Low pH	*High pH*
Acids $HA \rightleftharpoons H^+ + A^-$	Spectrum of HA	Spectrum of A^-
Bases $B + H^+ \rightleftharpoons HB^+$	Spectrum of HB^+	Spectrum of B

At intermediate pH (near to the value of pK_a) the spectrum will be a combination of those of the protonated and unprotonated forms. The ratio of the concentrations of these two species (and hence their contribution to the spectrum) is given by

$$pH = pK_a + \log_{10} \frac{[A^-]}{[HA]} \quad \text{or} \quad pH = pK_a' + \log_{10} \frac{[B]}{[HB^+]}$$

To find pK_a we need to run at least three spectra (*a*) in a buffer solution of accurately known pH (near to pK_a and determined by use of a pH meter), (*b*) in a solution on the acid side at least two pH units away from pK_a, (*c*) in a solution on the alkaline side at least two pH units away from pK_a. The separation of two pH units ensures that contamination by the other species is less than 1%.

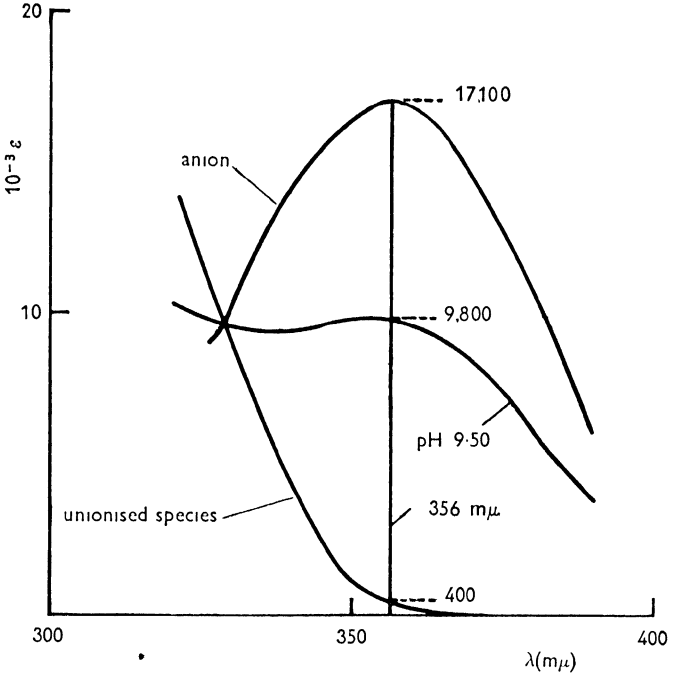


FIG. 6. Determination of the ionisation constant of the phenolic alkaloid numismine

Fig. 6 shows such a series of spectra for the alkaloid numismine,[44] the ionisation involved being that of a weakly acidic phenol grouping. It is easy to show that

$$pK_a = pH \text{ (of buffer)} + \log_{10} \frac{\varepsilon_{A^-} - \varepsilon}{\varepsilon - \varepsilon_{HA}}$$

where ε_{HA} and ε_{A^-} are the extinction coefficients for the phenol and its anion and ε is the apparent extinction coefficient measured in the buffer solution, all at the same wavelength. In this particular case, taking the wavelength 356 mμ, ε_{HA} (that is, the extinction coefficient for numismine determined in 0·1N HCl) is 400, ε_{A^-} (determined in 0·2N NaOH)

is 17,100 and ε (determined in a buffer of pH 9·50) is 9,800. Substitution in the above equation gives $pK_a = 9{\cdot}39$. The determination may then be repeated in a series of buffers of slightly different pH, the average value of pK_a being calculated. Albert and Serjeant[43] give an excellent account of the experimental procedure and of the complications which arise when a compound has two chromophoric acid or basic functions, the pK_a's of which lie close together.

If the absorption curves of an unionised molecule and of its ion intersect (as in Fig. 6), then it is clear that the absorption curves of incompletely ionised mixtures must also pass through the point (or points) of intersection. Such points are called *isosbestic points* and their presence is a useful though not infallible indication that the equilibrium in question involves only two absorbing species.

Charge transfer complexes. This name is given to molecular complexes formed between an electron donating and an electron accepting molecule, a classical example being the complexes of aromatic hydrocarbons with trinitrobenzene or picric acid. The spectra of such complexes show additional absorption not present in the spectra of the components (e.g. a band at 365 mμ for the naphthalene-trinitrobenzene complex). It is possible to determine the equilibrium constants for complex formation by measuring the intensity of the 'charge transfer absorption' in solutions of different compositions, although there are certain complications since charge transfer absorption may arise during a chance collisional encounter of donor and acceptor. According to the theory of charge transfer spectra[2,45] electron transfer from the donor to the acceptor is more complete in the excited state than in the ground state, and the wavelength of the absorption can, in fact, be correlated with electron affinity of the acceptor and the ionisation potential of the donor.

There is a formal similarity between the excitation process responsible for the spectra of the above complexes and the intramolecular charge transfer which occurs in the electronic excitation of α, β–unsaturated ketones (p. 140, footnote) and substituted benzenes. The absorptions of many inorganic ions are also of this type and some of the 'non-conjugated interactions' mentioned on p. 153 have their origin in charge transfer absorptions involving a donor and an acceptor group within the same molecule.

Molecular weight determination. If an unknown compound contains an isolated characteristic chromophore for which the extinction coefficient is known, then the equation on p. 25 can be applied to calculate the molecular weight of the unknown from an optical density

measurement at a wavelength at which the remainder of the molecule does not absorb. For example, picrates (e.g. amine picrates) have ε 13,440 at 380 mμ and the molecular weight of a monobasic amine can therefore be determined from a measurement on its picrate.[46] Similarly the molecular weights of oligosaccharides can be determined from the spectra of their osazones. Essentially the method is a type of functional group assay; further details of this approach may be found in Chapter 7.

The Literature of Electronic Spectra

This chapter can form only an introduction to the applications of ultraviolet and visible spectroscopy, and many reviews exist to augment it. Of general interest are two reviews by Braude,[47,48] who pioneered the empirical correlation of spectra, and the books by Jaffé and Orchin,[1] Gillam and Stern,[9] Rao,[49] Scott[50] and Beaven *et al.*[8] A large amount of information is contained in an article in 'Houben-Weyl' by Pestemer and Brück.[51] Theoretical aspects of spectroscopy are covered in textbooks of quantum chemistry (e.g. ref. 4), in the books by Barrow[5] and Jaffé and Orchin,[1] and in articles by Mason,[2] Duncan,[3] Matsen[3] and Kuhn.[35] An annual series[52] which provides reviews of theoretical aspects is also of interest.

The successful use of ultraviolet spectra in structure elucidation depends on the availability of data for model compounds and searching the literature for this has hitherto been difficult. A partial solution was achieved by the publication of *Ultraviolet and Visible Absorption Spectra. Indexes for 1930–1954 and for 1955–1959* (Hershenson[53]). These indexes give references (not data) for spectra recorded in graphical form, but they ignore the great wealth of numerical data published. This was remedied by the appearance of *Organic Electronic Spectral Data*,[54] a series listing (in order of molecular formulae) values of λ_{max} and ε_{max} taken from both graphical and numerical data. Other useful sources are the *Catalog of Ultraviolet Spectral Data*[55] of the American Petroleum Institute and the *Sadtler Ultraviolet Spectra*.[56]

Many reviews and compilations of data on specific classes of compounds are available: natural polyacetylenes,[57] carotenoids and the Vitamin A group,[16,17] visual pigments,[58] steroids,[19,59] aromatic compounds,[60] condensed nuclear aromatic hydrocarbons,[61] azulenes,[28] tropolones,[62] dyes,[35,36] heterocyclic compounds,[63,64] sulphur compounds,[1,65,66] acridines,[67] pteridines,[68] flavonoids,[69,70] indole alkaloids,[71] vitamins, hormones and coenzymes[72] and nucleic acid components.[73]

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Chapter 5

L. M. Jackman

MAGNETIC RESONANCE SPECTROSCOPY

Introduction—The Behaviour of the Electron and Atomic Nuclei in a Uniform Magnetic Field

The title of this chapter embraces two powerful types of absorption spectroscopy [viz. nuclear magnetic resonance (n.m.r.) and electron spin resonance† (e.s.r.)] which are currently finding increasing use in the investigation of molecular structure. Although the precise fields of application of the two techniques are quite divorced, they are, nevertheless, conveniently considered together because the fundamental principles of each have many features in common.

The electron, and the nuclei of many isotopes, possess a mechanical spin, and, as they are also associated with an electric charge, this motion endows them with a magnetic moment. In other words, these particles resemble tiny bar magnets. In a uniform magnetic field each orientation of the magnetic dipole will correspond to a certain potential energy. However, because of the microphysical nature of the system, the number of orientations is restricted by a quantum condition. The number of possible orientations is expressed by a spin number which may assume the values $0, \frac{1}{2}, 1, \frac{3}{2} \ldots$, and is symbolised by S ($=\frac{1}{2}$ only) for the electron and I for atomic nuclei. Nuclei, such as ^{12}C and ^{16}O, which do not have a magnetic moment have a spin number of zero ($I=0$). Particles with a spin number of one half may take up just two orientations in a uniform magnetic field. Representatives of this class are the electron ($S=\frac{1}{2}$), the proton, ^{19}F, and ^{31}P ($I=\frac{1}{2}$). ^{2}D and ^{14}N have spin numbers of unity and these nuclei can adopt three orientations. Each orientation or state corresponds to a certain energy which is a function of the spin number, the magnetic moment, and the strength of the applied field. Transitions between adjacent energy states are

† Sometimes known as electron paramagnetic resonance (e.p.r.).

permissible and will occur if the system is subjected to electromagnetic radiation of the appropriate frequency (ν) such that $h\nu$ is equal to the energy difference between the two states. The energy difference, and hence the resonance frequency, is directly proportional to the field acting on the electron or nucleus. Transitions of this type are illustrated diagrammatically, for a nucleus with $I=\frac{1}{2}$, in Fig. 1.

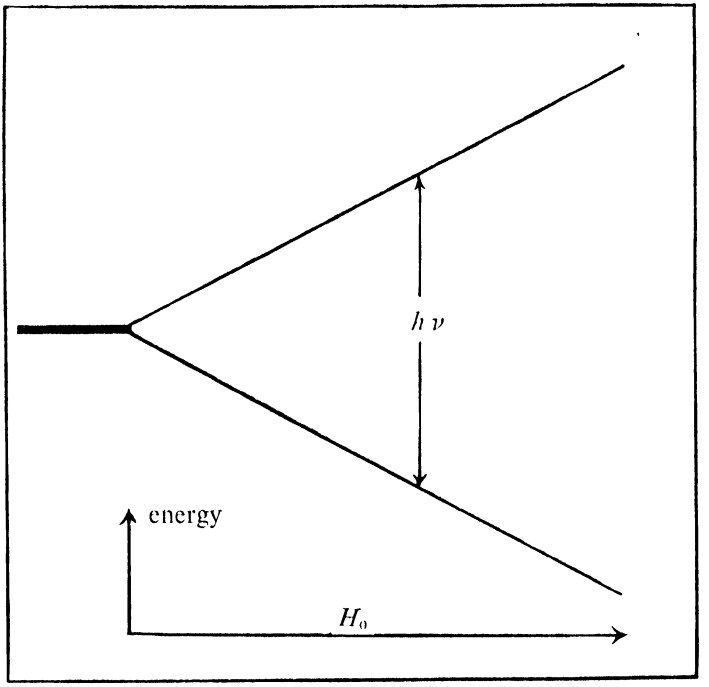


FIG. 1. The splitting of nuclear spin energy levels ($I=\frac{1}{2}$) in a uniform magnetic field (H_0)

For reasons which will become apparent later it is desirable to employ applied fields of the order of 10^4 gauss, for which value the characteristic or resonance frequencies of nuclei lie in the radio-frequency region. The electron has a much larger magnetic moment than those of nuclei, and its resonance frequency in fields of 10^4 gauss is in the microwave region.

The Spectroscopic Method (n.m.r.)

When a system of atomic nuclei is placed in a uniform magnetic field and irradiated at the resonance frequency, transitions between adjacent energy levels result in a net absorption of energy by the nuclear system because, although transitions from high energy states to low energy states have the same probability as those in the reverse direction, there is initially, in the presence of the applied magnetic field, a small but finite excess of nuclei in lower energy states. Nuclear magnetic resonance spectroscopy is therefore a type of absorption spectroscopy. The same considerations hold for electron spin resonance spectroscopy but a further discussion of this technique is deferred to the last section of this chapter.

The importance of nuclear magnetic resonance spectroscopy in organic chemistry derives from the fact that the apparent resonance frequency of a nucleus is to some extent dependent on molecular environment. This dependence is a consequence of the magnetic shielding of nuclei by the extra-nuclear electrons. The applied magnetic field causes induced electron circulations about one or more atomic nuclei (in much the same way as a change in magnetic flux induces a current in a closed loop of wire) and these circulations give rise to small secondary fields which may either augment or oppose the applied field at the nucleus, depending on the structure of the molecule. These secondary fields are very weak, so that variation of resonance frequency with structure is extremely small. For protons, the variation of resonance frequencies encountered in common organic molecules extends over a range of only fifteen parts per million.

In order to observe such small changes in frequency, certain conditions must be fulfilled. It is necessary that the strength of the applied field should be as high as possible, for the strength of the secondary fields and hence the variation of resonance frequencies are proportional to it. At the same time, it is vital that the applied field should be highly uniform (homogeneous) for otherwise different molecules will experience different applied fields. At present the maximum field strength obtainable with a homogeneity of 1 in 10^8 is approximately 14,000 gauss. Finally, high resolution nuclear magnetic resonance spectroscopy is restricted to liquids and gases. In solids, neighbouring nuclear dipoles make a variable contribution to the applied field at a nucleus, producing an effect which is similar to using an inhomogeneous field. In liquids and gases, molecular motions average to zero the effect of neighbouring nuclei.

The practical method of high resolution nuclear magnetic resonance spectroscopy may briefly be described as follows. The substance, either as a pure liquid or solution, is contained in a cylindrical sample tube, which is inserted into a probe placed between the pole pieces of a magnet. The probe is coupled to both a radiofrequency generator and receiver, and functions so as to deliver radiation at a constant radiofrequency to the sample, and to detect the absorption signal thus produced. Scanning of the spectrum is achieved by linearly varying the applied field over the small range covering the resonances of the nuclei under observation. This is done by means of subsidiary coils. For purely technical reasons, it is more convenient to vary the field rather than the frequency of the generator.

It is only feasible to examine the nuclei of one isotope by such scanning. If other isotopes are to be studied it is necessary to employ a different radiofrequency generator and probe or, with instruments using an electromagnet, to reset the applied field to a different strength.

If the spectrum is scanned from low to high fields, the nuclei which are poorly shielded by surrounding electrons come into resonance first. In practice the spectrum can be scanned in either direction and is displayed on an oscilloscope or recorded; in each case the field strength is the abscissa. It is important to realise that the resonance frequency is proportional to field strength so that the scan can be expressed either in gauss or in cycles per second (c/s).

With all but the most recently developed spectrometers, random fluctuations in the applied field make it impossible to scan the spectrum in a strictly linear fashion. For this reason each spectrum is usually calibrated by the so-called side band technique. Subjection of either the radiofrequency field or the applied magnetic field to an audiofrequency sinusoidal e.m.f. causes each absorption signal to be flanked by a pair of satellite signals, each separated from the true signal by the frequency of the applied e.m.f. The side bands can thus be used to calibrate the chart paper in cycles per second. In practice a variable frequency audio-oscillator is used and several side bands from one of the strongest signals are introduced at convenient intervals throughout the spectrum so that the linearity of the scan can be checked. Fig. 2 shows a spectrum which has been calibrated by side bands from the strong signal at the high field end of the spectrum.

As it is not feasible to determine the magnitude of the field experienced by the sample with the desired accuracy of 0·1 milligauss, it is necessary to express line positions in a nuclear resonance spectrum relative to some arbitrary zero. It is important that the 'zero' should be readily

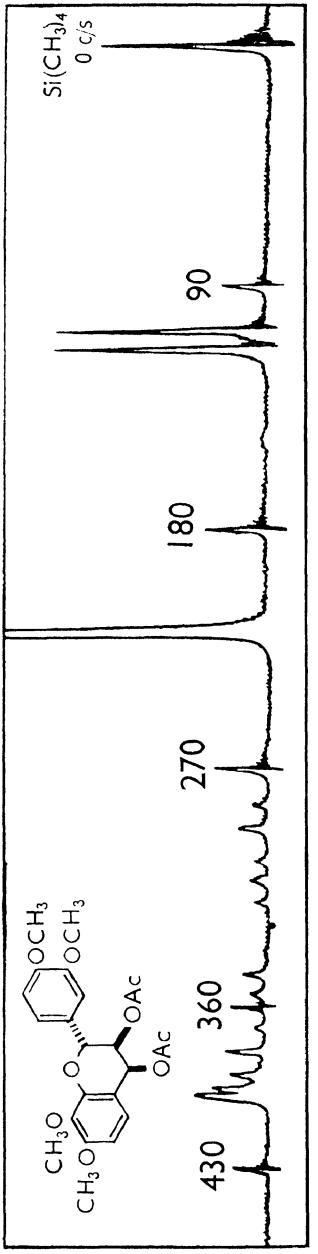


FIG. 2. A spectrum calibrated by the side band technique

identifiable in every spectrum and this is achieved by including, in each sample for investigation, a reference substance which will give a sharp, invariant, and readily recognisable absorption line. The separations, or *chemical shifts* as they are generally called, of other lines in the spectrum are then measured from this zero. This procedure as applied to proton spectroscopy will now be outlined in detail; it is of paramount importance and should be thoroughly understood by all intending users of the method.

The reference substance usually employed in proton spectroscopy is tetramethylsilane [$Si(CH_3)_4$]. It has the advantage that its single sharp absorption signal is found at a higher field than almost all the common absorptions of protons in organic molecules. In addition it is chemically inert and magnetically isotropic, and experience has shown that it gives a reliable measure of the magnetic shielding of protons in all systems commonly encountered by the organic chemist. The reference substance must be directly added to the liquid or solution under investigation. Calibration by the side band technique then permits measurement of the separation of each line in the spectrum of the sample from the reference line. The separations so obtained are in cycles per second and it must be remembered that they are proportional to the strength of the applied field, so that values determined with two spectrometers operating at different field strengths will not be the same. It is therefore

necessary to convert the values in c/s to dimensionless, field-independent, units by dividing by the approximate resonance frequency of the proton at the field strength used (i.e. by the frequency of the radiofrequency generator of the spectrometer). As the separations in c/s usually have values of 0–600 c/s, whereas the resonance frequency of the proton is of the order of 60×10^6 c/s, it is convenient to quote the dimensionless units as parts per million (p.p.m.). A common practice is to assign the line of tetramethylsilane as 10 p.p.m.[1] The values then obtained are called τ (tau)-values. Equation (1) gives the conversion of separations measured in c/s to τ-values.

$$\tau = 10 - \frac{\text{separation in c/s} \times 10^6}{\text{frequency of R.F. generator}} \qquad (1)$$

In the rare cases in which the separations are those of signals at higher fields than that of tetramethylsilane, the sign of the second term in equation (1) is made positive. It should be noted that poorly shielded protons have small τ-values; a few very acidic protons even have negative values. The convention of τ-values is not yet internationally recognised but it is commonly used by organic chemists and it will be employed as the unit for proton chemical shifts throughout the remainder of this chapter.

τ-Values are reliable only if determined from measurements made on dilute solutions in suitable solvents such as carbon tetrachloride, chloroform, and deuterochloroform. Other solvents are sometimes used but the chemical shift data thus obtained do not always correlate well with those obtained with the above solvents. This is particularly true of aromatic solvents. In general, satisfactory proton spectra can be obtained with 10–50 mg of compound in 0·3 ml of solvent.

The intensities of absorption bands are given directly by band areas and are proportional to the numbers of nuclei which produce them. *Thus a proton spectrum can be used to count the numbers of protons in different environments in an organic molecule.* The determination of intensities requires some care and an analysis of factors affecting the accuracy has been given by Williams.[2] By adhering to the techniques outlined by Williams, an accuracy of one proton in a hundred may be obtained with spectrometers equipped with electronic integrators.

Proton Nuclear Magnetic Resonance

The chemical shift. The majority of applications of spectroscopic methods to problems in structural organic chemistry involve the use of empirical correlations derived from a considerable body of experi-

mental data. This is also true of proton magnetic resonance spectroscopy although, as it is a comparatively new method, the number of reliable correlations is nowhere near as large as, for instance, in infrared spectroscopy. Furthermore, the theory of proton chemical shifts suggests that purely empirical chemical shifts will frequently fail when applied to molecules of some complexity. In structural work the use of chemical shift data requires considerable caution and a blend of empirical experience with theoretical understanding. A structural argument based solely on chemical shift data is usually acceptable only if data for closely related model compounds are available.

A preliminary idea of the general range of proton chemical shifts may be obtained by reference to the chart (Fig. 3). It is obvious that the ranges indicated in this chart are far too wide to be of much use for precise structural assignments. The chart may however be used for a preliminary classification of bands in a spectrum prior to applying the more accurate correlations outlined below.

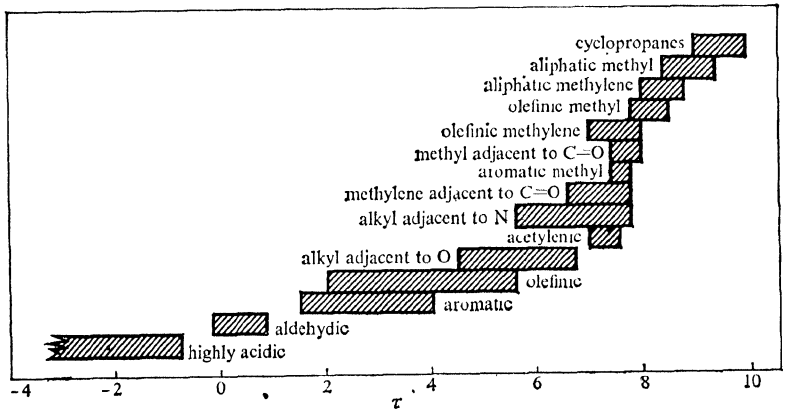


FIG. 3. Chart of approximate chemical shifts of protons in organic molecules (τ-values)

Methyl groups generally give rise to readily identifiable signals in an n.m.r. spectrum. Because a methyl group is rapidly rotating about a threefold axis of symmetry, its three protons are magnetically equivalent; the resultant absorption signal corresponds in intensity to three protons. Table 1 lists τ-values of methyl groups in different environments. Many of the entries refer to single substances and require no comment. Methyl groups attached to aliphatic chains absorb consistently near 9·1. In complex alicyclic systems methyl groups absorb over the range 8·9–9·4. The presence of electronegative substituents attached

to the same carbon atom as a methyl group shifts the τ-value of the latter into the region 8·5–9·0. In the absence of electron-attracting β-substituents, methyl groups attached to unconjugated double bonds absorb near 8·3 but in the presence of β-substituents such as carbonyl and carboxyl groups this figure drops to 7·7–8·0, the precise value depending on the geometric configuration of the double bond.[3,4,5] The

TABLE 1. τ-Values of methyl groups, CH_3–X*

Substituent (X)	τ	*Substituent* (X)	τ
H	9·77	I	7·84
Alkyl	9·1		
$\begin{matrix} & R^1 \\ & / \\ C & -OR^2 \\ & \backslash \\ & R^3 \end{matrix}$	8·5–8·9	$\begin{matrix} & R^1 \\ & / \\ N & \\ & \backslash \\ & R^2 \end{matrix}$	7·8
OH	6·62	CHO	7·83
O–Alkyl	6·7	CO–Alkyl	7·9
O–Acyl	6·3	CO_2–Alkyl	8·0
O–Phenyl	5·74	$CR^1{:}CR^2R^3$	8·35
Cl	6·95	Aromatic	7·70
Br	7·32	Pyridine	7·70
		$C \equiv CH$	8·30

* $R^1 = R^2 = R^3$ = Alkyl

methyl group of isoprene absorbs at 8·2 and the in-chain methyl groups in polyenes at 8·05.[6] In aromatic systems *o*–carboxy, *o*–carbonyl, and *o*–nitro substituents cause a paramagnetic shift (i.e. a shift to lower τ-values) of about 0·2–0·3 p.p.m.; the proximity of other aromatic rings causes similar shifts.

Methylene groups in acyclic saturated hydrocarbons usually absorb near 8·75. In most cyclic systems the value is lower by about 0·2 p.p.m.; a notable exception is provided by the methylene groups of cyclopropanes which absorb in the range 9·7–10·0 unless the ring carries an electron-attracting substituent. The shielding of the protons of a methylene group in acyclic systems is the sum of the individual contributions of its two substituents. Shoolery[7] has derived a set of values for the shielding contributions of substituents which, inserted into equation (2) (p. 176), reproduce well the observed τ-values of methylene groups in acyclic systems. Shoolery's numbers are given in Table 2. Equation (2) can be extended to acyclic protons of the type –$\dot{C}$H– but is then much less accurate. The σ-values in Table 2 fail to reproduce the τ-values of methylene groups in cyclic systems. Thus the predicted value for $-CH_2-CH_2-O-$ is 6·94 whereas the appropriate methylene group in tetrahydrofuran absorbs at 6·37 and in propylene oxide at 7·71.

$$\tau \text{ (Methylene)} = 9{\cdot}77 - \sigma_a - \sigma_b \qquad (2)$$

σ_a and σ_b are the shielding contributions (Table 2) of the two substituents.

TABLE 2. Shoolery's shielding contributions

Substituent	σ
Cl	2·53
Br	2·33
I	1·82
NR^1R^2	1·57
OR	2·36
SR	1·64
Ph	1·83
CR=O	1·70
$CR^1=CR^2R^3$	1·32
C≡CH	1·44
C≡N	1·70
CH_3 and aliphatic CH_2	0·47

The identification, in the spectrum of a compound, of protons in CH_3–, $-CH_2-$ or -CH– groups can often be achieved by examining the spectra of closely related transformation products. For example the assignment of a band, equivalent in intensity to three protons, at 8·3 to an olefinic methyl group is confirmed if the band moves to the 9·0 region on hydrogenation of the appropriate double bond. Similarly bands due to epoxides may be identified by examining the spectra of the related diols or of the alcohols derived by reductions with lithium aluminium hydride.[8,9] In the case of a primary epoxide, reduction generates a new C–methyl group absorbing near 8·80. A particularly valuable method used for assigning bands arising from protons adjacent to hydroxyl groups makes use of the fact that acylation of the hydroxyl group causes such bands to move to lower τ-values. For the systems $-CH_2OH$ and $\rangle CHOH$, the shifts on acetylation are 0·50 and 1·0–1·15 p.p.m. respectively, in both acyclic and cyclic structures. Acylation of amines is attended by similar shifts and quaternary salt formation causes shifts of the order of 1·0 p.p.m. to lower fields.

It will be seen from the chart (Fig. 3) that *olefinic protons* absorb in a fairly characteristic region of the spectrum and there are many examples in which proton magnetic resonance has been used to establish the existence or non-existence of mono-, di- and tri-substituted carbon-carbon double bonds in organic molecules. Here again assignments can be verified by comparing the spectrum of the olefin with that of its hydrogenation product. The τ-values of olefinic protons are very sensitive to the degree and nature of substitution of the double bond. These shifts are summarised in Table 3.[10] It should be noted that with

certain substituents the shifts observed for β-protons depend on whether the substituents are *cis* or *trans* to the β-proton.[3,4,5,10,11]

TABLE 3

Chemical shifts (p.p.m. relative to ethylene, $\tau = 4{\cdot}67$) of the olefinic protons of monosubstituted ethylenes, $\overset{\beta}{C}H_2 = \overset{\alpha}{C}H - X$.

Substituent (X)	α–H	β–H (*cis*)	β–H (*trans*)
F	−0·84	1·30	0·96
Cl	−0·97	−0·11	−0·19
Br	−1·16	−0·70	−0·55
CN	−0·20	−0·72	−0·58
OMe	−1·10	1·43	1·29
COMe	−0·81	−0·59	−0·21
Ph	−1·36	0·12	−0·38
CH_3	−0·39	0·47	0·39
CH_2CH_3	−0·43	0·49	0·40
$CH(CH_3)_2$	−0·34	0·56	0·48

Aromatic protons absorb at considerably lower τ-values than olefinic protons. The reason for this is well understood and will be discussed at the end of this section. The general range for aromatic protons is 2·0–3·5, the precise position depending on the number, nature, and orientation of substituents.[12] The ring protons of heterocyclic aromatic compounds also absorb in this region provided they are not attached to carbon atoms adjacent to a heteroatom. Thus the β- and γ-protons of pyridine absorb at 3·01 and 2·74, respectively, whereas the α-protons absorb at 1·5.[13] Similar values are found in other aromatic heterocycles.[14] The marked shift between the α- and β-protons in furan has been useful for establishing the position of substitution of the furan ring in several bitter principles possessing this structural feature.[15]

Acetylenic protons absorb between 7·3 and 7·8, depending on the nature of substitution. Conjugation, for instance, results in absorption at the lower end of this range. In general, *allenic protons* absorb about 0·4 p.p.m. higher than their olefinic counterparts although at present few examples are available.[16]

The *protons of aldehydes* absorb in a most characteristic region of the spectrum, viz. 0·0–0·7 p.p.m., and n.m.r. spectroscopy therefore provides a most convenient method of demonstrating the existence of an aldehyde group in a molecule. The corresponding 2,4–dinitrophenylhydrazones and semicarbazones absorb in the regions 2·2–2·6 and 2·8–3·2, respectively.[17]

The foregoing discussion is a brief survey of the chemical shifts of the more common types of carbon-bound protons. More detailed correlations are available[18,19,20,21] and much data can be found in the recent literature. Much less information is available for protons

attached to elements other than carbon. For instance there are very few examples of the use of n.m.r. for the characterisation of hydroxyl and amino groups. The reason for this is that the τ-values of such protons are most sensitive to the degree of intramolecular and intermolecular hydrogen bonding (n.m.r. can, in fact, be used to study hydrogen bonding; details may be found in ref. 55). Thus the τ-values of hydroxylic protons depend critically on concentration, temperature, and the nature of the solvent. Indeed, by varying these conditions, it is possible to observe hydroxylic proton bands almost anywhere in the proton spectrum. For this reason it is often desirable to replace hydroxylic protons by deuterium in order to avoid wrongly assigning absorption arising from –OH to other structural features. The τ-values of strongly chelated hydroxylic protons do not usually vary with concentration or temperature since the structures containing such protons are essentially monomeric. Strongly chelated phenolic hydroxylic protons often give sharp lines in the region $-1{\cdot}0$ to $-6{\cdot}0$.

The spectroscopy of protons attached to nitrogen (^{14}N) is complicated by the magnetic and electric properties of the nitrogen nucleus. The character of signals arising from N–H protons in various situations has been considered by Roberts.[22]

Time-dependent effects. In many cases, a proton in a given system may experience more than one environment. This may arise because of chemical exchange; for example, in aqueous ethanol the hydroxylic protons of ethanol are continually exchanging with the protons of water. Interchange of environments may also result from the equilibration of a molecule between two or more conformations. In such cases the nature of the proton absorption will depend on the rate of interchange between the various environments.

Consider the situation in which a proton exchanges between two environments, symbolised by A and B, spending a fraction, n, of its time in environment B. If the average time the proton spends in each environment is *small* compared with the reciprocal of the difference (in c/s) between its resonance frequencies in each environment (ν_A and ν_B, where $\nu_A > \nu_B$), its absorption will be a singlet at $\nu_A - n(\nu_A - \nu_B)$. If the average time spent in A and B is *large* compared with $1/(\nu_A - \nu_B)$, the proton will give rise to two singlets, one at ν_A with intensity $(1-n)$, and the other at ν_B with intensity n. If the average time in each environment is of the same order as $1/(\nu_A - \nu_B)$, a broad absorption band results. An analysis of the shape of this band affords the rate of exchange between A and B. Thus it is possible to use nuclear magnetic resonance spectroscopy to study the rates and activation energies of

fast reactions (half-lives of the order of 0·01 sec), such as proton exchange and conformational interchange. The latter application is particularly interesting; by making measurements at suitably low temperatures, it is possible to evaluate the energy barriers for rotation about single bonds, and between conformations in flexible cyclic systems (see also p. 191).

Types of shielding. It was stated earlier that the magnetic shielding of protons in molecules arises from the circulations of electrons induced by the applied magnetic field. The shielding arising from such circulations may be classified in the following way[23]:

1. Local diamagnetic shielding
2. Neighbouring paramagnetic shielding†
3. Interatomic diamagnetic shielding†

Local diamagnetic shielding is associated with the circulation of electrons about the proton itself and is illustrated in Fig. 4. It is seen that the secondary magnetic field opposes the applied magnetic field at the nucleus so that, at a given radio frequency, the value of the latter which corresponds to resonance is slightly higher than for a bare proton. The magnitude of the secondary field and hence of the local diamagnetic shielding is clearly related to the electron density at the hydrogen atom. Thus this shielding effect will be controlled by inductive and mesomeric effects operating at the atom to which the hydrogen atom is attached. A consideration of the data in Tables 2 and 3 will show the importance of this shielding mechanism. The direct effect through space of neighbouring formal charges or electric dipoles may also alter the local diamagnetic shielding of a proton.[24,25,26]

Neighbouring paramagnetic shielding arises from the circulations of electrons about neighbouring atoms. It can be shown[19] that the secondary magnetic fields are only effective at nearby protons if they are anisotropic, i.e. if the ease with which the electrons circulate around the neighbouring atom depends on the orientation of the molecule with respect to the direction of the applied field. This may be illustrated by the neighbouring paramagnetic shielding of acetylenic protons by the circulations of π-electrons in the triple bond. Fig. 5 illustrates the circulations of π-electrons when the acetylene molecule is oriented with its molecular axis parallel to the direction of the applied field. In this orientation the circulations are readily induced within the

† The term 'paramagnetic' is derived from the fact that the circulations are restricted, resulting in a decrease of the total diamagnetic susceptibility. Conversely, the term 'diamagnetic' relates to an effect resulting in enhanced diamagnetic susceptibility.

annular π-molecular orbitals and the associated magnetic field is such as to shield the acetylenic protons. It is obvious that similar circulations are no longer possible when the molecule is oriented transverse to the applied field. The effect averaged over all possible orientations must therefore result in a shielding of the acetylenic protons as is evident from their τ-values (*ca.* 7·4) which are high for such acidic (i.e. low

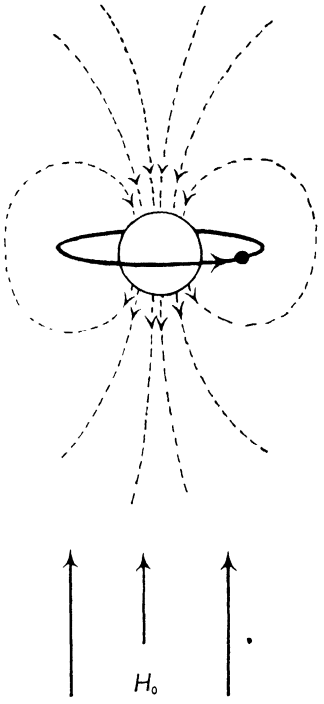


FIG. 4. The origin of diamagnetic shielding in an atom

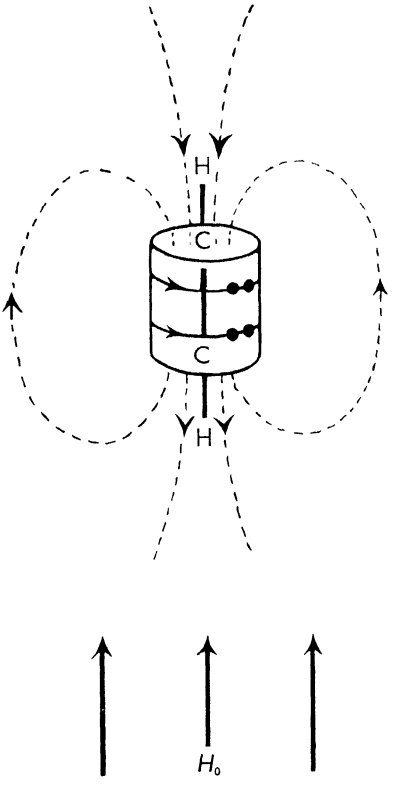


Fig. 5. The mechanism of the paramagnetic shielding of the protons in acetylene

local diamagnetic shielding) protons. It should be noted that if in a complex molecule a proton is situated above the centre of the triple bond it will be deshielded by the same neighbouring paramagnetic effect. Neighbouring paramagnetic shielding also contributes to the observed τ-values of aldehydic[19] and olefinic protons,[18,21] although it is less easy to present a physical picture of the induced circulations,

structural analysis since it provides definite evidence regarding the sequential arrangement of proton-bearing carbon atoms in a molecule. Examples at the end of this chapter will serve to illustrate this point. In addition to this type of information it is frequently possible to obtain

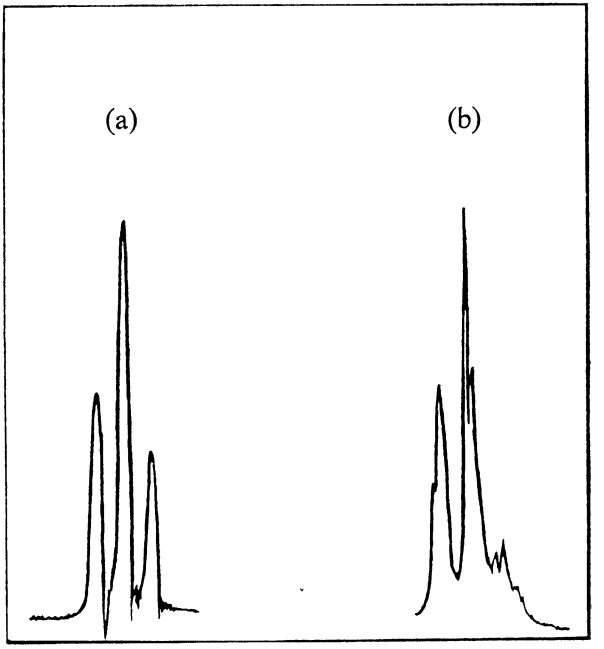


FIG. 13. Spectra (at 40 Mc/s) of the methyl protons in ethyl groups:
(*a*) Diethyl ether (chemical shift between methyl and methylene protons considerably greater than *J*)
(*b*) *n*–Butyraldehyde (chemical shift similar in magnitude to *J*)

stereochemical information from the magnitudes of spin-spin coupling constants (*J*) where these can be obtained directly from the spectra (as for $A_nX_m \ldots$ systems) or indirectly by analytical methods (as for $A_nB_m \ldots$ etc.).

The theory of spin-spin coupling for protons is reasonably well understood.[35,36] The existence of a coupling constant between two protons requires delocalisation of electrons over the intervening bonds between them. The degree of delocalisation in a system of single (σ) bonds depends principally on bond angles, and the magnitude of spin-spin coupling constants is intimately related to details of stereochemistry. Fig. 14 shows the relation of the coupling constant between two protons attached to the same carbon atom and the H–C–H

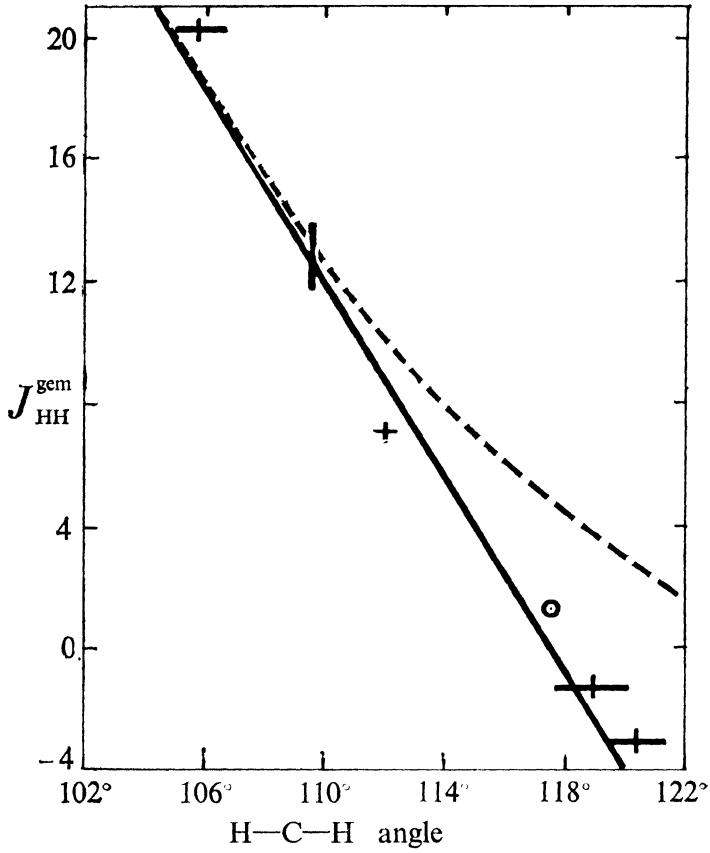


FIG. 14. Correlation of the H – C – H angle with J_{HH} (after Banwell and Sheppard[10]). The broken line represents a theoretical relation.

angle.[37,38] Because of the uncertainties in the determination of bond angles it is not yet clear whether the magnitude of the coupling constant is solely dependent on the angle, which however must certainly be the major factor.

A very useful relation between the coupling constants and geometry has been established for the interaction between two protons separated by a carbon-carbon single bond as in ethane. Calculations by Karplus[39] have shown that in such systems the coupling constant is a function of the dihedral angle† between the two C–H bonds, and Conroy[40] has

† The dihedral angle is the angle between the projections of the two C–H bonds on a plane which is normal to the carbon-carbon single bond. In the staggered conformation of ethane this angle is either 60° or 180°.

established the empirical curve shown in Fig. 15. This relation is particularly useful for studying stereochemistry in saturated six-membered rings. In a rigid cyclohexane ring the dihedral angle assumes values of 60°, 60°, and 180° for di-equatorial, equatorial-axial, and di-axial relations between the interacting protons and the corresponding coupling constants are 2–4, 2–4, 6–13 c/s respectively. Thus if the coupling constants of a proton with those of an adjacent methylene group can be determined, the configuration of the former is immediately established. Whether the relation in Fig. 15 is also valid for systems such as cyclohexene, cyclohexanone, and five-membered rings, in which small distortions of valence angles occur, has yet to be determined.

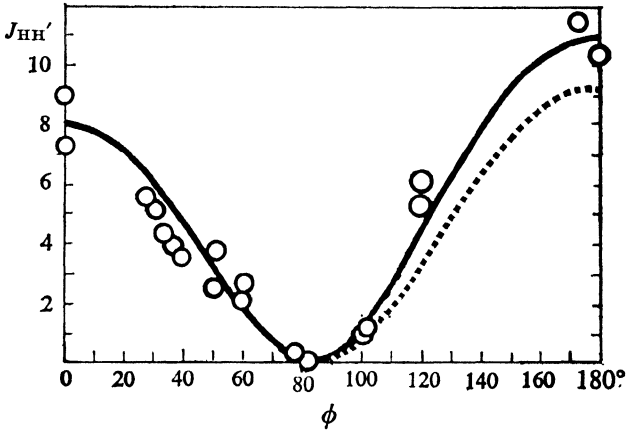
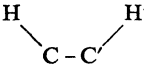


FIG. 15. Correlation of the dihedral angle, ϕ, in the system

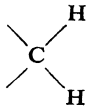
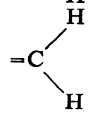
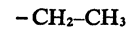
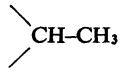
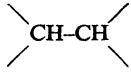
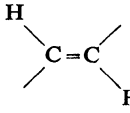
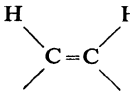
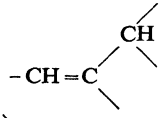
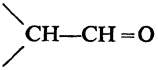
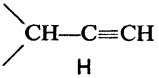
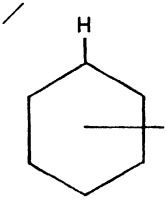


with $J_{HH'}$ (after Conroy[21])

If the cyclohexane ring exists as a rapidly exchanging mixture of the two chair conformations, the observed coupling constants will be the averages of the values for each conformation, suitably weighted according to their relative populations (cf. p. 178). A similar situation prevails with simple substituted ethanes, although at low temperatures it is sometimes possible to observe discrete signals from one or more of the three possible conformations.[41]

The coupling constants between protons of 1,2-disubstituted ethylenes can be used to establish the configuration of the double bond as the *trans*-coupling constant is always appreciably larger than the *cis*-coupling constant although the absolute magnitudes of each depend on the nature of the substituents.[38,42]

TABLE 4. Spin-spin coupling constants

System	*J* (*c*/*s*)
	12–15†
	−3·2 to +1·6‡§
	6·7–7·2
	5·0–7·0
	0·0–14·0†
	12·0–18·0
 acyclic	5·0–12·0
cyclic	3·0–10·0
	−3·5–0·0§
	0·0–3·0
	2·0–3·0
 *o*	7·0–10·0
m	2·0–3·0
p	0·0–1·0

† See, however, p. 190.
‡ Dependent on β-substituents.[10]
§ It is sometimes possible to obtain the relative signs of coupling constants from the mathematical analysis of complete spin systems. Absolute signs are not known.

Although coupling between protons separated by two or more carbon–carbon single bonds is rarely observed,[43] small interactions across three or four carbon atoms are frequently encountered in unsaturated[44] and aromatic[45] systems. There is some evidence that the coupling of an olefinic proton with a *cis*–allylic proton is greater than with a *trans*–allylic proton.[4,11]

Table 4 shows expected ranges for the values of coupling constants between protons in a number of common situations.

Examples of Applications

Many applications of nuclear magnetic resonance spectroscopy in structural work merely involve the demonstration of the presence or absence of certain groups. Thus the method is well suited for detecting the following groups: C–methyl (8·5–9·5, saturated; 7·9–8·4, olefinic), R_2N–methyl (7·7–7·9, aliphatic amine; 7·0–7·2, aromatic amines; 6·9–7·2, amides) RO–methyl (6·6–6·8 aliphatic; 6·1–6·4, aromatic), R–CO_2–methyl (6·0–6·4), –CO–methyl (7·8–8·0, aliphatic; 7·3–7·5, aromatic), olefin (see Table 3), aromatic (1·8–3·8), and aldehyde (1·0–0·0). Although such applications are very important, the real power of the method derives from its ability to define complete sequences of groups even in complex molecules. In order to introduce the reader to the more sophisticated uses of proton spectroscopy three examples will now be discussed in detail.

Example 1. The situation often arises in which the structure of one member of a class of closely related compounds is known and the problem consists of elucidating the structures of the remaining compounds. In such circumstances comparison of the nuclear magnetic resonance spectra of the unknown compounds with that of the known members is sometimes sufficient for the solution of the problem. An elegant example of this type of application is provided by determination of the structure of the alkaloid lunine ($C_{16}H_{17}O_4N$) reported by Goodwin, Shoolery, and Johnson.[46] The structure of a companion alkaloid, lunacrine, has been shown to be (3) (in fact, part of the structural proof rests on nuclear magnetic resonance evidence) and its spectrum is reproduced in Fig. 16a. The spectrum of lunine (Fig. 16b) is very similar in many respects and the two alkaloids must have many structural features in common. The bands *f* and *g* are associated with the methine and methyl protons, respectively, of the isopropyl group; the two methyl groups are not conformationally equivalent and coupling with the methine proton therefore gives rise to two separate doublets. The eight-line band *e* (AB segment of an ABX spin system)

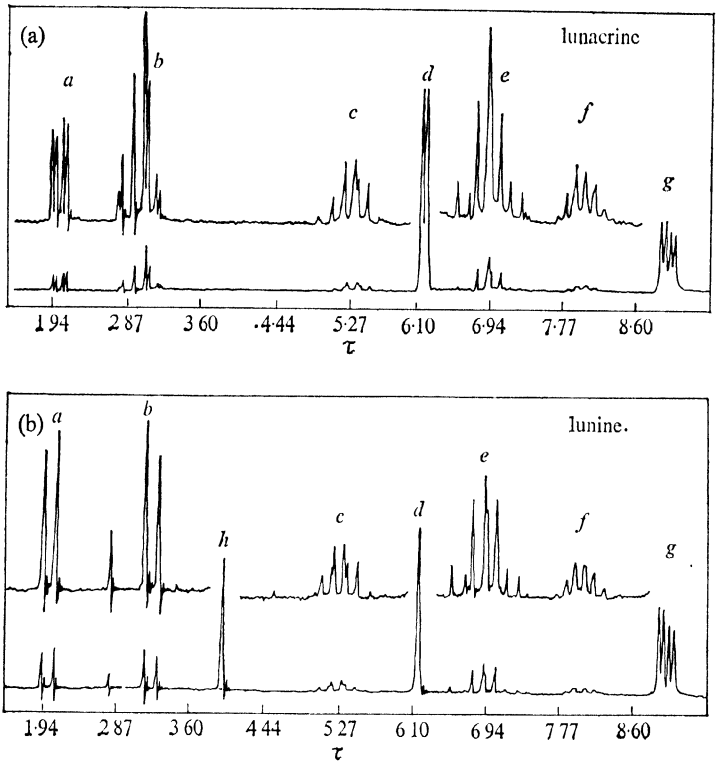
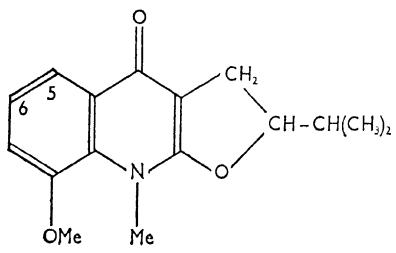
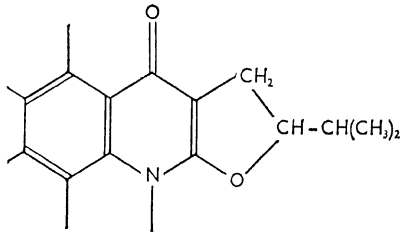


FIG. 16. Spectra (at 60 Mc/s in deuterochloroform) of (*a*) lunacrine; (*b*) lunine

and the band *c* are due, respectively, to the methylene and methine protons of the dihydrofuran ring. The partial structure (4) for lunine may therefore be written.



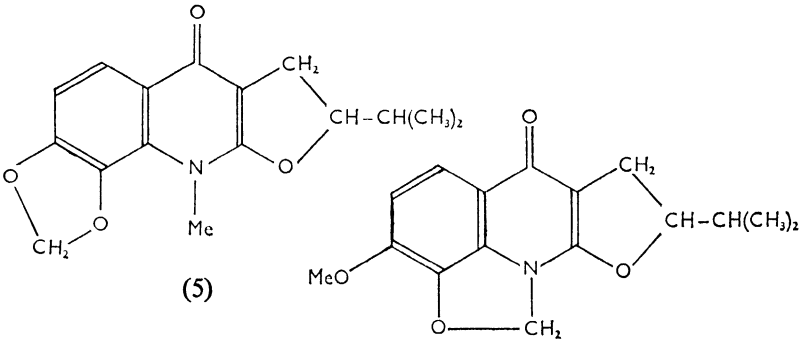
(3)



(4)

The three aromatic protons of lunacrine give rise to an ABX system

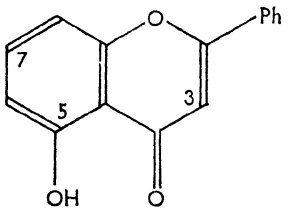
(*a* and *b*). The X-nucleus (region *a*) is the proton at $C_{(5)}$; being in close proximity to the carbonyl group it is deshielded by the strong long range shielding effect of the latter. The corresponding regions in the spectrum of lunine contain a pair of doublets ($J \sim 8{\cdot}5$ c/s) arising from two mutually coupled protons, one of which corresponds to the $C_{(5)}$ proton in lunacrine. The coupling constant is typical of an *o*-interaction, so that lunine must have hydrogen at $C_{(5)}$ and $C_{(6)}$, and substituents at $C_{(7)}$ and $C_{(8)}$. The partial structure at this stage accounts for all but $C_2H_5O_2$ of the molecular formula. The remaining bands *d* and *h* are equivalent to three and two protons, respectively. The singlet *d* must arise from either O–methyl or N–methyl, both absorptions having almost the same τ-value in lunacrine. The τ-value of the band *h* is consistent with the presence of a methylenedioxy group[47] in lunine which therefore most probably has the structure (5), although the structure (6) cannot be eliminated by the n.m.r. evidence. It is important to note that, in their analysis of the spectra of lunacrine and lunine, Goodman, Shoolery, and Johnson made use of the spectra of well chosen model compounds to check their assignments.



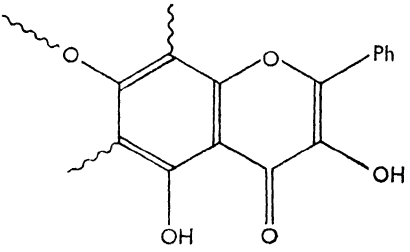
Example 2. A nuclear magnetic resonance spectrum rarely provides sufficient evidence to permit the elucidation of the structure of an organic molecule. In general, the information is used in conjunction with other physical data and deductions based on chemical transformations. An example of this type of application is now discussed.

Sericetin,[48] a substance isolated from the root bark of *Mundulea sericea*, has the molecular formula $C_{25}H_{24}O_5$ [the molecular weight was

determined by mass spectrometry (Chapter 9)]. It forms a mono-acetate, and its infrared spectrum indicates that it contains two phenolic or enolic hydroxyl groups. Consideration of the ultraviolet and infrared spectra of sericetin and its derivatives suggests that the compound is a 5–hydroxyflavone. Alkaline fusion or oxidation with alkaline hydrogen peroxide of sericetin yields benzoic acid so that the part structure (7) may be written.



(7)

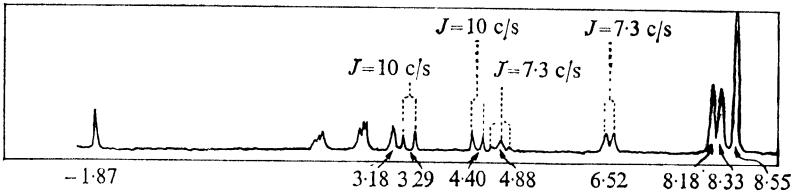


(8)

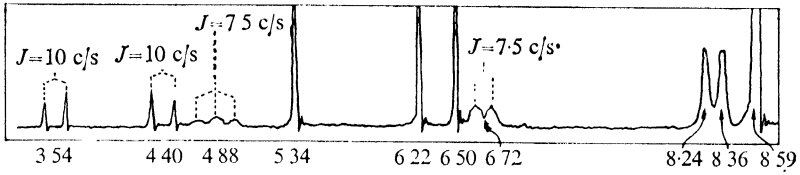
The second hydroxyl is almost certainly in the 3-position as the compound is stable to alkaline hydrolysis. On biogenetic grounds the fifth oxygen is most probably in the 7-position, an assumption which is supported by ultraviolet light absorption data for sericetin. The nuclear magnetic resonance study of the compound thus begins with part structure (8). The spectrum of sericetin is shown in Fig. 17a and confirms some of the features of (8). The band at $-1{\cdot}87$ clearly arises from the strongly chelated 5–hydroxyl group (p. 178). The position of the band at 3·18 is temperature-dependent and it is absent in the spectrum of a solution of sericetin which has been shaken with deuterium oxide. This absorption is therefore caused by the proton of the 3–hydroxyl group. The complex band between 2·0 and 3·0 is equivalent to five protons, and its position and general appearance are characteristic of a phenyl group the *o*-protons of which are deshielded by the substituent (in this case the double bond of the flavone system). The remaining bands in the spectrum are associated with the unknown portion ($C_{10}H_{17}$) of the structure.

The two sharp doublets at 3·29 and 4·40 are each equivalent in area to one proton and have exactly the same separation (10 c/s) which is independent of the strength of the applied field. They must therefore arise from a pair of spin-spin coupled protons. Their τ-values are in the olefinic region of the spectrum and the coupling constant suggests that the two protons belong to a *cis*-disubstituted double bond, leading

(*a*) sericetin



(*b*) sericetinol dimethylether (17)



(*c*) isosericetinol dimethylether (18)

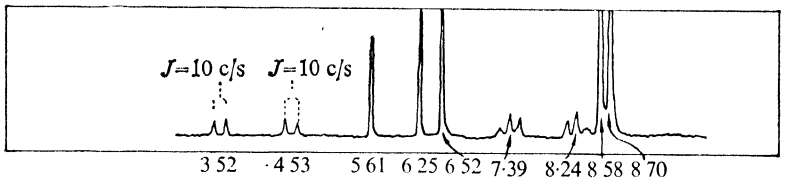
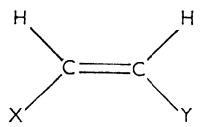
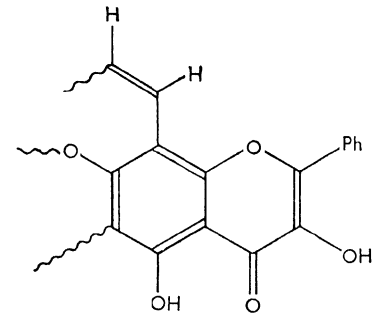


FIG. 17. Spectra (at 56·4 Mc/s in deuterochloroform) of sericetin and two transformation products

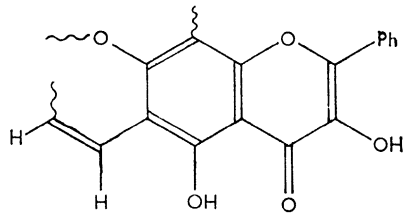


(9)

to the structure (9) in which the elements X and Y do not carry protons. The precise position of the two bands (cf. Table 3) suggests conjugation of the double bond with an aromatic ring so that (9) can be expanded to (10a or b). The broad triplet at 4·88 must also arise from one olefinic proton.

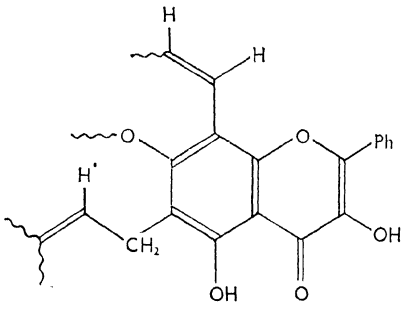


(10a)

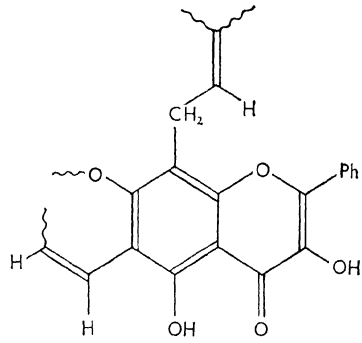


(10b)

The spacing of the triplet is equal to that of the broad doublet at 6·52 which is equivalent to two protons [a simple example of situation (3), p. 184]. The second double bond must therefore be attached to a methylene group. The τ-value of the methylene group requires (Shoolery's rules) that it is also attached to an aromatic ring [partial structures (11a or b)]. The two broad bands at 8·33 and 8·18 are

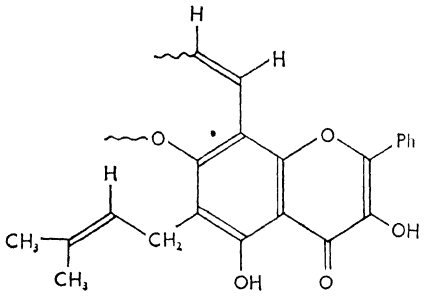


(11a)

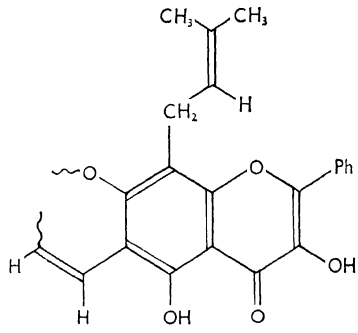


(11b)

each equivalent to three protons and their τ-values lead to their assignment to methyl groups attached to an olefinic double bond. Accordingly the partial structures (11a or b) can be expanded to (12a or b). The width of the lines from all the protons of the β,β-di-

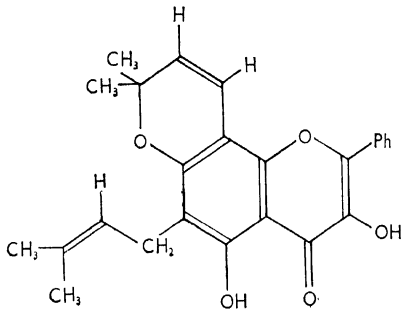


(12a)

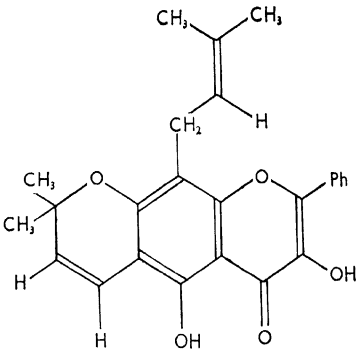


(12b)

methylallyl residue arises from small couplings across the double bond (Table 4, p. 192). The remaining line in the spectrum of sericetin is a singlet at 8·55 equivalent to six protons and must arise from two equivalent methyl groups attached to a fully substituted carbon atom;



(13a)



(13b)

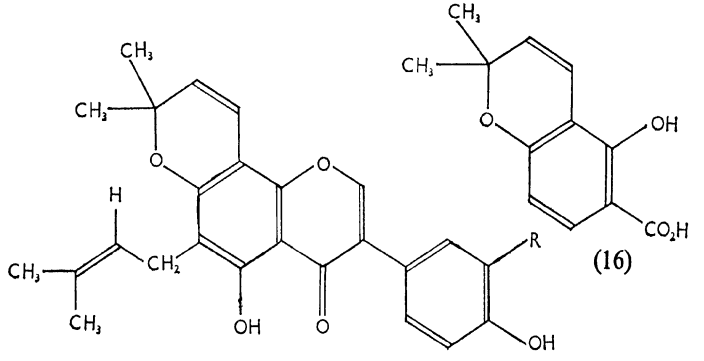
incorporation of this feature leads to structures (13a) and (13b) for sericetin. These assignments are well substantiated by the comparison (Table 5) of the spectrum of sericetin with the spectra of the model compounds (14), (15) and (16). The only significant deviation involves

TABLE 5

Chemical shift and coupling constants for sericetin and model compounds

	$\diagdown$C(Me)$_2$	=C(Me)$_2$	-CH$_2$—CH=		-CH=CH-	
Sericetin (13a or b)	8·55	8·33, 8·18	6·52 ($J\sim$7·0 c/s)	4·88	4·40 (J=10 c/s)	3·29
Osajin (14)	8·51	8·32, 8·18	6·66 ($J\sim$7·0 c/s)	4·87	4·40 (J=10 c/s)	3·32
Pomiferin (15)	8·54	8·33, 8·19	6·66 ($J\sim$7·0 c/s)	†	†	†
β-Tubaic acid (16)	8·51	— —	—	—	4·43 (J=10 c/s)	3·30

† The low solubility of pomiferin precluded observation of these absorptions.

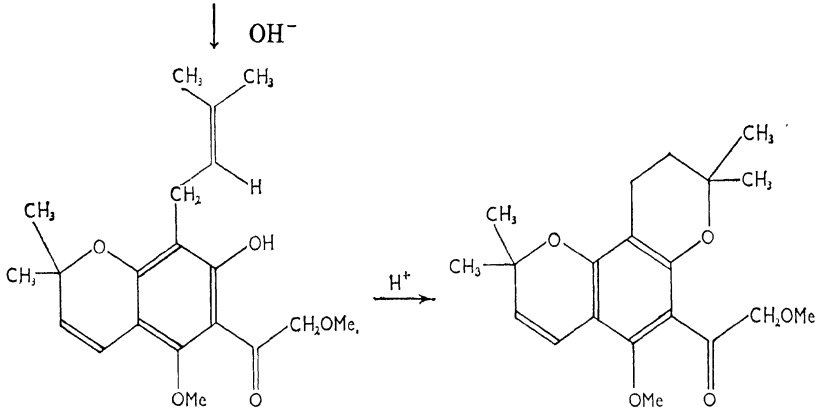


(16)

(14) R=H
(15) R=OH

the τ-value of the methylene group of the β,β-dimethylallyl residue which is 0·14 p.p.m. lower in sericetin, suggesting that sericetin has the structure (13b) rather than (13a). That (13b) is the correct structure is proved by the observation that the compound (17), prepared by alkaline cleavage of the dimethyl ether of sericetin, undergoes a mild acid-catalysed cyclisation to (18). The structures of these transformation products are substantiated by their nuclear magnetic resonance spectra (Fig. 17b and c), and the reader should confirm that this is so by assigning the various absorptions in these spectra.

(13b) → dimethylether

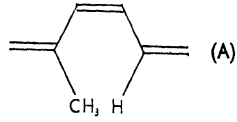


(17) (18)

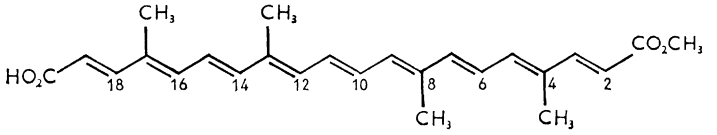
Example 3. As both long range shielding and spin-spin interaction depend on molecular geometry it follows that nuclear magnetic resonance spectroscopy is of considerable use in the investigation of stereochemical problems. An example is now given in which both chemical shift and spin-spin coupling data are used to establish the stereochemistry of a compound.

Bixin, a mono-methyl ester, was the first carotenoid in which geometric isomerism was encountered and the naturally occurring pigment is a geometric isomer of the synthetic 'all-*trans*' carotenoid (19). Light absorption data have shown that bixin is a mono unhindered† *cis*-isomer

† i.e. it does not contain the system (A) in which the steric hindrance encountered by the methyl group would prevent co-planarity of the double bonds (see Chapter 4).



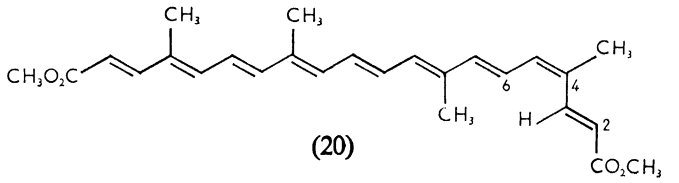
(A)



(19)

of (19) thus excluding *cis*–6- and *cis*–14-double bonds. The central *cis*-isomer has been excluded by synthesis. The problem of the stereochemistry of bixin may therefore be considered in two parts,[49] the first being the assignment of a *cis*-configuration to the 2,3-, 4,5- or 8,9-double bond in the dimethyl ester, methyl bixin, and the second, the location of the *cis*-double bond with respect to the carbomethoxy group in the naturally occurring half ester. (In methyl bixin, the 2,3-, 4,5- and 8,9-double bonds are respectively identical with the 18,19-, 16,17- and 12,13-bonds since the terminal groups are the same; the second problem arises because this is no longer true in bixin.)

The olefinic regions of the spectra of 'natural' methyl bixin and its 'all–*trans*' isomer are reproduced in Figs. 18b and 18a, respectively. The majority of the olefinic protons give rise to the broad band near 3·45 in each spectrum. However, the spectrum of 'all–*trans*' methyl bixin also exhibits two doublets (J = 15·8 c/s), each equivalent to two protons, at 4·12 and 2·61. These bands clearly arise from the pairs of protons of the two terminal double bonds since all other olefinic protons occur in groups of three or four and would give rise to more complicated multiplets. Studies[18] of α,β-unsaturated acids have shown that the α-olefinic proton absorbs at higher fields than the β-proton. The spectrum of 'natural' methyl bixin exhibits the doublet, equivalent



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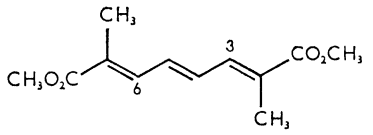
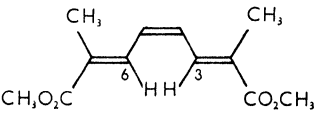
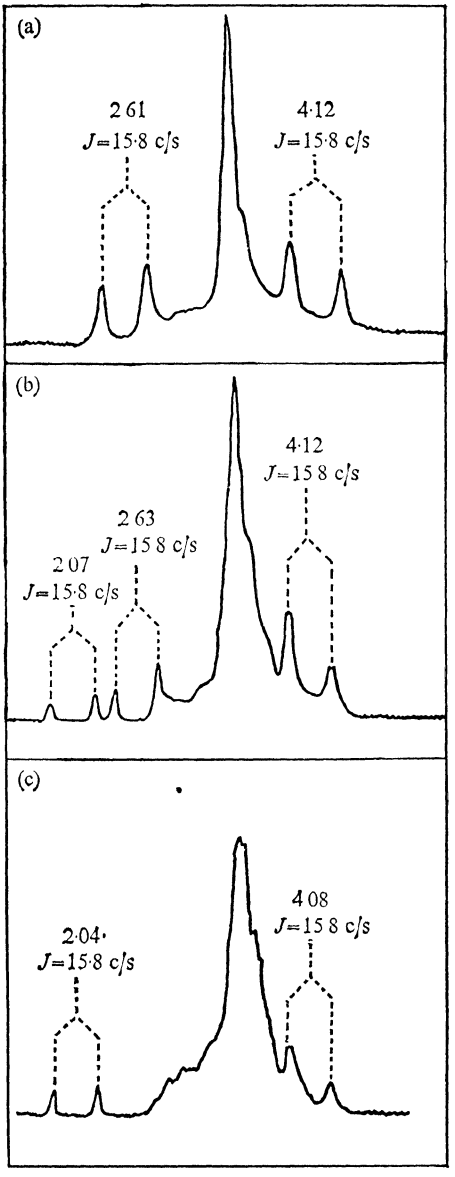


(21)



(22)

to two protons, at 4·12, but that at 2·63 is of half the intensity and in addition there is another doublet (J = 15·8 c/s) at 2·07. It follows that the presence of the *cis*-double bond causes a significant change in the shielding of the β-proton of one of the terminal double bonds. However, both terminal bonds still have the *trans*-configurations as the coupling constants have the same values as observed for the 'all-*trans*' isomer. It is unlikely that a *cis*-configuration of the 8,9-double bond could cause a significant change in the shielding of the 3-proton, and 'natural' methyl bixin must therefore be represented by (20).

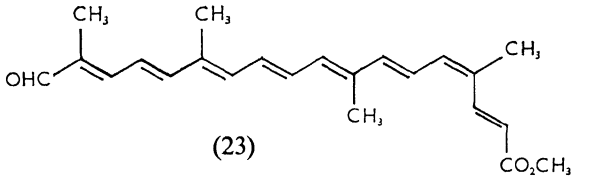


Inspection of formula (20) shows that the *cis*-configuration of the 4,5-double bond places the 3-proton in the plane of and in close proximity to the 6,7-double bond thus explaining the shift to lower fields of the absorption of this proton in the *cis*-isomer. Confirmation of this deshielding effect is provided by the spectra of the two triene esters (21) and (22) in which the 3- and 6-protons in the *cis*-isomer (22) absorb

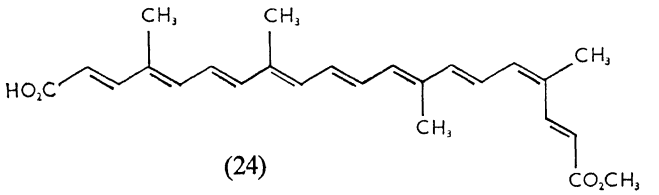
FIG. 18. Spectra (at 40 Mc/s in deuterochloroform) of
(*a*) methyl 'all-*trans*' bixin
(*b*) 'natural' methyl bixin
(*c*) *cis*-apo-1-norbixinal methyl ester

at a field lower by 0·42 p.p.m. than observed for the same protons in the *trans*-isomer.

The location of the *cis*-double bond with respect to the carbomethoxy group in 'natural' bixin follows directly from the spectrum of *cis*–apo–1–norbixinal methyl ester (23) prepared by oxidation of natural



(23)



(24)

bixin.[50] The spectrum (Fig. 18c) shows doublets at 4·08 and 2·04 indicating the retention of the α,β-unsaturated ester function adjacent to the *cis*-double bond. Bixin is therefore (24).

Electron Spin Resonance

Since electron spin resonance occurs only if unpaired electrons are present, the application of this type of spectroscopy in organic chemistry is confined to the study of free radicals and of certain organometallic compounds. The method is therefore of limited applicability and should be considered as a highly specialised technique rather than a routine aid in structural analysis. Nevertheless, within its sphere of application this form of spectroscopy is extremely powerful and it has already added considerably to the understanding of the chemistry of free radicals.

The experimental technique is very similar in principle to that of nuclear magnetic resonance spectroscopy. As already stated, spin transitions of the electron in homogeneous fields of the order of 3,000–13,000 gauss require microwave frequencies (9,000–36,000 Mc/s) and conventional radiofrequency techniques are replaced by those used in radar methods. The reader is referred to more extensive texts for experimental details.[51,52] It should be noted, however, that for reasons of sensitivity it is often convenient to modulate at audiofrequency the

field experienced by the sample, in such a way that the first derivative of the absorption signal, rather than the absorption signal itself, is recorded or displayed. Fig. 19 shows a typical electron resonance absorption signal and its first derivative.

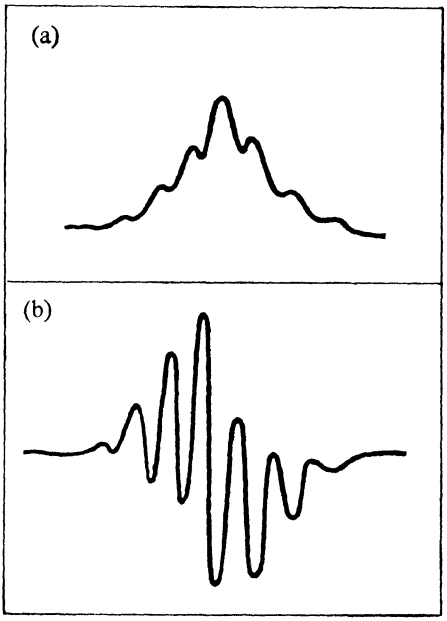


FIG. 19. An electron spin resonance absorption spectrum (*a*) and its first derivative (*b*) (after Ingram[52])

The resonance frequency of an electron is given by equation (3)

$$h\nu = g\beta H_0 \tag{3}$$

in which β is the Bohr magneton and H_0 is the strength of the applied field. The quantity g is the Landé spectroscopic splitting factor and has the value 2·0023 for a free electron. In atoms and molecules mixing of spin momentum with orbital momentum may cause g to depart from this value. However such mixing is invariably small for organic free radicals so that the value of g is of little interest. In other words, in the study of organic free radicals there is no counterpart of the chemical shift in nuclear magnetic resonance spectroscopy. As the resonance frequency of the electron remains relatively constant for organic free radicals, electron spin resonance spectroscopy provides a most convenient method for detecting free radicals. With the equipment at present available minimum concentrations of about 10^{12} free radicals

per gramme can be detected and quantitative determinations of concentrations are usually made by comparing the intensities of the signals with those from known concentrations of free radicals.

In addition to the detection of free radicals much interesting information concerning the electronic structures of free radicals can be obtained from effects analogous to spin-spin coupling in nuclear magnetic resonance.

The interaction of the spins of two unpaired electrons in the same molecule can and does occur. Such an effect is of course confined to molecules containing two (or more) unpaired electrons. If the two unpaired electrons occupy well separated sites in a molecule, little or no interaction occurs, and each gives an electron resonance signal as though it belonged to a separate monoradical. Molecules of this type are called biradicals. If on the other hand the two electrons are sufficiently close, as in molecules in the triplet state, interaction becomes important. After many unsuccessful attempts to observe the electron resonance spectra of organic molecules in the triplet state, this has recently been achieved for photo-excited naphthalene in solid solution in a single crystal of durene[53] and also in a rigid glass solution in glycerol.[56]

The electron spin may also interact with the spins of magnetic nuclei. Such interactions may be divided into two types. The first is an anisotropic interaction, the physical picture of which is the direct interaction, through space, of the electron magnetic dipole with nuclear magnetic

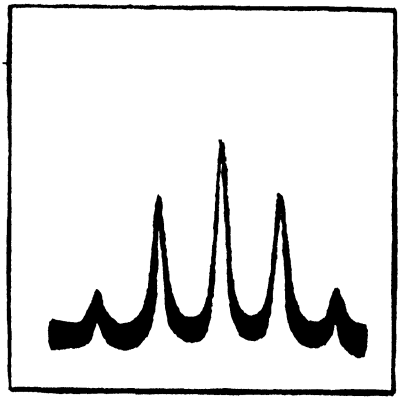
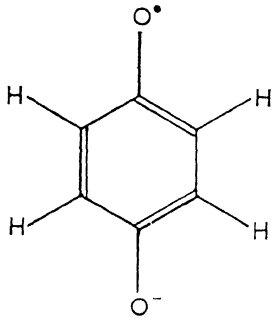


FIG. 20. Electron spin resonance spectrum (absorption) of *p*-benzosemiquinone anion (25) (after Venkataraman and Fraenkel, *J. Amer. Chem. Soc.*, 1955, **77**, 2707)

dipoles. This interaction only produces appreciable fine structure in single crystals. In other solid samples it causes line broadening, and in the liquid state it is generally averaged to zero by random thermal motion.

In addition to the anisotropic interaction there is an isotropic coupling which, like spin-spin coupling in nuclear resonance spectroscopy,



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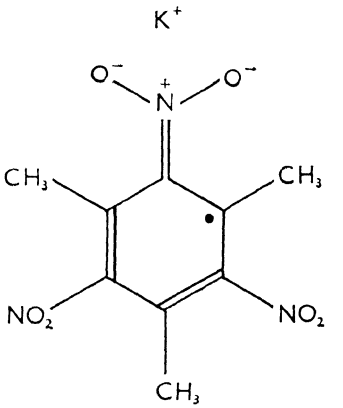

(26)

is not affected by thermal molecular motion. This coupling gives rise to the characteristic hyperfine splittings of many electron spin absorption signals. The rules governing these splittings are precisely the same as those formulated above for nuclear spin-spin coupling, but, because the unpaired electron may often interact with a large number of nuclei, very complicated spectra are sometimes observed. Fig. 20 shows the

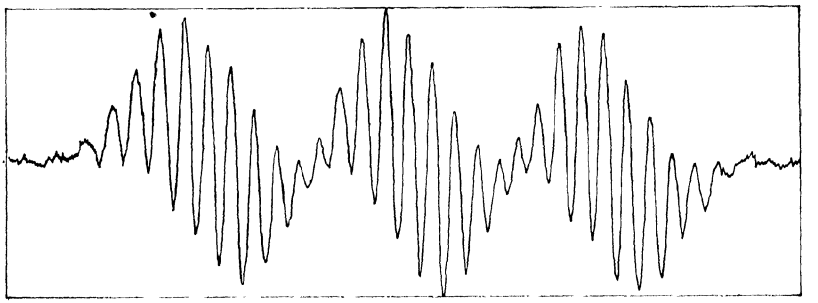

FIG. 21. Electron spin resonance spectrum (derivative) of the potassium salt of trinitromesitylene in 1,2-dimethoxyethane (after Ward, *J. Amer. Chem. Soc.*, 1961, **83**, 1296)

spectrum of *p*-benzosemiquinone anion (25) in which, by symmetry, the electron is coupled equally to four protons and it is seen that this spectrum can be predicted by rule 3 (p. 184). Fig. 21 shows the spectrum of the salt (26) resulting from the reaction of trinitromesitylene with potassium. In this spectrum the major triplet arises from coupling with one nitrogen nucleus ($I=1$, see footnote on p. 187) and the secondary splittings arise from interactions with the nine protons.

The magnitudes (of the order of gauss) of the hyperfine interactions give a direct measure of the unpaired electron densities at the nuclei concerned. Values obtained in this way may be compared with those derived by quantum mechanical methods and it is encouraging that the more refined theoretical procedures[54] can account well for the observed results.

In addition to its use in studies of stable free radicals, electron spin resonance spectroscopy is finding wide application in the investigations of transient radicals produced by ultraviolet, X- and γ-ray irradiation, and in polymerisation and pyrolytic (carbonisation) reactions. In all these studies the transient radicals are trapped in a solid matrix which is initially present, formed during the reaction, or produced by very low temperatures. In this way it is proving possible to study the structures of many unstable molecules and to investigate the characteristics of a number of radical-forming reactions. Furthermore it is also possible to study, *in vivo*, biological processes which involve free radicals. A detailed account of these very interesting aspects lies outside the scope of this chapter and the reader is referred to an excellent survey given by Ingram.[52]

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Chapter 6

J. C. P. Schwarz

OPTICAL ROTATION

Introduction

The rotation of the plane of polarisation of plane polarised light was the first optical property of organic compounds to be interpreted in terms of molecular structure. This historical precedence accounts for the rather curious term 'optical *activity*'. The recognition that the optical activity of solutions is related to molecular asymmetry† (Pasteur, 1848) was of great importance in the development of organic chemistry as it led to the postulation of tetrahedrally directed carbon valencies (van't Hoff and Le Bel, 1874). Later the qualitative and quantitative study of optical rotation played a vital part in the consolidation of stereochemical theory and in the elucidation of configurations.[1] Many stereochemical problems can now be solved by other physical methods (see Chapter 1) or by chemical methods based on our increasing understanding of reaction mechanisms, but optical rotation evidence still retains much of its importance and is sometimes indispensable. Thus optical rotation is the only common physical property which can be used to distinguish between enantiomorphous molecules, a problem which is particularly important in work on natural products and in the elucidation of reaction mechanisms. Although the main applications of optical rotation measurements lie in the realm of stereochemistry, the method can also give other structural information, e.g. it may help to decide between two possible positions for a substituent. In addition, it is useful as a criterion of purity and as an analytical tool.

The use of optical rotation is obviously restricted to compounds

† Strictly speaking, the term *dissymmetry* is more appropriate (ref. 1, p. 12). The condition for optical activity is that the molecule and its mirror image must not be superimposable. This requires that certain, though not all, symmetry elements must be absent (pp. 222 and 283).

whose molecules are asymmetric.† Furthermore, it is clear that the optically inactive mixtures of enantiomers which are produced when asymmetric compounds are synthesised from optically inactive reactants must be resolved before optical activity can be demonstrated and measured. This process is often tedious, and it may be uneconomical to attempt it if the problem in hand can be solved by other physical or chemical methods. For this reason, optical rotation has been most widely employed in the chemistry of natural products (e.g. steroids, terpenoids, alkaloids, carbohydrates and proteins); most of these have asymmetric molecules and occur in nature in one of their enantiomorphous forms.

The magnitude of optical rotations varies with the wavelength of the light used; this variation is known as optical rotatory dispersion (O.R.D. or R.D.). Until about 1950 nearly all optical rotations determined by organic chemists were measured at a single wavelength, usually that of the sodium D-line (*ca.* 589 mμ), largely because measurements in the ultraviolet region involved tedious photographic techniques. However, photoelectric spectropolarimeters have now become available and recent work, particularly by Djerassi and his school, has brilliantly illustrated that rotatory dispersion measurements are an exceedingly valuable structural tool. Djerassi has referred to the first commercial photoelectric spectropolarimeter (O. C. Rudolph and Sons) as 'the prince who woke the sleeping beauty of rotatory dispersion'. In this chapter recent work on rotatory dispersion and earlier studies using measurements at a single wavelength are both discussed. The relative importance of the latter approach is likely to diminish but many of the general principles employed will probably remain useful.

Even more recently, photoelectric instruments have been developed for the measurement of circular dichroism, an absorption phenomenon closely related to optical rotation (see p. 218). Circular dichroism is likely to assume considerable importance in structural work and it is therefore considered briefly in this chapter.

Djerassi[2] and Klyne[3,4] have written authoritative reviews on rotatory dispersion. The applications of measurements at a single wavelength have also been reviewed.[5,6] These reviews provide extensive literature references and give fuller details of most of the applications discussed here. The use of circular dichroism has also been reviewed.[7,19]

† This is not quite true. Substances which are normally optically inactive become optically active in a magnetic field (Faraday effect). However, 'magneto-optical rotations' have so far found little application in organic chemistry; one inherent difficulty is that, in solution measurements, both solvent and solute show optical activity.

THE MEASUREMENT OF OPTICAL ROTATIONS

Units and conventions. Optical rotations are generally measured in solution and the *specific rotation* ([α]) of a compound is defined as

$$[\alpha] = \frac{100\,\alpha}{lc}$$

where α is the observed rotation, l is the length of the sample tube in decimetres and c is the concentration of the solution in g/100 ml. The wavelength of the light and the temperature are often indicated by subscripts and superscripts respectively, and the solvent and concentration are usually given in brackets, e.g. $[\alpha]_D^{17} + 35°$ (c 2 in $CHCl_3$). In quantitative comparisons involving compounds of different molecular weight, it is usually necessary to use *molecular rotations*, defined by

$$[\mathrm{M}] \text{ or } [\phi] = \frac{\alpha \times \mathrm{M.Wt.}}{100}$$

The symbol [φ] has recently been suggested to replace [M]. In some publications, particularly in carbohydrate chemistry, the product is not divided by 100.

Classification of rotatory dispersion curves. Djerassi and Klyne have suggested that rotatory dispersion curves (i.e. plots of optical rotation against wavelength) should be classified into two main types—plain curves and Cotton effect curves.† Compounds which do not have an optically active absorption band (see p. 218) within the region studied generally give curves which show no maxima or minima.‡ Such curves are known as *plain curves*; they are further classified as *positive* or *negative* depending on whether the rotation of the compound becomes more positive or more negative with decreasing wavelength (see Fig. 1). When the measurements are extended into the region of an optically active absorption band, curves showing maxima and minima are obtained. Such curves are called *Cotton effect curves*; the maxima and minima are known as peaks and troughs (to avoid confusion with the salient features of absorption curves) and they may be collectively referred to as *extrema*. Curves which have only one peak and trough are called *single Cotton effect curves*. They are classified as *positive* or *negative* according to whether the extremum at longer wavelength is a peak or a trough (Fig. 2). The algebraic difference between the mole-

† See ref. 2–4 for additional suggestions regarding the presentation of rotatory dispersion data in the literature.

‡ Occasionally curves with a single smooth maximum or minimum may be obtained (cf. Fig. 9, p. 235).

cular rotations at the peak and the trough is known as the molecular amplitude (*a*).† Curves which have more than one extremum of the same type are called *multiple Cotton effect curves* (Fig. 7, p. 230).

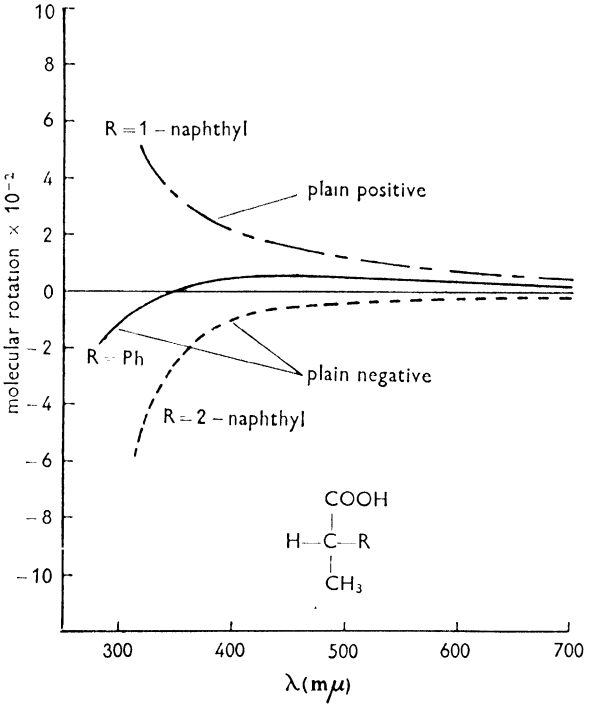


FIG. 1. Plain curves. The rotatory dispersion curves of some substituted propionic acids in 0·1N sodium hydroxide (from Sjöberg[21]).

Apparatus. Instruments for the measurement of optical rotation are called polarimeters or, if the wavelength can be varied, spectropolarimeters. Considerable advances have recently been made in their design and convenient photoelectric polarimeters are now available in addition to the classical visual and photographic instruments. Some of the modern instruments have facilities for the automatic recording of rotatory dispersion curves or for continuous recording at a single wavelength. The various commercial instruments differ greatly and the following account is confined to a few general principles (for details see refs. 2, 4 and 8).

† Recently,[14] it has been suggested that, for convenience, the molecular amplitude should be defined as the above difference divided by 100.

A schematic diagram of the essential parts of a polarimeter is given in Fig. 3. For work at a single wavelength in the visible region, the *light source* may be a sodium discharge lamp or a tungsten lamp with suitable filters. In spectropolarimetry, a monochromator is used to

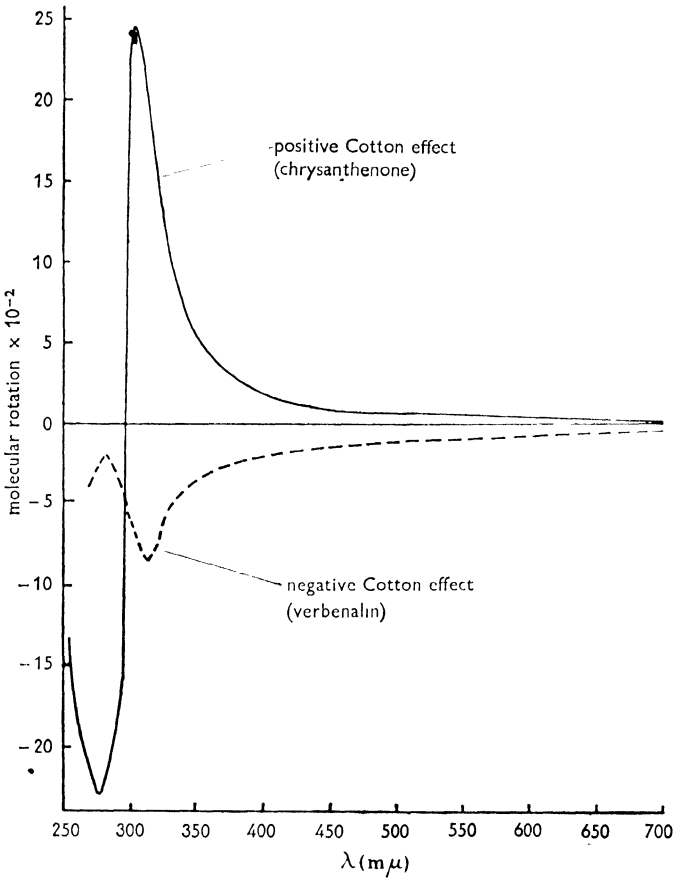


FIG. 2. Cotton effect curves. The rotatory dispersion curves of two naturally-occurring ketones: chrysanthenone (in iso-octane) and verbenalin (in water) (from Moscowitz, Mislow, Glass and Djerassi[16]).

isolate light of variable wavelength from a source giving a continuous spectrum. By passage through the *polariser* (usually a prism of a doubly refracting material such as quartz or calcite) the light becomes plane polarised, i.e. its vibrations are restricted to one plane, the orientation of which is determined by the orientation of the polariser. After traversing the sample, the light reaches the *analyser*, a prism

similar to the polariser. The *detector* may be a photomultiplier or, in the visible region, the eye.

The intensity of the light falling on the detector varies as the analyser is rotated. It is a minimum (ideally zero except in regions of absorption) when the 'plane of transmission' of the analyser is at 90° to the plane of polarisation of the light incident on it. In the absence of an optically active sample, this minimum position gives the zero setting of the polarimeter†; this setting is best determined with the sample tube full of solvent. The sample tube is then filled with a solution of the material

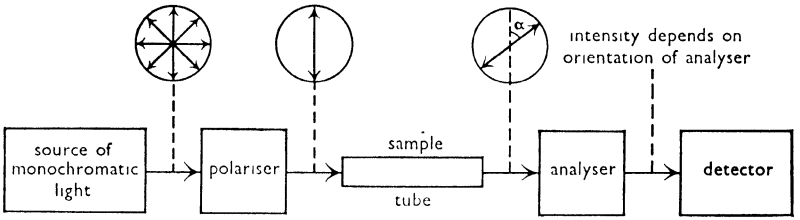


FIG. 3. Essential components of a polarimeter (the circles represent cross-sections of the light beam as viewed from the analyser, the lines indicating the planes of polarisation—in the above case the sample is dextrorotatory).

and the angle through which the analyser must be rotated until the light is again extinguished gives the optical rotation of the solution. In some recording instruments the analyser or polariser is turned by a servo-motor; in others they are fixed and the rotation of the specimen is compensated electrically using the Faraday effect (the production of optical activity in normally inactive materials by a magnetic field). In spectropolarimetry, measurements are carried out at a number of wavelengths until the required range has been covered.

To obtain the maximum information from rotatory dispersion measurements, it is usually desirable to extend them as far into the ultraviolet as possible. On many instruments the lower wavelength limit is *ca.* 250–270 mμ (some recent instruments, however, penetrate below 200 mμ), but measurements may become unreliable or impossible even above the usual limit if the solution is strongly absorbing.

Sample requirements. The amount of material required for rotation measurements depends on the size of the specific rotation of the compound at the wavelength (or wavelengths) of the measurements, on the

† In practice, the direct determination of the precise position of the minimum is difficult. It is therefore located indirectly, on the principle that equal angular displacements on either side of the minimum position give equal signals at the detector; the exact manner in which this 'equal angle' principle is applied depends on the type of instrument.

polarimeter and on the accuracy required. Errors as high as 10% can often be tolerated in structural work using rotatory dispersion, but these would be serious for most analytical applications or in data which are to be subjected to detailed mathematical analysis. For work with visual polarimeters (sensitivity $\sim 0{\cdot}01°$) quantities between 5–200 mg are generally used. Photoelectric polarimeters may have sensitivities in the visible region higher than 0·001°, but sensitivities in the ultraviolet are generally lower, especially with absorbing solutions. However, since rotations in the ultraviolet are usually higher than in the visible, 1–5 mg are often sufficient for rotatory dispersion measurements.

Other factors. Specific rotations vary with temperature, concentration and solvent. Concentration effects are usually small for concentrations below $\sim 2\%$ and temperature variations of a few degrees are generally without significance except in very precise work. Unusually pronounced effects of concentration or temperature can often be ascribed to changes in association or ionisation equilibria; temperature effects may also be caused by changes in conformation. Optical rotation, like some of the other physical methods (Chapter 1), can be used for studying such changes. Solvent effects can sometimes be explained in similar terms, but more subtle factors, such as the effect of solvation on the light absorption of the compound (see below) are also involved. Tetra-*O*-methyl D-mannono-γ-lactone provides an outstanding example of solvent effects on specific rotations at the D-line ($[\alpha]_D^{20} = +65°$ in water; $-10°$ in chloroform; $-36°$ in ether; $-49°$ in benzene; *c*, 1–2%).

Solvent effects are difficult to predict and rotations used for structural comparisons within a group should be determined in the same solvent. Water, chloroform and ethanol are common solvents for work at the D-line; water, methanol and dioxan are often used for rotatory dispersion measurements.

Analytical applications of optical rotation measurements. Like other physical properties which depend on the concentration of a solute, optical rotation can be used for quantitative analysis. An example of industrial importance is the determination of sucrose (saccharimetry). Rotations at the D-line have been widely used for analytical purposes; measurements in the ultraviolet (where rotations may be 5–100 times larger) give increased sensitivity. Rotation measurements at more than one wavelength provide a method for the analysis of mixtures of substances whose rotatory dispersion curves differ significantly.[3,4] Rotation or rotatory dispersion measurements can also be used as criteria of purity and identity.

Reactions in which optically active substances take part usually involve changes in rotation, and rotation measurements have therefore proved useful in the determination of reaction rates and in the quantitative study of equilibria. The 'inversion' of sucrose (a hydrolysis) and the 'mutarotation' of glucose (an epimerisation) are two reactions which owe both their name and their early popularity with physical chemists to the rotational changes which accompany them. Such changes in rotation are often very useful in preparative work. For example, acid hydrolysis of 1, 2:3, 5-di-O-isopropylidene D-xylose ($[\phi]_D = +28°$, in water) gives first the 1, 2-mono-O-isopropylidene compound ($[\phi]_D = -36°$) and then D-xylose (equilibrium $[\phi]_D = +30°$); in the preparation of the mono-isopropylidene compound, the reaction mixture is therefore worked up when the rotation has reached the maximum negative value.

THE THEORY OF OPTICAL ROTATION

Paradoxically, although optical rotation was the first physical property to be used in structural work, its fundamental theory is far from simple. The present account is therefore confined to a brief statement of those theoretical features which are essential for the understanding of structural applications. The reader is referred elsewhere[2,9-11,34,35] for details of the classical and quantum mechanical theories of optical rotation.

Plane and circularly polarised light. A beam of light is associated with varying electric and magnetic fields. In a beam of *plane polarised light*, the electric vector is restricted to one plane and its magnitude at a given point in the beam varies periodically. In a beam of *circularly polarised light*, the magnitude of the vector remains unchanged, but its direction at a given point rotates either clockwise (right circularly polarised) or anticlockwise (left circularly polarised light). Crudely speaking, plane polarised light corresponds to an ordinary wave in one plane, while circularly polarised light corresponds to movement along a right or left handed helix. It can readily be proved (see ref. 1, chapter 14, ref. 2, chapter 12, or textbooks of physics) that

(*a*) plane polarised light is equivalent to the sum of right and left circularly polarised components of equal amplitude (the separation of the components can, in fact, be accomplished experimentally), and

(*b*) rotation of the plane of plane polarised light results when the two circularly polarised components are propagated with unequal velocity, i.e. when the medium has a different refractive index for right and left circularly polarised light (circular birefringence).

The relation between optical activity and light absorption. It can be shown that the refractive index of a medium for unpolarised light is quantitatively related to the wavelengths and intensities of its absorptions in the visible and ultraviolet regions. Similarly, the optical rotation of a medium is quantitatively related to the *differences* in the intensities of absorption of left and right circularly polarised light at various wavelengths. These differences are known as the *circular dichroism* of the absorption bands (see also p. 232). Absorption bands which show circular dichroism and which therefore play a part in determining the optical rotation are known as *optically active absorption bands*. Such absorptions are provided by chromophores (p. 127) which are either intrinsically asymmetric or which become asymmetric by interaction with an asymmetric molecular environment.† Hexahelicene (formula 1, p. 222), in which the chromophore extends over the entire asymmetric molecule, is an example of the former type; the carbonyl group exemplifies a symmetrical absorbing group which can become 'optically active' in an asymmetric environment.

The composite nature of optical rotation. Formally, *the optical rotation of a compound at any wavelength can be regarded as the algebraic sum of contributions due to the various optically active absorption bands.* These contributions are known as *partial rotations.*‡ As indicated above, their individual magnitude and sign depend on the circular dichroism of the particular absorption band and this, in turn, depends on the nature of the chromophore and of its asymmetric environment.

A *purely theoretical picture* of the wavelength dependence of the partial rotation associated with a *single* optically active absorption band is shown in Fig. 4. The heavy line gives the magnitude of the partial rotation and the dotted line represents the circular dichroism of the absorption band. The circular dichroism is, of course, confined to the

† In theory,[35] the 'rotational strength' of the electronic transition is given by $\rho\mu \cos\theta$, where ρ and μ are the electric and magnetic transition moments (p. 129) and θ is the angle between the directions of the moments. The former moment corresponds to a linear displacement of the electron and the latter to a circular displacement (as in the $n \rightarrow \pi^*$ transition of ketones where the lone-pair *p*-orbital and the π^*-orbital are perpendicular to each other). Combination of the two displacements is, of course, equivalent to a helical displacement of the electron.

The $n \rightarrow \pi^*$ transition of ketones is an example of a 'magnetic dipole' transition which becomes optically active when interaction of the carbonyl group with its molecular environment provides a suitably directed electric transition moment. By contrast, inherently asymmetric chromophores usually are extended π-electron systems (e.g. dienes, etc.) having 'electric dipole' transitions which derive some magnetic dipole character from the twisting of the chromophore.

‡ The partial rotation associated with an absorption band must not be confused with the 'contribution of an asymmetric centre', a quite different and much less satisfactory concept; see p. 237.

region of absorption,† but the partial rotation contribution is appreciable even at wavelengths far from this region. The spectacular changes in rotation in the region of absorption and the related circular dichroism are together referred to as the *Cotton effect*. For a symmetrical band, the wavelength (λ_i) at which the rotation curve crosses the wavelength axis lies halfway between the wavelengths of the peak and the trough and coincides approximately with the wavelength of maximum absorption. Outside the region of absorption, the magnitude (ϕ_i) of the partial

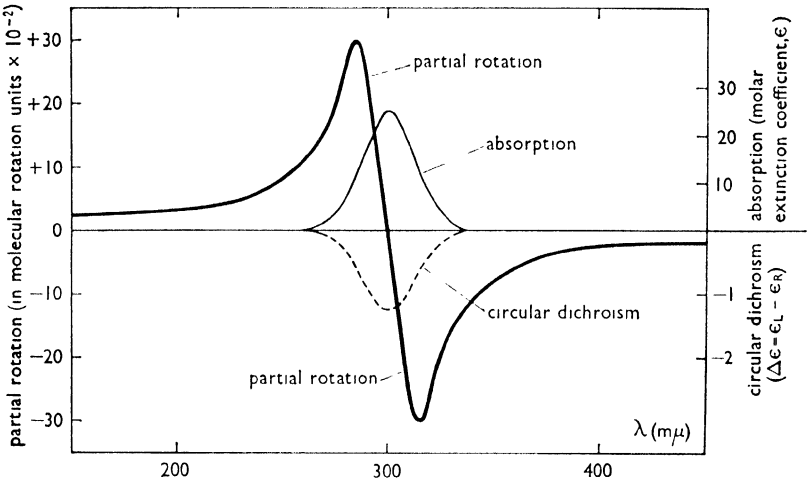


FIG. 4. Wavelength dependence of the partial rotation, circular dichroism and absorption associated with a single optically active chromophore (e.g. carbonyl group) with $\lambda_{max} = 300$ mμ and a negative Cotton effect (idealised).

rotation decreases with the wavelength (λ) of observation according to an equation known as the Drude equation: $\phi_i = K_i/(\lambda^2 - \lambda_i^2)$, where K_i is a constant. A much more complex equation is required to describe the shape of the partial rotation curve in or near the region of absorption (see ref. 2, p. 164).

In practice, any optically active molecule will have a considerable number of optically active absorption bands. Many (or even all) of these bands will be associated with saturated portions of the molecule

† However, the shape of the circular dichroism curve need not follow the shape of the absorption curve exactly. Thus the wavelengths of the maxima need not coincide precisely and circular dichroism often shows more fine structure than ordinary absorption. Furthermore, circular dichroism, unlike absorption, can be negative (see Fig. 4 and p. 233).

which absorb only in the experimentally inaccessible far-ultraviolet region (i.e. $\lambda_i < 200$ mμ), but nevertheless each of them will, in general, make a contribution to the total rotation at any wavelength. The experimentally observed rotatory dispersion curve can therefore be regarded as the sum of a number of partial rotation curves of the type shown in Fig. 4. In practice, one or two absorption bands, whose wavelengths lie closest to the wavelength at which the rotation is measured, often provide the greatest partial rotations but, even so, the contributions of other bands (e.g. those in the far-ultraviolet) may be far from negligible. In general any group in the molecule can contribute to the optical rotation in two ways: (*a*) as the site of an absorption band, (*b*) as part of the asymmetric environment of other absorbing groups (the latter role is known as *vicinal action*). Optical rotation is therefore related to structure in a less direct way than, say, infrared or ultraviolet absorption.

The Correlation of Structure and Optical Rotation

The most common application of rotation measurements is in deciding between a relatively small number of structural possibilities, often only two. These possibilities may differ in the point of attachment of a group (e.g. p. 226), in the configuration of one or more asymmetric centres (e.g. p. 226) or in conformation (e.g. p. 229). In molecules which contain two or more asymmetric centres, optical rotation evidence can be used both to determine the configuration of the centres relative to each other and to elucidate the absolute configuration of the molecule. The latter problem involves relating the configuration of one of the centres to that of a compound whose configuration has already been related to D-glyceraldehyde, the absolute configuration of which is known (see ref. 6, where other methods for the correlation of configurations are also discussed).

The decision between the structural possibilities is usually based on comparison of the observed 'rotatory behaviour' with that predicted for the various structures. Aspects of 'rotatory behaviour' which have been correlated with structure include (*a*) the presence or absence of optical activity, (*b*) the sign, shape and amplitude of Cotton effect curves, (*c*) circular dichroism, (*d*) the trend of plain curves, (*e*) rotations at a single wavelength (e.g. at the D-line) and (*f*) the changes in rotation which occur when a compound is structurally modified. Examples of each of these approaches are discussed below.

Predictions regarding the rotatory behaviour of hypothetical struc-

tures could, in principle, be obtained from calculations based on classical or quantum mechanical theories of optical rotation, but at present such an approach is greatly restricted, although encouraging progress has recently been made.[34,35] In practice, therefore, the rotatory behaviour of the various possible structures is predicted from that of closely similar compounds, i.e. empirical analogies are used. This approach is similar to that employed with many of the other methods discussed in this book (e.g. absorption spectra). However, since optical rotation is often very sensitive to small changes in structure, special care must be taken to ensure that the analogies are valid to the degree of approximation required to distinguish the structural alternatives. The validity of regarding two structures as analogous can usually be assessed by comparing the rotatory behaviour of a number of related compounds of known structure. The resulting correlations are often formulated into 'rules'; but these may be subject to exceptions, particularly when applied to novel structures. Unless there is a considerable margin of safety, the conclusions should be regarded only as a prelude to further chemical work.

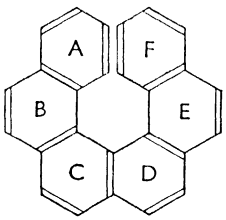
One important complication must be mentioned. Many organic molecules exist as mixtures of two or more conformations and the rotations of these conformations may differ in magnitude and even in sign. For example, acyclic compounds often have much lower rotations than related cyclic compounds (compare hexitols with the parent sugars) and this is largely due to the mutual cancellation of the rotations of the various conformations. If the rotatory behaviour of one compound is to be predicted by analogy with another, then the conformational situations must be identical for both compounds, i.e. either the two compounds must be conformationally homogeneous, or the relative proportions of the various conformations must be similar. Polycyclic compounds with *trans*-fused rings usually (though not always) exist in a single readily predictable conformation and this is one reason why steroids and polycyclic terpenes provide a particularly favourable field for correlations based on optical rotation. In the case of monocyclic or acyclic compounds, conformational uncertainties may frustrate attempts to correlate optical rotation and structure. However, if suitable analogies are available, the dependence of rotatory behaviour on conformation can be put to good use in studying conformational equilibria.

As mentioned above, the correlation of rotatory behaviour with structure has been approached in a number of ways. These will now be considered in turn.

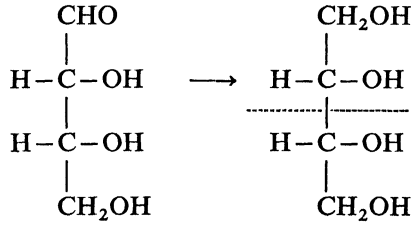
The Presence or Absence of Optical Activity

If a compound shows optical activity in solution, its molecule cannot have a plane or centre of symmetry, or a four-fold inversion axis (p. 283). The mere presence of optical activity can therefore give useful information about structure. The demonstration that a compound is optically inactive and non-resolvable may also be used as structural evidence, although it must be remembered that this does not necessarily show that the molecule has one of the above elements of symmetry; ready racemisation may make resolution impossible, as in the case of tertiary amines ($R^1R^2R^3N$).

These considerations played an important part in classical work on the elucidation of valency distributions; for example, the optical activity of compounds of the type *Cabde* [e.g. $CH_3CH(OH)COOH$] showed that the valencies of the central carbon atom cannot lie in one plane. Another classical application was the demonstration that suitably substituted diphenyls exist in a non-planar conformation, with restricted rotation about the central single bond.[1] More recently, optical activity has been used to show that certain hydrocarbons, e.g. hexahelicene (1), have a non-planar structure (rings A and F overlap, hence the molecule is either a right-handed or left-handed spiral).



(1)



(2) (3)

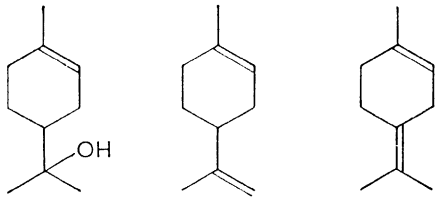
Optical activity is also valuable in the investigation of reaction mechanisms. Racemisation during reaction suggests that a symmetrical intermediate or transition state is involved (e.g. the carbonium ion $R^1R^2R^3C^+$ formed in the solvolysis of tertiary halides; cf. ref. 1, chapter 13). If racemisation does not occur, the methods described in the following sections may be applied to determine whether inversion or retention of configuration has taken place.

In suitable cases the presence or absence of optical activity provides an excellent method for the elucidation of configurations. Thus reduction of D-erythrose (2) gives an optically inactive alcohol; this fixes the relative configuration of the two asymmetric carbon atoms in erythrose

although it does not give any information about the absolute configuration. Emil Fischer's brilliant work on the stereochemistry of the hexoses made frequent use of this approach.

The presence of optical activity may also help to decide between structural possibilities. For example, dehydration of α-terpineol (4) with acetic anhydride gives limonene, which therefore has the structure (5) or (6); the second alternative can however be excluded as limonene is optically active.

When rotations at the D-line are very small, measurements at shorter wavelengths have proved useful for demonstrating the presence of optical activity.



(4) (5) (6)

Cotton Effect Curves

The method outlined in the previous section is limited in scope and a more quantitative approach is generally necessary, especially in work on complex molecules. As mentioned above, the optical rotation at any wavelength may be considered as the sum of a number of partial rotations each of which is associated with an absorption band. When the structure or stereochemistry of a molecule is altered, each of these partial rotations may be affected; this obviously complicates the quantitative correlation of optical rotation and structure. These complications can, however, be largely avoided if the rotation can be measured at wavelengths within the region of an optically active absorption band. The rotatory changes associated with the Cotton effect of this band can then be observed clearly above the general 'background rotation' due to contributions from the other absorption bands. The ketonic carbonyl group (weak absorption at *ca.* 280–300 mμ) is a particularly suitable group for this purpose and Fig. 5 shows the characteristic Cotton effect rotatory dispersion curves of some steroid ketones (see also Fig. 2).

It has been shown, particularly by Djerassi and his school, that the sign, shape and amplitude (p. 213) of such Cotton effect curves can be readily correlated with the structural and stereochemical environment

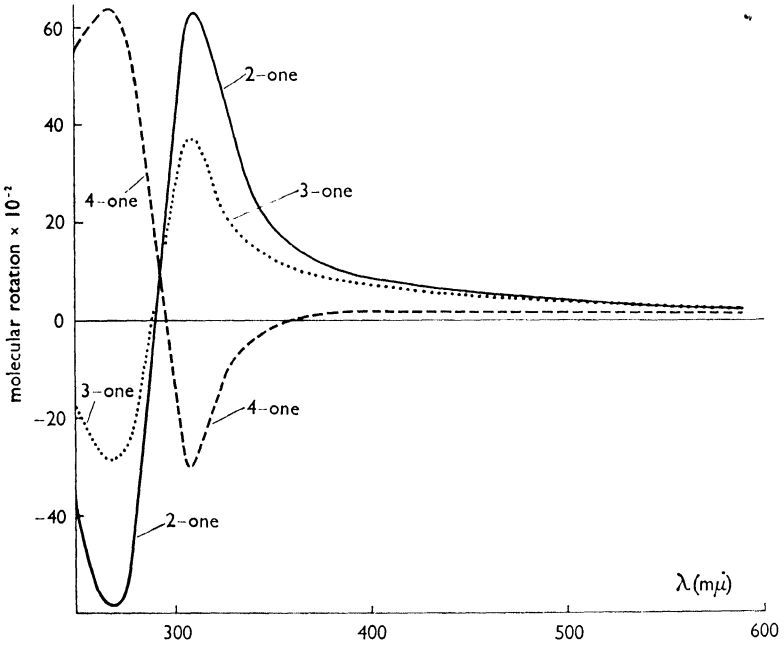
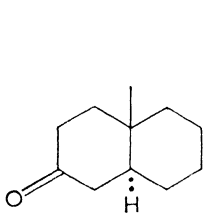


FIG. 5. Rotatory dispersion curves of 5α-cholestan-2-one, 5α-cholestan-3-one and 5α-cholestan-4-one (in methanol; from Djerassi *et al.*, *J. Amer. Chem. Soc.*, 1956, **78**, 3163)

of the keto group.[2,3,4,14] As might be expected, the nature of the Cotton effect curve depends mainly on the immediate environment of the keto group; changes at some distance from the absorbing function generally have only a minor effect. For example, if the keto group is in a terminal ring, the sign of the Cotton effect curve is generally determined by the



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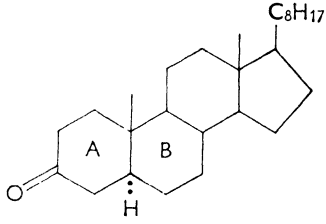
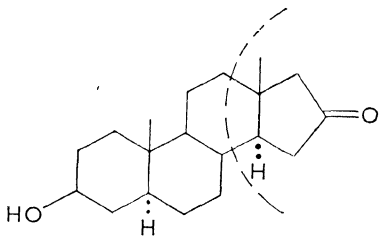


(8)

nature of the terminal unit; thus the *trans*-decalone (7) gives a positive Cotton effect curve rather similar to that of the A/B-*trans* steroid ketone, 5α–cholestan–3–one (8).† Analogies on which structural arguments can be based (cf. p. 221) are therefore usually not difficult to find. Each terminal unit can, of course, exist in two enantiomeric forms



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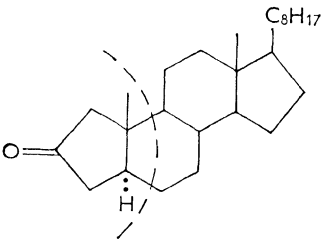
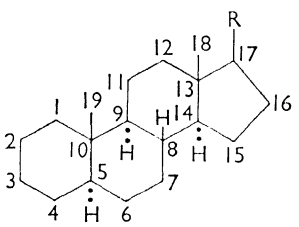


(10)

which give rotatory dispersion curves of opposite sign; a striking illustration of this is that the compounds (9) and (10) give Cotton effect curves which are almost perfect mirror images of each other.

Since steroid and terpenoid ketones played the leading role in establishing Cotton effect curves as a structural tool, a few examples from this field have been chosen to illustrate the types of application. Absorbing functions other than the keto group are briefly discussed later.

For reference, some conventions used in steroid chemistry will first be summarised. The steroid nucleus is numbered as shown in formula (11). In the natural steroids the two angular methyl groups at $C_{(10)}$ and $C_{(13)}$ project to the front of the molecule; such groups are designated as β–orientated and their orientation is indicated by solid or bold bonds. Bonds which project to the rear are referred to as



(11)

α–orientated and are indicated by dotted or broken lines (e.g. the C—H

† However, such similarity between poly- and bi-cyclic analogues is not always found; in particular, conformational differences may cause discrepancies.

bond at $C_{(5)}$ in formula 11). A similar stereochemical convention is used for terpenes and other cyclic compounds.

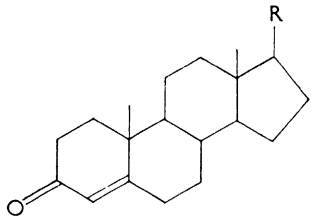
Many steroids carry β–orientated side chains at $C_{(17)}$. For example, the hydrocarbon 5α–cholestane, ketonic derivatives of which are discussed below, has the structure (11, R $= -CHMe-(CH_2)_3-CHMe_2$). In 5α–compounds the A/B ring junction is *trans*; A/B–*cis* compounds in which the hydrogen at $C_{(5)}$ is β–orientated are also common.

The location of carbonyl groups. The Cotton effect curves of steroids with a keto group in various positions show characteristic differences in sign and amplitude; these are usually little affected by the presence of other substituents unless the immediate environment of the keto groups is altered. Rotatory dispersion measurements can therefore be used for locating carbonyl groups in steroids and in other compounds (e.g. triterpenes) provided suitable analogies are available (infrared spectroscopy may be used in conjunction, e.g. to distinguish between keto groups in 5– and 6–membered rings; p. 64). The effect of the position of the carbonyl group is illustrated in Fig. 5 (p. 224), which shows the rotatory dispersion curves of 5α–cholestan–2–one, 5α–cholestan–3–one and 5α–cholestan–4–one, ketonic derivatives of (11, R $= C_8H_{17}$). The 2–ketone gives a positive Cotton effect curve of fairly large amplitude, the 3–ketone gives a positive curve of somewhat smaller amplitude† and the 4–ketone gives a negative curve.

Stereochemical applications. Cotton effect curves also provide a powerful tool for the investigation of stereochemical detail. For example, a common problem in steroid chemistry is that of distinguishing between the two reduction products (13) and (14) of unsaturated ketones of the type (12). This can now be solved readily by analogy with 5α–cholestan–3–one (13, R $= C_8H_{17}$) and 5β–cholestan–3–one (14, R $= C_8H_{17}$) which give positive and negative Cotton effect curves respectively (see also Fig. 8, p. 233).

One of the most important applications of rotatory dispersion measurements is the determination of absolute configurations. The procedure generally used is to compare the sign of the Cotton effect curve of a suitable ketonic derivative of the compound with that of an appropriate steroid analogue or of some other ketone whose absolute configuration is known. Commonly, as in the example in the next

† The 2–ketone and the 3–ketone can be distinguished unambiguously by adding a drop of hydrochloric acid to the methanolic solution of the ketone in the polarimeter tube. The curve of a 3–ketone then shows a considerable decrease in amplitude while that of a 2–ketone is hardly affected. This difference arises because, under the above conditions, the two ketones form ketals to different extents. This procedure forms a useful adjunct to rotatory dispersion measurements.



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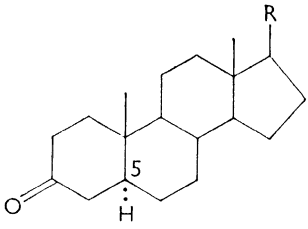

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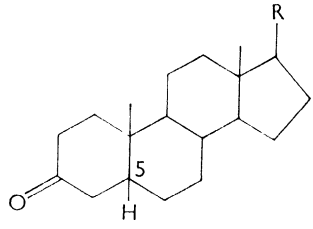
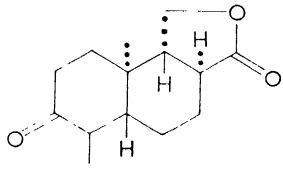


(14)

paragraph, the keto group is in a terminal ring; as noted earlier, the sign of the Cotton effect curve of such ketones is nearly always determined by the nature and configuration of the terminal unit.

The sesquiterpene, iresin, has been transformed into a ketone which



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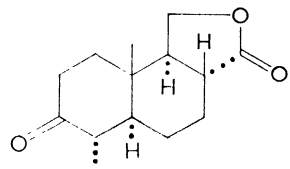

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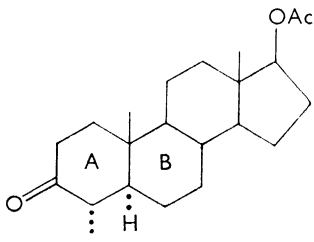
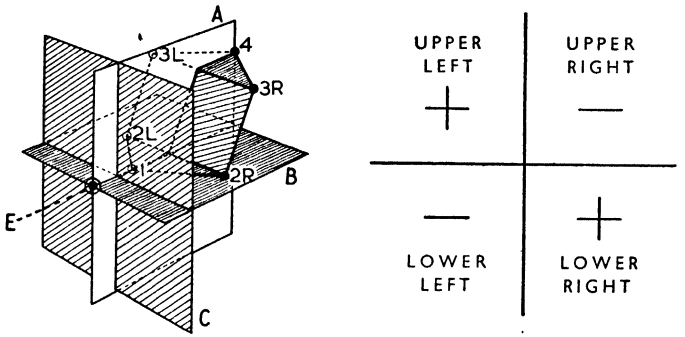


(17)

is known to be either (15) or the enantiomer (16). This ketone has a negative Cotton effect curve while the steroid analogue (17) has a positive curve.† The ketone therefore has the configuration (15), and iresin (unlike most other sesquiterpenes) must have the opposite absolute configuration to that of the A/B ring junction in the steroids.

This method of determining absolute configurations has been applied with outstanding success to a wide range of sesqui-, di- and tri-terpenes.[2,3]

The Octant Rule. All the examples discussed above depended on direct analogy. In the case of cyclohexanones, however, enough data have been accumulated to allow the formulation of a rule which enables the sign and approximate amplitude of the Cotton effect curve to be predicted from consideration of the disposition of the substituents relative to the keto group. This rule is not only very useful in practice



(*a*) The Octants (from Klyne[3]) (*b*) Contributions of groups in the far Octants

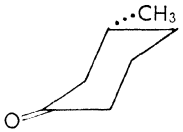
FIG. 6. The Octant Rule

but it is also of theoretical interest as it partly bridges the gap between arguments depending purely on analogy and predictions based on the theories of optical rotation.[34,35] Only a brief account of the rule can be given here and the reader is referred to key papers[12,14] or to reviews[2,3] for further information and examples.

To apply the rule, the cyclohexanone ring is divided into eight octants by three planes, as shown in Fig. 6. One of these passes through the oxygen, $C_{(1)}$ and $C_{(4)}$, another through $C_{(1)}$, $C_{(2R)}$ and $C_{(2L)}$ (the equatorial substituents at $C_{(2R)}$ and $C_{(2L)}$ lie approximately in this plane) and the third bisects the carbonyl bond and lies perpendicular

† The lactone and ester functions in these compounds do not interfere with the observation of the Cotton effects of the keto group as the carbonyl absorption › carboxylic acid derivatives lies at much shorter wavelengths (p. 143).

to the other two. For most purposes the four octants in front of the third plane can be neglected (the observer is assumed to be looking along the O=C bond). According to the Octant Rule, substituents (including additional rings) lying behind the third plane contribute to the amplitude of the Cotton effect as follows: (*a*) substituents lying in the far upper left and far lower right octants make positive contributions, (*b*) substituents lying in the far lower left and far upper right octants make negative contributions, (*c*) substituents lying in any of the three planes make no contribution. Taking a very simple example, we should expect the 3-methylcyclohexanone having the absolute configuration and conformation shown in (18) to have a



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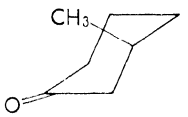

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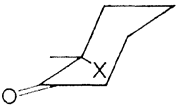

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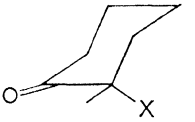


(21)

positive Cotton effect. In more complex cases it is often necessary to assess the relative importance of various contributions. This sometimes leads to uncertainties but in the great majority of cases the Octant Rule leads to correct predictions of the sign and approximate amplitude of the Cotton effect.[12,14] In fact the rule can be extended, with some complications, to cyclopentanones[13] and certain other ketones.

The Octant Rule is obviously of great value when no direct analogies are available.[2,3,14] We will consider a simple example of its application to a conformational problem. The 3-methylcyclohexanone mentioned above could also exist in the alternative conformation (19) which would be expected to have a negative Cotton effect. The observed positive Cotton effect therefore suggests that the molecule prefers the conformation (18). This approach has been used not only to distinguish between alternative chair conformations of monocyclic ketones and *cis*-decalones[2,3,14] but also to show that the terminal rings in certain polycyclic compounds assume a conformation other than the chair.[14]

One particular example of the Octant Rule deserves special mention. In axial α-chloro and α-bromoketones (but not fluoroketones) the octant contribution of the halogen generally far outweighs the contributions of the other substituents. Haloketones containing the structural arrangements (20) and (21) therefore have negative and positive Cotton effects respectively. This 'Axial Haloketone Rule' has proved very useful for assignments of configuration and conformation.[2] As

would be expected from the Octant Rule, the introduction of an equatorial halogen substituent does not have any profound effect on the amplitude of the Cotton effect of the parent ketone.

α, β–unsaturated ketones. The chromophore C=C–C=O is associated with weak absorption above 300 mμ and strong absorption at shorter wavelengths (cf. p. 139). In optically active molecules the former gives rise to a multiple Cotton effect curve, exemplified in Fig. 7 which shows the curve for the hormone testosterone (22, R = CH_3). Such curves

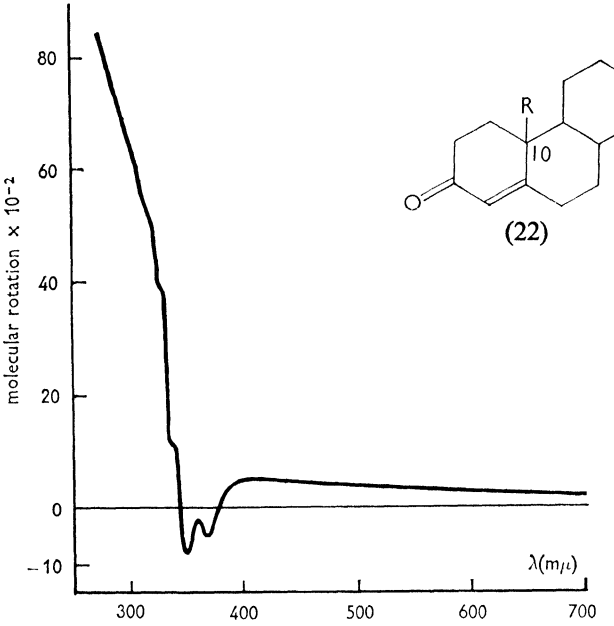


FIG. 7. Rotatory dispersion curve of testosterone (in dioxan; from Djerassi *et al.*, *J. Amer. Chem. Soc.*, 1956, **78**, 6377)

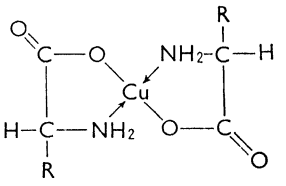
have proved useful in structural work; for example, a 19–nortestosterone whose configuration at $C_{(10)}$ was uncertain was shown to be (22, R = H) on the basis of the similarity of its rotatory dispersion curve to that of testosterone.

Recent improvements in technique have made it possible to observe the Cotton effects associated with the intense short-wavelength band[15]; the rising curve in Fig. 7 in fact heralds a positive Cotton effect at shorter wavelengths. These latter Cotton effects have very high amplitudes, a characteristic associated with chromophores which are inherently asymmetric (the C=C and C=O being in different planes;

cf. p. 218). In such cases the sign and amplitude of the Cotton effect depend on the direction and extent to which the chromophore is 'skewed' by the molecular environment.[15,16,35] Similar considerations hold for the Cotton effects of skewed diene chromophores and theoretical calculations based on skewed models have given a satisfactory account of the observed dispersion curves.[17] Like the Octant Rule, such considerations provide a powerful tool for the investigation of conformational detail.

It may be noted that there is no rigid dividing line between such inherently asymmetric chromophores and the asymmetrically perturbed symmetrical chromophore covered by the Octant Rule. Thus the α–haloketones discussed earlier may be considered as occupying an intermediate position and the same is true for certain β, γ–unsaturated ketones in which the orbitals of the double bond and the carbonyl group overlap.[16,35]

Absorbing functions other than the carbonyl group. Although ketones have received the most attention, Cotton effect curves have also been observed for other compounds which absorb in the visible or near-ultraviolet region and some of the generalisations obtained have proved useful in structural work.[2,3] For example the copper complexes (e.g. 23) of α–amino acids and α–hydroxy acids show Cotton effect curves in



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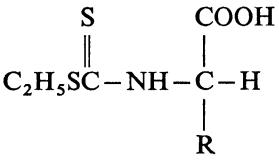

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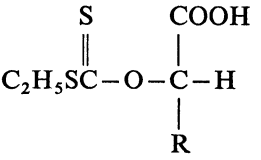


(25)

the visible region associated with the absorption of the metal ion; these played an important part in classical work relating the natural α–amino acids to each other and to α–amino and α–hydroxy acids from carbohydrates. More recently Djerassi has shown that the Cotton effect curves given by the dithiocarbamates (24) of α–amino acids and the

dithiocarbonates (25) of α–hydroxy acids can be used for similar correlations.

Compounds giving Cotton effect curves of structural interest include thioketones, carbonic acid derivatives containing a C=S group, nitrites, nitro and nitroso compounds, disulphides, azides, metal complexes, skewed dienes[17] (see above) and non-planar diphenyls.[18] The last two provide examples of inherently asymmetric chromophores. In many cases, for the purpose of structural correlations, the absorbing functions have been deliberately introduced into compounds which would otherwise give only plain dispersion curves. The compounds (24) and (25) exemplify suitable derivatives of amines and alcohols; carboxylic acids† may be converted into acylthioureas or nitrosoamides and olefines into osmates.

The following requirements should be kept in mind when an absorbing function is to be introduced for the purpose of configurational correlations based on Cotton effect curves. The absorption should lie in the experimentally accessible region of the spectrum and, unless the specific rotations are very high (as for inherently asymmetric chromophores), the absorption should be weak since measurements in the region of absorption will otherwise become impossible. Furthermore, the absorbing function should be close to the asymmetric centres whose configuration is being investigated and its orientation with respect to these centres should not be obscured by undue conformational mobility. One of the great advantages of Cotton effect curves as a stereochemical tool is that it is possible to explore various regions of the molecule in turn by introducing absorbing groups at various points!

Circular Dichroism

As mentioned earlier (p. 223) the value of Cotton effect curves lies in the fact that, in the region of wavelengths in which a particular chromophore absorbs, its partial rotation tends to overshadow the partial rotations of the other optically active absorption bands. Nevertheless the 'background rotation' due to the other bands often complicates the interpretation of the results, particularly from a quantitative point of view, and in complex cases even the sign of the Cotton effect associated with a particular absorption band may remain in doubt. The recent development of a commercial, photoelectric instrument (Jouan, Paris) for the measurement of circular dichroism (C.D., cf.

† The extended range of some recently developed spectropolarimeters enables the Cotton effects associated with the absorption of carboxylic acids, esters and amides (at ~200 mμ) to be observed directly.

p. 218) is therefore of great interest. As noted earlier, circular dichroism is the difference between the intensities of absorption of left and right circularly polarised light. The circular dichroism associated with an absorption band is confined to the region of absorption and complications due to overlap are therefore greatly reduced (see p. 219 and Fig. 4).

With the Jouan instrument the difference between the molar extinction coefficients for left and right circularly polarised light ($\Delta\varepsilon = \varepsilon_L - \varepsilon_R$) can be measured directly. The sign of $\Delta\varepsilon$ is the same as the sign of the Cotton effect rotatory dispersion curve of the compound;

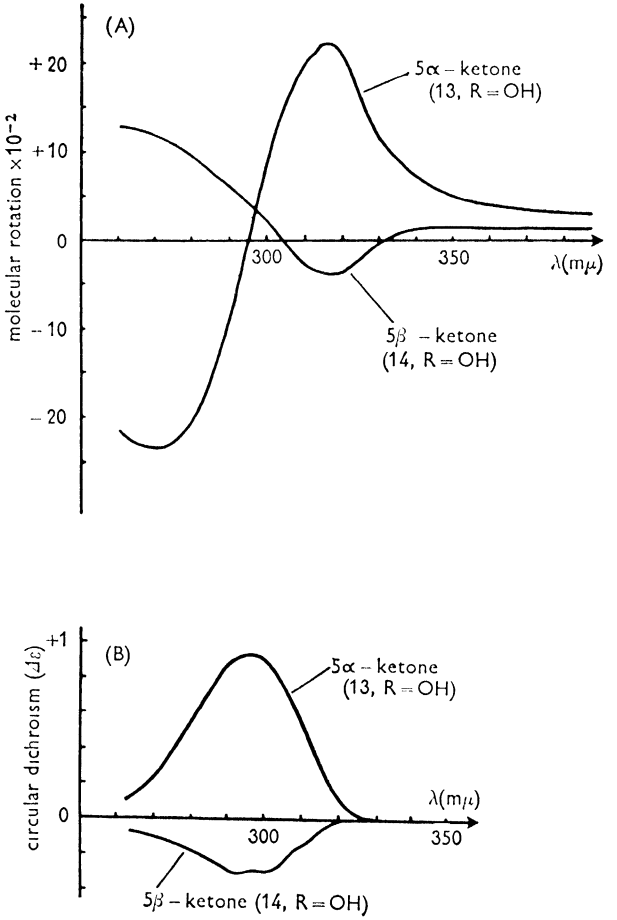


FIG. 8. Rotatory dispersion (A) and circular dichroism (B) of typical 5α- and 5β-steroid 3-ketones (13 and 14, R = OH) (in dioxan; from Legrand and Mathieu, *Bull. Soc. chim. France*, 1961, 1679)

this is illustrated in Fig. 8 for two steroid 3-ketones which differ in configuration at $C_{(5)}$ (cf. p. 226). Measurements of circular dichroism can therefore be used in the same way as rotatory dispersion curves in the investigation of structure, configuration and conformation. However, for the reasons given above, circular dichroism curves are easier to interpret and their detailed quantitative exploitation is likely to provide results of great importance. Refs. 7, 19 and 35 give accounts of some typical structural and theoretical applications.

Plain Curves

For many compounds it is impossible to observe Cotton effect rotatory dispersion curves or to measure circular dichroism, either because the optically active absorption bands all lie in the far-ultraviolet or because the intensity of the absorption frustrates measurements within the absorption band. In such cases it is often profitable to prepare derivatives with suitable absorption bands (p. 232). Alternatively structural arguments may be based on measurements outside regions of absorption, i.e. on plain rotatory dispersion curves or even—as in most classical work—on rotations at a single wavelength.

Outside regions of absorption the various partial rotations can be represented by Drude terms—$K_i/(\lambda^2-\lambda_i^2)$—and the observed rotation will be the sum of the Drude terms associated with the various absorption bands (cf. p. 219). The trend (up or down) of a plain curve at shorter wavelengths is in most cases determined by the sign of the Cotton effect of the absorption band of longest wavelength. In favourable cases the values of K_i and λ_i for this band can be obtained by mathematical analysis of the curve into Drude terms.[20] However, such mathematical analysis is mainly of historical interest; it is time-consuming, requires measurements of fairly high accuracy and can lead to spurious results (e.g. λ_i values which do not correspond to absorption bands).

Nevertheless, plain rotatory dispersion curves are often more informative than rotations at a single wavelength.[21] For example, (+)-α-phenoxypropionic acid (26) and the corresponding amide give the plain dispersion curves shown in Fig. 9. Although the rotations at the D-line (589 mμ) are of opposite sign, both curves show an upward trend at shorter wavelengths. This suggests that the Cotton effect of the asymmetric absorption band of longest wavelength is positive in both compounds. The configurational similarity is therefore apparent from the trend of the dispersion curves but not from the rotations at the D-line. The negative rotation of the amide at longer wavelengths

is probably due to a predominating contribution from an absorption band lying in the more distant ultraviolet.

Mathematical analysis of plain curves has given interesting information about the helical chain conformations of polymer molecules, e.g.

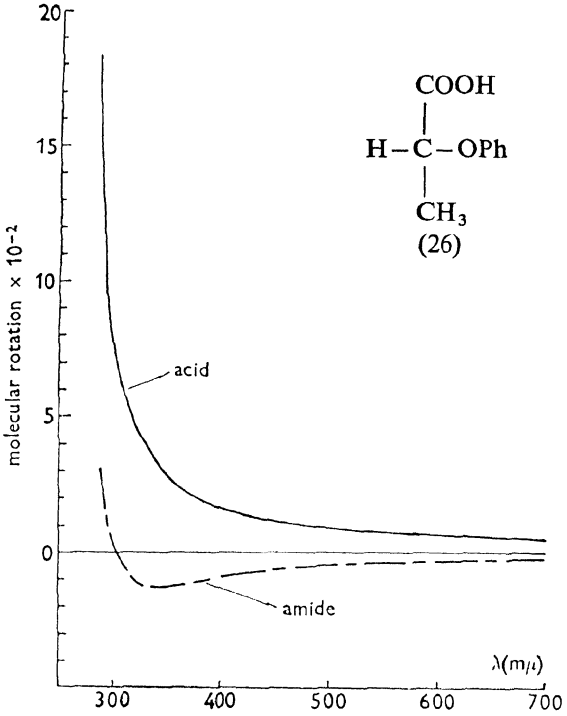


FIG. 9. Rotatory dispersion curves of (+)-α-phenoxypropionic acid (26) and its amide (in methanol; from Sjöberg[21])

proteins (see ref. 2, chapter 17, and refs. 22 and 35). Another method of approach to polymer problems depends on the observation that symmetrical dye molecules bound to asymmetric polymers give Cotton effect curves.[23] In addition it has recently proved possible to observe extrema below 230 mμ associated with the Cotton effects of the amide absorptions of proteins.[24]

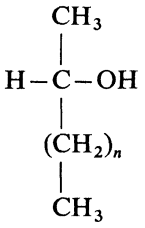
ROTATIONS AT A SINGLE WAVELENGTH

Until 1950 nearly all the work on optical rotation was confined to measurements at a single wavelength, usually that of the sodium D-line (589 mμ). The wavelength of the D-line is remote from the

absorption wavelengths of most organic compounds, and for this reason the various partial rotations which contribute to the observed rotations are often of comparable magnitude (p. 220). Consequently D-line rotations are less satisfactory as a structural tool than measurements in or near regions of absorption (p. 223). However, despite their limitations, it seems likely that D-line rotations will continue to be of value in structural work, especially in cases where the D-line rotations of suitable reference compounds are already in the literature. The possibility of applying the methods discussed below to rotation measurements at shorter wavelengths should also be borne in mind, particularly for compounds which do not give Cotton effect curves. At 300 mμ, for example, both the observed rotation and the relative magnitude of the partial rotation associated with the absorption band of longest wavelength are generally much greater than at the D-line.

The methods of approach which have been used in extracting structural information from D-line rotations can roughly be divided into two classes: (*a*) arguments based directly on the sign and magnitude of the rotation, and (*b*) arguments based on comparison of the shifts in rotation which occur when two compounds are structurally modified in a particular way. The latter course is generally more reliable. Examples of both approaches will now be discussed.

Sign and magnitude of the rotation. It is usually found that two compounds which differ only at some distance from their asymmetric centres give molecular rotations of the same sign and approximate magnitude provided their absolute configuration is the same (Tschugaeff's Distance Rule). This generalisation can be used for correlating the configurations of members of a homologous series; thus secondary



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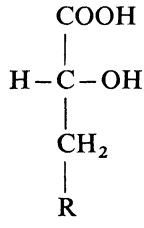


(28)

alcohols of the formula and configuration (27, $n>0$) are all dextrorotatory at the D-line (other examples are given in refs. 5 and 6). Complications are most likely to arise when the structural differences involve a substituent which absorbs in the near-ultraviolet. For example,

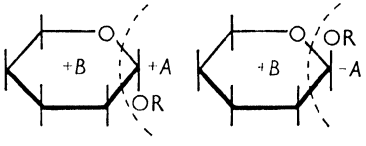
although the hydroxy acids (28, R = H, methyl, ethyl, propyl, etc.) are all laevorotatory, the acid (28, R = phenyl) is dextrorotatory. Rotatory dispersion measurements will often clarify such situations (cf. p. 234).

A more versatile method of applying D-line rotations is provided by an empirical theory, recently derived by Brewster,[25] which can be used to predict the sign and approximate magnitude of the rotations of a considerable number of compounds from a comparatively small number of empirical parameters. This treatment relates optical rotation to the spatial distribution of polarisability and a valuable feature is that it recognises the importance of conformation in determining optical rotation. Details cannot be discussed here. The theory, which incorporates many earlier generalisations, can be applied to both acyclic and cyclic compounds, including polysubstituted systems such as the carbohydrates. Despite its limitations,[26] it has proved useful in determining configurations; the conclusions should however be regarded with caution, particularly when groups which absorb in the near-ultraviolet are present.

Another method of applying optical rotation measurements is based on the 'Rule of Optical Superposition' (van't Hoff, 1894). This states that the rotation of a molecule which contains several asymmetric centres is the algebraic sum of constant rotatory contributions associated with the various centres,† the contribution of each centre being positive or negative according to its configuration. Modern theories of optical rotation show that the concept of the 'contribution of an asymmetric centre' is fundamentally unsound except in certain special circumstances[26] and arguments based on the Rule should be used with great caution unless the asymmetric centres are far apart.

The use and limitations of the Rule of Superposition can be illustrated by reference to Hudson's work on sugars and glycosides.[5,27] Hudson analysed the molecular rotations ($[\phi]_\alpha$ and $[\phi]_\beta$) of the ano-

α–anomer (29)



β–anomer (30)

meric forms (29 and 30) of an aldose or aldoside in terms of two contributions (*A* and *B*) associated with the anomeric centre and the remainder of the molecule respectively. If the anomers are derived from

† The term 'contribution' used in this sense must not be confused with the partial rotation associated with an absorption band (p. 218).

a D-sugar, then $[\phi]_\alpha = B+A$ and $[\phi]_\beta = B-A$. Hence $[\phi]_\alpha-[\phi]_\beta = 2A$ and $[\phi]_\alpha+[\phi]_\beta = 2B$. According to the Rule of Superposition, the magnitude of A should be independent of changes in configuration and minor changes in structure in the sugar residue (Hudson's First Rule) and the magnitude of B should be independent of the nature of R (Hudson's Second Rule).

Hudson found that the A values calculated from the two methyl D-glucopyranosides ($A_{OMe} = 188$) and from the two methyl D-galactopyranosides ($A_{OMe} = 190$) were very similar, but that the A value calculated from the two methyl D-mannopyranosides was appreciably different ($A_{OMe} = 145$); this difference is related to the fact that in mannose the configuration at $C_{(2)}$ is different from that in glucose and galactose.† Later work revealed further imperfections in Hudson's Rules; for example, the B value for a particular sugar residue calculated from the molecular rotations of the anomeric phenyl glycosides (R = Ph) was different from that obtained from the anomers of the sugar or of the methyl glycoside (R = H or Me). Despite such limitations calculations based on Hudson's Rules can often be used to determine anomeric configurations in glycosides and disaccharides, since differences in rotation between anomers are usually larger than the departures from the Rules.

The molecular rotation of a compound which consists of two asymmetric portions can sometimes be calculated very roughly by adding the molecular rotations of compounds resembling these portions. For example, Klyne[5] has found that the molecular rotations of steroid glycosides can be calculated by adding the molecular rotation of the parent steroid alcohol to that of the corresponding α- or β-methyl glycoside. The results are only approximate but can be used to determine the anomeric configuration of such glycosides.

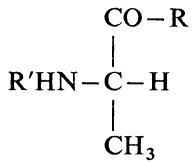
The Rule of Shift (Displacement Rule). This Rule, which was formulated by Freudenberg around 1930, can be stated as follows: *Like structural changes in like molecular surroundings cause similar shifts in molecular rotation.*‡ The sign and (in favourable cases) the magnitude of such shifts can therefore be correlated with the environment of the

† As the Rule of Superposition is fundamentally unsound, it seems preferable to regard the similarity of the A_{OMe} values of the glucosides and galactosides as an example of the Rule of Shift (above). The different value shown by compounds with the mannose configuration is then seen as a logical result of the different steric environment of $C_{(1)}$ in these compounds.

‡ A more precise but longer version of the Rule is: The shifts in molecular rotation which occur when two related molecules are altered in the same way are of the same sign (and often of similar magnitude) if the stereochemical environment of the change is similar in both molecules.

site of the change in much the same way as the sign and amplitude of a Cotton effect curve can be correlated with the environment of the absorbing function (although the Rule of Shift is rather less reliable). In most of the early applications of the Rule, the 'structural change' consisted of the modification of a functional group (e.g. the conversion of an acid to an amide). However the rule can often be extended to alterations involving the introduction of a new asymmetric centre or the inversion of an existing one (e.g. see footnote on p. 238).

Freudenberg himself applied the Rule of Shift to the important problem of correlating the configuration of derivatives (31) of the natural amino acid (+)-alanine with derivatives (32) of L(+)-lactic acid. When the group R was varied (e.g. from NHC_6H_5 to OPh, NMe_2 or OEt) the shifts were usually in the same direction in the two series, suggesting that (+)-alanine also has the L-configuration, as shown in (31). This was later confirmed by other evidence (see also p. 231).



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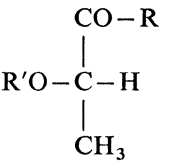

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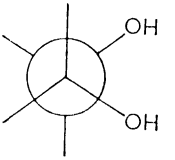


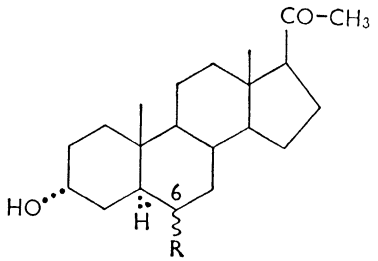
(34)

A typical variant of the Rule of Shift is Hudson's Amide Rule which, like certain other rules,[5] can be used to correlate the configurations of hydroxy acids of the type (33). The Amide Rule states that the difference obtained by subtracting the molecular rotation of a hydroxy acid from that of its amide will be positive if the acid has the D-configuration on $C_{(2)}$. Further, in the case of amides of aldonic acids [33, R = $-(CHOH)_nCH_2OH$], the magnitude of the molecular rotation shift is largely determined by the configuration at $C_{(3)}$; the more distant asymmetric centres have a much smaller effect. Such rules have proved very useful in the elucidation of the configurations of unusual sugars, such as those from antibiotics.

A striking illustration of the application of the Rule of Shift to conformational studies is provided by the work of Reeves[28] who was able to assign preferred conformations to pyranose sugar derivatives on the basis of the observation that glycol systems having the orientation (34) show an increase in rotation when they are converted into cuprammonium complexes; the mirror-image arrangement shows a decrease.

The method of molecular rotation differences. This method,[5,29] which is of considerable value in steroid and terpenoid chemistry, is essentially based on the Rule of Shift. It was devised by Barton who pointed out that, to a first approximation, the molecular rotation shift which occurs when a group is introduced into a steroid depends only on the position and orientation of the group and on the configuration of the steroid nucleus. The shifts are only slightly affected by the nature of any other substituent present, *provided that* this is sufficiently far from the new substituent to avoid 'vicinal action' between them. As long as this condition is fulfilled, the molecular rotation of a steroid derivative can be calculated by adding increments characteristic of the various functional groups to the molecular rotation of the appropriate nucleus. Tables[5,29] have been constructed giving the molecular rotation increments (Δ values) which result when double bonds or hydroxyl, acetoxyl or keto groups are introduced at various positions. Although these tables must be used with care, they have often proved useful for deciding between structural and configurational possibilities.

The method of molecular rotation differences can be illustrated by its application to Lieberman's Compound A31, one of a series of ketosteroids isolated from human urine. This compound had been shown to have the structure (35, R = OH) but, as indicated by the wavy line, the stereochemistry at $C_{(6)}$ remained uncertain. The molecular rotations of the compound and of the parent (35, R = H) are $+351°$ and $+309°$ respectively; hence the shift Δ for the introduction of the hydroxyl group is $+42°$. According to the tables, the average shifts for the introduction of 6α– and 6β–hydroxyl groups are $+55°$ and $-50°$ respectively. Barton and Klyne therefore suggested that the 6–hydroxyl group in Compound A31 is α–orientated; this was later confirmed by synthesis.

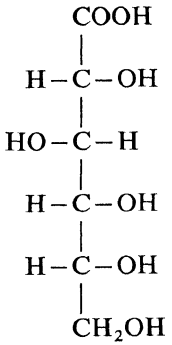


(35)

The method of molecular rotation differences has been extended to

di- and tri-terpenoids and to other compounds containing polycyclic systems. Klyne[30] has noted that the sign and approximate magnitude of the molecular rotation shift resulting from an alteration in a terminal ring is mainly determined by the nature of the terminal ring and the configuration of the terminal ring junction. Important assignments of configuration were made on this basis, using reasoning closely similar to that employed some years later with Cotton effect curves (p. 227). The latter generally provide a more powerful tool for the determination of absolute configurations but the present method remains important for determining the configurations of hydroxyl substituents in alicyclic compounds; a number of empirical rules for predicting rotation shifts caused by the introduction or modification of hydroxyl groups may be found in refs. 30 and 31.

The Lactone Rule. Hudson found that lactones formed from aldonic acids have a positive rotation if the lactone-forming hydroxyl is D-orientated. This generalisation is known as Hudson's Lactone Rule. For example, both the 1, 4 and 1, 5 lactones of D-gluconic acid (36) have positive rotations; by contrast D–galactono–1, 4–lactone (37) has a negative rotation. Klyne[32] has devised a more general form of the



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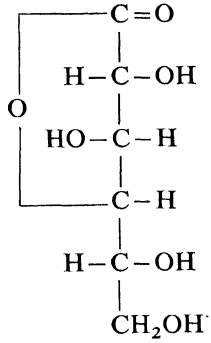

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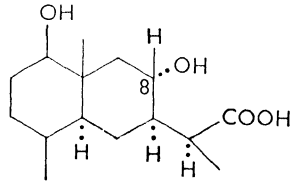

(38)

Lactone Rule which considers the sign of the difference $[\phi]_{\text{lactone}} - [\phi]_{\text{hydroxy acid}}$.† In this form the Rule can be applied to polycyclic compounds; for example, hexahydro–ψ–santonic acid (38) incurs a negative shift in molecular rotation on lactonisation indicating that the hydroxyl at $C_{(8)}$ has the configuration shown.

† Even this version of the Lactone Rule is not strictly analogous to the Rules of Shift which have been discussed above.

Summary

The rotatory behaviour of optically active compounds provides a powerful structural tool, particularly for the investigation of stereochemical problems. The principal disadvantage of the method is that the relationship between optical rotation and structure is often rather complex. For this reason empirical arguments, based on close analogy, are usually employed, though predictions from fundamental theory have recently shown promise, particularly for inherently asymmetric chromophores.

Several different types of approach have been developed; the choice between the various methods depends on the problem and the analogies available. Rotatory dispersion data generally provide a more powerful method than measurements at a single wavelength, particularly when Cotton effect curves are obtained. Measurements of circular dichroism can be even more informative.

In many cases the conclusions are only tentative, encouraging and guiding further chemical work rather than replacing it. Most of the generalisations and 'Rules' are based on a limited number of compounds and must therefore be used with care. In a classic paper[33] Freudenberg wrote: 'A critical mind is as important to success as the Rules themselves.' This is a wholesome maxim.

The writer is grateful to Professor E. L. Hirst and Professor W. Klyne for advice and encouragement.

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Chapter 7

C. T. Greenwood

THE DETERMINATION OF MOLECULAR WEIGHT

Introduction

The most fundamental physical characteristic of any organic compound is its molecular weight. For the organic chemist, the commonest application of molecular weight measurements is in the determination of molecular formulae. Molecular weight determinations are also widely used in industry, e.g. for characterising the fractions obtained during the distillation of petroleum. It is perhaps hardly necessary to stress that the complete characterisation of a natural or synthetic polymer must entail a knowledge of its average molecular weight; the biological activity and the industrial utility of polymers are both very dependent on this quantity.

Methods of measuring molecular weight are therefore very important. A large number of techniques are available; however, these are not universally applicable and the molecular size under investigation primarily determines which of them are suitable. For this reason, this chapter discusses in turn the methods appropriate to particular ranges of molecular weight. Inevitably, this involves some overlap in experimental techniques between different sections, but it is hoped that the advantages and limitations of the different techniques will be made more apparent.

The great majority of molecular weight methods involve measurements on solutions, and the theoretical basis of these methods is usually derived on the assumption that the solutions are ideal. All solutions behave ideally at infinite dilution, but at finite concentrations this may not hold. Deviations from ideal behaviour (e.g. Raoult's law) can be very large for polymers, but, even for compounds of low molecular weight, they are more common than might first be thought. For example, polar compounds, e.g. *o*-dinitrobenzene, may show departures

of 5–10% at quite low concentrations (<0·1 molal). In practice, therefore, molecular weight determinations on polymers are made at various concentrations and the results extrapolated to infinite dilution, and the same procedure should be adopted for compounds of low molecular weight if accuracy is important.

The degree of accuracy required for the determination of molecular formulae varies greatly. Modest accuracy is sufficient to decide between multiples such as C_7H_6O and $C_{14}H_{12}O_2$ or even between $C_5H_{10}O_5$ and $C_6H_{12}O_6$. Far greater accuracy is often required when the differences in composition between alternative formulae fall within the limits of analytical precision. For example, the formulae $C_{28}H_{50}$ (C, 87·0; H, 13·0%) and $C_{29}H_{52}$ (C, 86·9; H, 13·1%) differ in molecular weight by less than 4%. Such problems are often encountered in natural product chemistry with compounds of molecular weight above 300, and then mass spectrometry (p. 255 and Chapter 9) may be the only molecular weight technique which provides sufficient accuracy.

Although a method may be suitable theoretically, it may be inapplicable in practice because the sample has unsatisfactory solubility, volatility or stability characteristics, or because it undergoes molecular association or dissociation. Solution methods are obviously inapplicable if an adequate solvent cannot be found. Solubility difficulties are generally less at higher temperatures (e.g. boiling-point methods suffer less than freezing-point methods) but decomposition of the sample may then occur. Methods which involve vaporisation (e.g. vapour density determinations and mass spectrometry) are also limited by stability considerations. Association (particularly by hydrogen bonding) and dissociation are not uncommon,† for example, carboxylic acids tend to dimerise in hydrocarbon solvents and to ionise in aqueous solution. Solute–solute association by hydrogen bonding can often be minimised by using solvents which themselves participate in hydrogen bonding (e.g. dioxan and other ethers, cf. p. 60). Solubility, stability and association difficulties may often be overcome by converting the sample into a derivative (e.g. esterification of an acid).

It is not possible to make general recommendations as to which are the best molecular weight methods in a particular range; the accuracy and reliability of the methods depend not only on their principle but —to a great extent—on the degree of sophistication of the particular experimental arrangement and on the nature of the compound being examined. It is to be noted that, for molecular weights below 1000, a

† Molecular weight methods are valuable for investigating such phenomena, but this aspect lies outside the scope of this chapter (see ref. 1 for details).

considerable number of methods are often equally applicable and the final choice may then depend on the available equipment.

Molecular weight averages. An important complication arises when the sample contains molecules of different sizes, i.e. there is a distribution of molecular weight, as is the case for nearly all polymers. An *average* value for the molecular weight is then obtained. There are different types of average, and the actual average obtained depends on the molecular weight method used. It is essential to appreciate this fact. For example, a molecular weight of 500,000 determined for a polymer by osmotic pressure measurements is not necessarily incompatible with a value of 1,000,000 obtained on the same sample by the light-scattering method.

There are a large number of molecular weight averages, but the most important are as follows:

1. *Number-average molecular weight* ($\overline{M}_n$). This is the average obtained from all methods involving colligative properties: these effectively count the number of molecules present.

Taking osmotic pressure measurements as an example, the osmotic pressure (Π) is related to the number of moles of solute (n) by

$$\Pi = nRT/V$$

where V is the volume of solvent. By definition, therefore, the observed osmotic pressure (Π_{total}) for a sample with a molecular weight distribution is related to the number-average molecular weight ($\overline{M}_n$) by

$$\Pi_{total} = (w/\overline{M}_n)\,(RT/V) \tag{1}$$

where w is the total weight of solute. Now assume that the sample consists of i species, and that for the ith species there are n_i moles of molecular weight M_i. For this species it follows that

$$\Pi_i = n_i\,(RT/V).$$

The total osmotic pressure (Π_{total}) will be then the sum of the osmotic pressures of all the molecular species, i.e.

$$\Pi_{total} = \sum_{i=1}^{\infty} \Pi_i = (RT/V) \sum_{i=1}^{\infty} n_i. \tag{2}$$

The total weight (w) of the sample will be the sum of the weights of each molecular species,

$$w = \sum_{i=1}^{\infty} w_i = \sum_{i=1}^{\infty} n_i M_i,$$

and hence equation (1) can be rewritten as

$$\Pi_{total} = (\sum_{i=1}^{\infty} n_i M_i / \overline{M}_n)\,(RT/V). \tag{3}$$

A comparison of (2) and (3) then gives an expression for the number-average molecular weight,

$$\overline{M}_n = \sum_{i=1}^{\infty} n_i M_i \,/ \sum_{i=1}^{\infty} n_i.$$

The number-average molecular weight is simply the arithmetic mean, and it is affected to an equal extent by both large and small molecules (cf. below for the weight-average molecular weight).

2. *Weight-average molecular weight* ($\overline{M}_w$). This is the average obtained from methods which depend on the *weight* of the molecules present and not their number. Taking light scattering as an example, the turbidity (τ) is related to the molecular weight (M) by the relation, $\tau = H'wM$, where H' is a constant and w is the sample weight in a given volume of solution. By reasoning similar to that used above, it is easily shown that the weight-average molecular weight is given by

$$\overline{M}_w = (\sum_{i=1}^{\infty} w_i M_i)/w,$$

or

$$\overline{M}_w = (\sum_{i=1}^{\infty} n_i M_i^2)/(\sum_{i=1}^{\infty} n_i M_i).$$

$\overline{M}_w$ is thus the sum of the products of the molecular weight of each class of molecule and its *weight-fraction* (w_i/w). The value of $\overline{M}_w$ is influenced considerably by high molecular weight material and in general $\overline{M}_w$ is greater than $\overline{M}_n$. (For a sample of uniform molecular weight, of course, $\overline{M}_w = \overline{M}_n$.) The ratio of $\overline{M}_w/\overline{M}_n$ provides a useful measure of the spread of the molecular weight distribution in any sample.

The influence of high and low molecular weights on $\overline{M}_n$ and $\overline{M}_w$ can be illustrated by a simple example. If equal weights of molecules with $M = 500{,}000$ and $M = 50{,}000$ are mixed, $\overline{M}_w = 275{,}000$ and $\overline{M}_n = 90{,}900$; if equal numbers of each species are mixed, $\overline{M}_w = 459{,}100$ and $\overline{M}_n = 275{,}000$.

3. *Other molecular weight averages.* Only two of these will be mentioned briefly:

(i) In viscosity measurements, if the viscosity is directly proportional to the molecular weight, a weight-average value is obtained, but the

relation, $[\eta] = KM^{\alpha}$, results in a *viscosity-average molecular weight* ($\overline{M}_v$), where

$$\overline{M}_v = [(\sum_{i=1}^{\infty} n_i M_i^{\alpha+1})/(\sum_{i=1}^{\infty} n_i M_i)]^{1/\alpha}$$

(ii) *The Z-average molecular weight* ($\overline{M}_z$) follows from the relation,

$$\overline{M}_z = (\sum_{i=1}^{\infty} n_i M_i^3)/(\sum_{i=1}^{\infty} n_i M_i^2).$$

This average, which emphasises higher molecular weights even more than $\overline{M}_w$, may be evaluated from sedimentation data.

Molecular Weights of up to 2,000

Typical compounds and the available methods for molecular weight determinations are as shown:

Typical compounds: Non-polymeric compounds; oligosaccharides, peptides; small synthetic polymers.

Methods: Vapour density; depression of vapour pressure (including isothermal distillation and thermoelectric methods); depression of freezing- or melting-point; elevation of boiling-point; X-ray diffraction; mass spectrometry; chemical or spectroscopic functional-group assay; pseudo-equilibrium method of ultracentrifugation.

Vapour density measurements. These methods are applicable only to compounds which volatilise without decomposition or polymerisation. The classical Dumas, Hofmann and Victor-Meyer techniques have all been modified so that sample weights of only a few mg are required. For example, in one technique, the vapour displaces its own volume of mercury. Another approach which is well suited to micro-determinations is to measure the pressure exerted when a known mass of substance is volatilised in a known volume at a given temperature.

Such methods are valuable for very volatile substances, since for these many of the other methods are inapplicable, but otherwise they have not been widely used. Accuracies as high as 2% can be obtained. Full details may be found elsewhere.[2,3]

Depression of vapour pressure. Molecular weights can be calculated from vapour pressure measurements using Raoult's law, i.e. $(p_0 - p)/p_0 = x$, where p and p_0 are the vapour pressures of solution and solvent, and x is the mole-fraction of solute. The lowering of vapour pressure, $p_0 - p$, is best measured directly using a differential manometer. Methods have been described which claim 2% accuracy for samples of only a few mg[2,3] but they are little used because of their

experimental complexity. However, indirect methods based on Raoult's law are of considerable importance.

When two solutions of different molar concentrations of solute in the same solvent are placed together in a closed system at constant temperature, there will be distillation of solvent from the low to the high concentration. This *isothermal distillation* forms the basis of several methods which are particularly suitable for micro-work; essentially, the molar concentration of a solution containing the unknown is determined with reference to one or more standard solutions of known molarity. In *isopiestic methods*, distillation is allowed to proceed until vapour equilibrium is achieved (isopiestic = of equal pressure), that is, until the molar concentrations of the two solutions are equal. A *dynamic method* in which the molecular weight is calculated directly from the rate of distillation is described on p. 259.

In a very simple method,[2,3,4] which is based on a procedure originally devised by Barger, the standard and unknown solutions are contained in two capillaries; these are placed inside a larger capillary which is then sealed (Fig. 1). The increase or decrease in the volumes of the

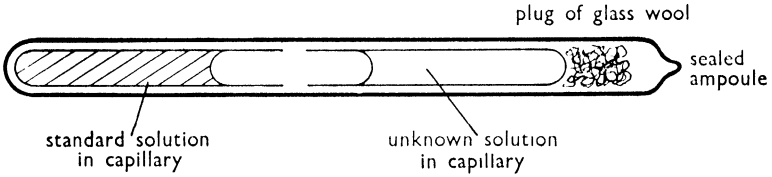


FIG. 1. Modified Barger apparatus for isothermal distillation

solutions is observed with a travelling microscope. If a series of standard solutions is used, it is possible, by graphical interpolation to zero change of volume, to find the molarity of standard solution which is isopiestic with the unknown.

An alternative approach[2,3] is to allow solutions containing known weights of the unknown and standard to equilibrate, and to determine the final volumes of the solutions either directly or by weighing. In one method, for example, the solutions are placed in platinum crucibles in a desiccator.

These methods require very little material and only simple apparatus. A wide variety of solvents can be used, e.g. ether, acetone, ethanol, methyl acetate, ethyl bromide and chloroform. Azobenzene is a useful standard. Accuracies of a few per cent have been obtained but the procedures are less satisfactory for molecular weights above 750. A minor

disadvantage is that the distillation is slow; several days may be required.

Another method which depends on differences in vapour pressure is to measure the elevation of temperature caused by the condensation of solvent vapour.[3] This forms the basis of the *thermoelectric methods.* These are empirical and have to be calibrated. The temperature changes are conveniently measured using thermocouples or thermistors.† For example, if a pair of matched thermistors—one in the solvent and the other in the solution—are incorporated in a Wheatstone-bridge circuit, an accuracy of 1% can be achieved with relatively simple apparatus. The method is particularly versatile as both organic and aqueous solutions can be used. Measurements can be made rapidly on small quantities, and the only difficulty is that accurate thermostatting is required. This would appear to be the most promising of the vapour pressure methods and apparatus is available commercially.

Depression of freezing- or melting-point.‡ Thermodynamically it can be shown that for an ideal solution, the depression of freezing-point (ΔT_f) is related to the mole-fraction of solute (x) by the relation,

$$\Delta T_f = T_0 - T = -\frac{RTT_0}{\Delta H} \ln (1-x) \qquad (4)$$

where T_0 and T are the freezing-points of the solvent and solution, and ΔH is the molar heat of fusion of the solvent.

For dilute solutions where ΔT_f and x are small, it follows that $\Delta T_f \propto x$. In cryoscopic measurements it is conventional to express concentrations in molalities, and the equation used in practice is $\Delta T_f = K_f\, w/M$, where K_f is the molal freezing-point depression constant, and w is the weight of solute of molecular weight M in 1000 g of solvent. The value of K_f can be calculated on the basis of equation (4), but it is more satisfactory to determine it experimentally using a standard substance. Approximate values of K_f for some useful solvents are shown below:

Solvent	*m.p.* (°C)	K_f (°C)	*Solvent*	*m.p.* (°C)	K_f (°C)
benzene	6	4·9	water	0	1·9
dioxan	12	4·6	acetic acid	17	3·8
nitrobenzene	6	6·8	naphthalene	80	7·0

Under optimum conditions, the cryoscopic method is more accurate than the boiling-point elevation method (see p. 252) for the same solvent,

† A thermistor is a device composed of resistance material with a high negative temperature coefficient of resistance.

‡ The freezing- and melting-points of a solution are identical.

as the molal constant for freezing-point depression is greater than the corresponding value for boiling-point elevation (compare above table with that on p. 253).

For the range of molecular weight under discussion, the apparatus of Beckmann (Fig. 2) is suitable. The freezing-point of the solvent is

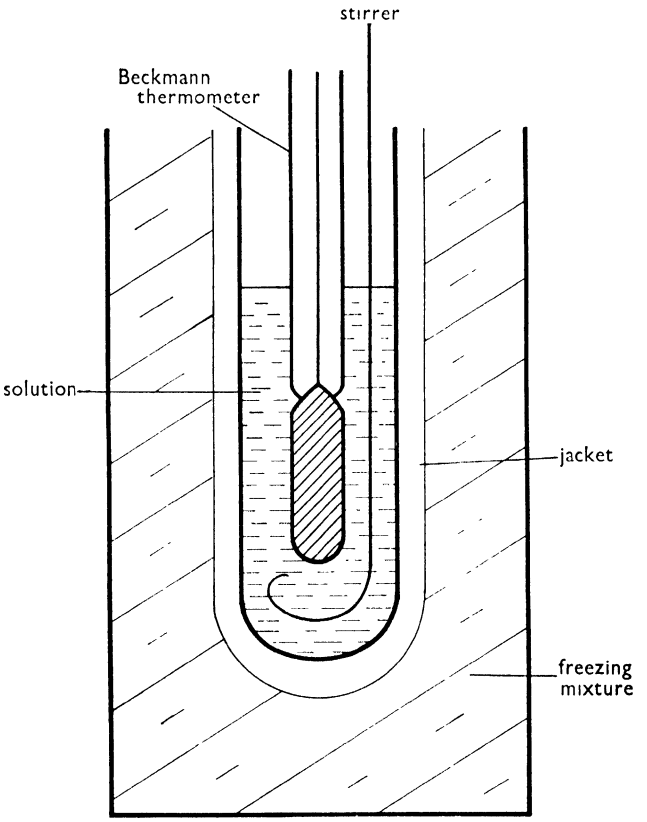


FIG. 2. Schematic diagram of a Beckmann-type freezing-point apparatus

measured, a known weight of sample is added and the freezing-point of the solution then determined. The addition of sample is then repeated several times. Deviations from ideal behaviour are very common, and in accurate work results should always be obtained at several concentrations, so that the resultant molecular weight values can be extrapolated to infinite dilution. The method is usually calibrated with a standard substance. With efficient stirring, an accuracy of about 2% is possible, and the method can be adapted to micro-quantities by

reducing the size of thermometer and apparatus. Details of the various types of apparatus may be found in refs. 2, 3 and 7.

The freezing-point depression method has the advantage that it can often deal with volatile, unstable, or very reactive substances. It is widely used, as the apparatus is simple and a large number of different types of compounds will dissolve sufficiently in the common cryoscopic solvents. However, the solute must not form solid solutions, or interfere with solvent-crystallisation, or associate or form molecular compounds with the solvent. The absence of these effects can best be checked by the agreement of results obtained in two different solvents. The sample must be free from contaminating solvents, especially water, and the solvent must also be carefully purified.

It should be noted that solvents with large K_f-values are not necessarily capable of yielding the most accurate results. A large K_f-value means that the heat of fusion is low and hence the stability of the measurements is poor. This may cause large random errors.

However, notwithstanding this fact, the most successful micro-method is that in which solvents with melting-points above room temperature, and having a freezing-point depression of some 5–20°C for a 10% solution, are used. Melting-points can then be determined in a capillary tube in the usual manner. Rast introduced the use of camphor as a solvent; the approximate molal K_f-values for this, and other similar solvents, are shown below:

Solvent	*m.p.* (°C)	K_f (°C)
camphor	176–180	37–40
camphene	49	31
bornylamine	164	41
cyclohexanol	25	43
cyclopentadecanone	66	21
dicyclopentadiene	32	46

Again, K_f should be determined empirically—particularly for camphor where the value depends on the source of the material. An accuracy of 2% can be achieved by this method in favourable cases if a very careful experimental technique is followed to ensure uniform solution (see ref. 2). The main disadvantage of camphor is that the high melting-point may cause decomposition of the solute. The absence of non-ideal behaviour must also be shown.

Elevation of boiling-point. A relation analogous to equation 4 (p. 250) holds for the elevation of boiling-point (ΔT_b) for an ideal solution, and when the solution is dilute and concentrations are expressed in molalities, then $\Delta T_b = K_b w/M$, where K_b is the molal boiling-point elevation constant, and w is the weight of solute of molecular weight M in 1000 g

of solvent. Approximate values of K_b for some useful solvents are as shown:

Solvent	*b.p.* (°C)	K_b (°C)	*Solvent*	*b.p.* (°C)	K_b (°C)
ethyl ether	35	2·2	ethanol	78	1·2
acetone	56	1·7	benzene	80	2·6
carbon tetrachloride	77	5·0	water	100	0·52

Experimentally, it is essential to avoid superheating, i.e. to measure

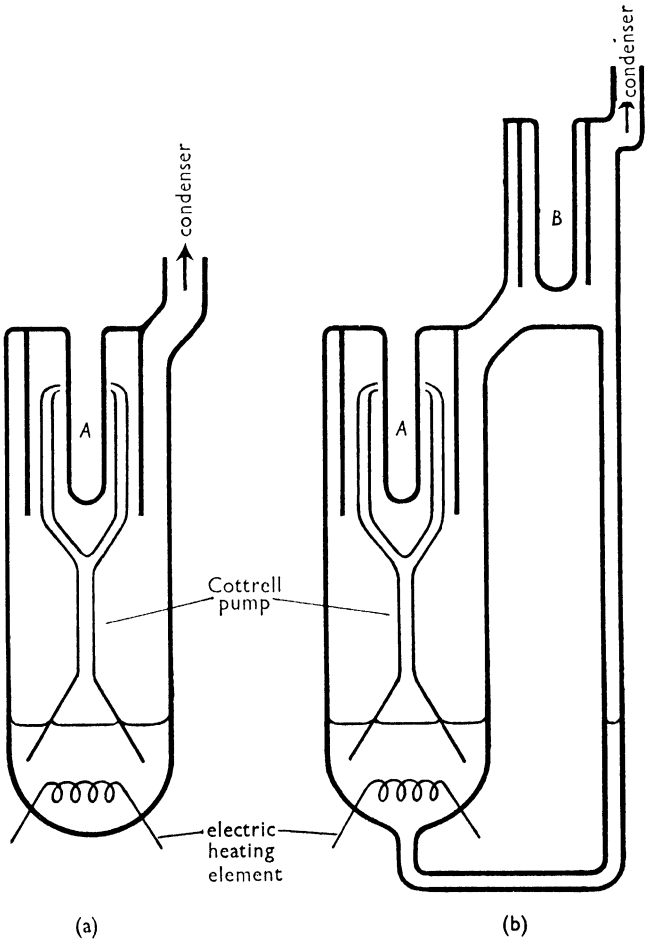


FIG. 3. Schematic diagram of (*a*) a simple ebulliometer and (*b*) a differential ebulliometer. *A* represents the well for the measuring device (e.g. thermometer) for measuring the boiling-point of the solution and *B* the corresponding feature for measuring the boiling-point of the solvent.

correct boiling temperatures.[2,3,7] This is achieved best by the use of a Cottrell-pump.† The temperature can be measured by Beckmann thermometers, but to avoid thermal lags, it is probably more satisfactory to use a differential vapour-pressure thermometer,[2, 3] thermocouples or thermistors.[3]

It is usual to measure temperature differences rather than the actual temperatures, and to calibrate the method with a standard substance, e.g. benzil, naphthalene, anthracene or hexamethyl benzene. The technique employs either two simple ebulliometers (see Fig. 3a) or a differential ebulliometer (see Fig. 3b). When twin ebulliometers are used, one is operated continuously with pure solvent, the other with solution (this compensates for changes in atmospheric pressure). The differential apparatus is probably more accurate as the temperature difference between the boiling solution and its own vapour is measured directly. In an apparatus of this type, corrections for the 'dead-space' can be eliminated by alternate additions of standard and unknown.

Many of the experimental arrangements can be modified for microwork.[2,3] For molecular weights of up to 1,000, an accuracy of 2% has been claimed using samples of 3–15 mg. The same accuracy for molecular weights of up to 2,000 has been achieved using 50–200 mg samples.

The boiling-point method is generally applicable to a wide range of materials. Solubility difficulties are less frequent than in the freezing-point method but the sample must not decompose or polymerise in the boiling solution. It must also have a low vapour pressure (i.e. the boiling-point must be at least 100° greater than that of the solvent) and be free from volatile contaminants. In accurate work, ebulliometric measurements should be carried out at several concentrations to establish whether the behaviour is ideal, and in more than one solvent to show that association effects are absent.

X-ray diffraction. The molecular weight of a *crystalline solid* can be determined directly by X-ray diffraction. It is shown in Chapter 8, p. 291 that the volume (V) of the unit cell of a crystal can be determined by diffraction measurements and that this is related to the molecular weight (M) by the equation, $ZM = \rho VN$, where ρ is the density of the solid, N is Avogadro's number and Z is the number of molecules in the unit cell.

The accuracy of molecular weight determinations by this method is potentially high as cell dimensions can be determined to within 0·1%.

† This is simply a funnel inverted in the boiling liquid which forces a stream of droplets of liquid interspersed with vapour on to the measuring device. The liquid drops will be necessarily in equilibrium with the vapour, and so the true boiling-point will be registered.

However, inaccuracies in the determination of the density of the solid may often reduce the overall accuracy of the method to about 2%. The main limitation is in the finding of Z (see Chapter 8). If the approximate molecular weight is known, Z can be determined, but for a complete unknown it cannot be found readily. Thus the method cannot distinguish, in general, between a monomer and an addition dimer. However, when the approximate molecular weight of a compound is known, the X-ray diffraction method is often very valuable for molecular weights of up to 2,000. It is applicable where many others fail, e.g. for substances which are insoluble in normal solvents, or have an insufficiently high vapour pressure for mass spectrometry. The technique is relatively simple; only a Weissenberg photograph and an oscillation photograph are required and the mathematics involved is that of Bragg's law. Furthermore, a few mg of sample are sufficient.

Mass spectrometry. Mass spectrometry is the most accurate method for measuring the molecular weight of a simple organic compound (see Chapter 9, p. 315 for an outline of this technique). A single-focusing instrument has an accuracy of about 0·2%, whilst that for a double-focusing instrument can be better than 0·01%. Only a few mg of sample are required, but the compound must volatilise without decomposition. This method is not in more general use simply because of the limited number of such specialised and expensive instruments.

Chemical or spectroscopic functional-group assay. Many organic compounds contain characteristic functional groups, and often reagents can be found which react specifically with these groups. If the number of such groups in the molecule (f) is known, then the molecular weight (M) can be determined by estimating the number of moles of specific reagent (R_{eq}) equivalent to a known weight (w) of the sample, i.e. $M = wf/R_{eq}$. The method is readily applicable to a wide range of compounds, and perhaps the simplest example is the determination of the equivalent weight, and hence the molecular weight, of an acid or base. The analysis can be extended to oligomers. Here, it is often referred to as 'end-group assay', as the functional group is usually at the end of the molecule.

Functional groups can be assayed by either chemical or physical methods. Obviously a wide range of *chemical* methods are applicable depending on the functional group; a complete list of these suitable for the micro- and semi-micro scale is given in ref. 2, chapter 17. The usual *physical* methods are infrared and ultraviolet spectroscopy. The application of these to end-group assay is dealt with elsewhere (see pp. 106 and 163).

Functional-group assay may be illustrated by reference to reducing oligosaccharides; for these compounds, the potential aldehyde group can be assayed by hypoiodite-oxidation, or by forming the osazone and measuring the ultraviolet absorption.

It should be noted that the success of any functional-group assay depends entirely on the completeness with which the specific reagent reacts with the functional group; side-reactions should not occur. Under ideal conditions, the accuracy of the method can be high, but it decreases with increase in molecular weight. An obvious limitation is that the number of functional groups per molecule must be known.

Pseudo-equilibrium method of ultracentrifugation. Recent advances in ultracentrifugation† have extended the general scope of the method to include low molecular weights. In the original techniques—developed for high polymers, see pp. 268–272—measurements were made of either (i) the rate of movement of the solute in high force-fields, i.e. the *sedimentation velocity*, or (ii) the distribution of solute at *sedimentation equilibrium*, i.e. when the centrifugal force was such that the movement of the solute to the bottom of the cell was counterbalanced exactly by the tendency to diffuse. Within the last few years, however, an experimental method has been developed by which molecular weights can be determined from measurements made during the approach to equilibrium. This *pseudo-equilibrium method*, which is based on earlier theoretical work by Archibald, is perhaps the most important recent development in ultracentrifugation.[5] The lower limit of molecular weights amenable to investigation has been extended to molecules as small as sucrose, raffinose or glycyl-L-leucine; such molecular weights can be measured to an accuracy of 1%.

Fig. 4 shows schematically the lay-out of a commercially-available ultracentrifuge.‡ In this instrument, a maximum routine running-speed of 60,000 r.p.m. is achieved by means of a geared electrical motor. This speed corresponds to a force-field of 250,000 g acting on the solution. Full details of the instrument and the experimental procedure are given in ref. 5. The changes in concentration occurring in the cell during ultracentrifugation are measured optically either by (i) *absorption methods*, in which a wavelength is chosen so that the solute absorbs more strongly than the solvent, or (ii) *Schlieren methods*, which give a photographic record of the refractive index gradient (this is proportional to

† An ultracentrifuge is simply a high-speed centrifuge in which the resultant force-field is sufficiently large to affect molecules in solution. The instrument was devised by Svedberg in 1924–6, but only in the last fifteen years has the instrumentation been developed sufficiently for the method to come into widespread use.

‡ From Spinco Division, Beckmann Instruments Inc., U.S.A.

the concentration gradient) (see p. 269 for details), or (iii) *interference methods*, in which a Rayleigh interferometer is incorporated, and the distortion of the interference fringes provides a very sensitive measure of concentration.

The pseudo-equilibrium method of ultracentrifugation depends on the fact that, although an extremely long time may be required to establish equilibrium conditions throughout the solution, the rate of

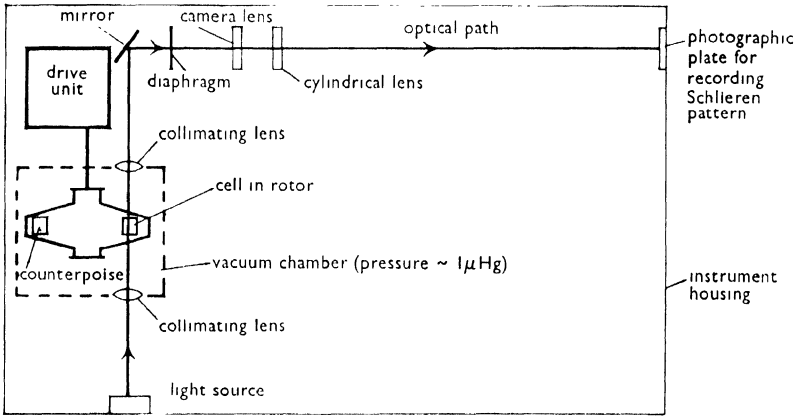


FIG. 4. Schematic diagram of an electrically-driven ultracentrifuge

transport of solute across the bottom of the cell and across the meniscus must at all times be zero! Hence at these points the rate of transport under the influence of the gravitational field must be equal and opposite in sign to the rate of transport by diffusion. It can be shown that this leads to the relations:

$$M_m = \frac{RT}{(1-\bar{V}\rho)\omega^2} \cdot \frac{(\delta c/\delta x)_m}{c_m x_m}$$

and

$$M_b = \frac{RT}{(1-\bar{V}\rho)\omega^2} \cdot \frac{(\delta c/\delta x)_b}{c_b x_b},$$

where m and b refer to conditions at the meniscus and bottom of the cell, and M, c, x and $\delta c/\delta x$ represent molecular weight, concentration of solute, distance from the axis of rotation and concentration gradient, respectively ($\bar{V}$, ρ and ω are defined on p. 268). For a sample of uniform molecular weight, $M_m = M_b$.

The type of photograph obtained using Schlieren optics is shown in Fig. 5. The vertical height of the curve above the base line gives $\delta c/\delta x$

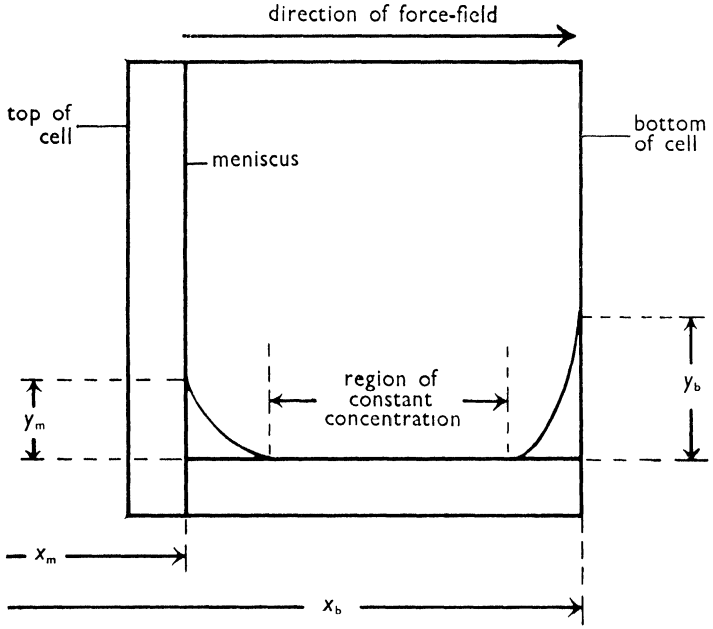


FIG. 5. Schlieren pattern obtained in the Archibald pseudo-equilibrium method of ultracentrifugation (x denotes distance from axis of rotation)

in arbitrary units, y_m and y_b corresponding to $(\delta c/\delta x)_m$ and $(\delta c/\delta x)_b$. The quantities c_m and c_b can be determined in the same arbitrary units by graphical integration using the relations:

$$c_m = c_0 - x_m^{-2} \int_{x_m}^{x_p} x^2 \, (\delta c/\delta x) \, . \, \mathrm{d}x$$

and

$$c_b = c_0 + x_b^{-2} \int_{x_p}^{x_b} x^2 \, (\delta c/\delta x) \, . \, \mathrm{d}x,$$

where x_p refers to any point in the region of constant concentration (c_0). Values of $\delta c/\delta x$ at various points in the cell are obtained directly from the curve. c_0 is determined (again in the same units) by creating a boundary between the solution and solvent in a 'synthetic boundary cell', the area $\int(\delta c/\delta x)\mathrm{d}x$ of the Schlieren curve for this boundary being equal to c_0 (see ref. 5 for details).

It is to be noted that—in contrast to all the other methods mentioned in this section—the Archibald method yields a weight-average molecular weight for a sample containing molecules of different sizes. The method has great potentialities, particularly for heat-labile, water-soluble mater-

ials. A small amount of sample is required (*ca.* 50 mg), and the accuracy is high. Although experimentally it is easier to deal with aqueous solutions, non-aqueous solutions can be examined. Furthermore, the method provides a useful test for purity as the values of M_m and M_b are very sensitive to inhomogeneity. It seems probable that the method will be utilised more in the future.

Molecular Weights in the Range 2,000–20,000

This range of molecular weights is the most difficult to investigate; the methods described in the first section are insufficiently sensitive without modification, whilst those described later for very large molecular weights are not always applicable. Both for the present range and for high polymers, sample preparation is very important. Particular care must be taken to remove impurities of low molecular weight (e.g. solvents or unreacted monomer) especially when the molecular weights are determined by number-average methods. When a solvent cannot be removed without decomposing the sample, its effect may be minimised by using a molecular weight method which employs the same solvent.

Typical compounds: Many condensation polymers, natural products including proteins, antibiotics, hemicelluloses, lignin degradation-products and gallotannins.

Methods: Depression of vapour pressure; depression of freezing-point; elevation of boiling-point; chemical or spectroscopic end-group assay; pseudo-equilibrium method of ultracentrifugation; surface pressure.

Depression of vapour pressure. A differential manometer (see p. 248) devised by Puddington can be used for measurements in this range.[3,6] However, a more convenient vapour pressure method is that involving isothermal distillation. For organic solvents, the dynamic method (p. 249) can be modified so that an accuracy of 5–10% is possible. Solution and solvent are sealed in an evacuated glass vessel, which is thermostatted to $\pm 0{\cdot}001$°C and the rate of distillation of solvent into solution then followed by measuring the changes in volume. It is found experimentally that the rate of distillation is linearly proportional to the mole-fraction of solute, and hence, after calibration with a standard solute, the molecular weight can be readily found. This method has proved particularly successful, for example, in the case of methylated and acetylated derivatives of hemicelluloses. Solvents such as benzene, carbon tetrachloride and 1,3-dioxolane can be used, and only a few hundred mg of sample are required. Measurements have to be made, of

course, at several concentrations and the apparent molecular weight extrapolated to infinite dilution.

Depression of freezing-point. Although the cryoscopic method has the theoretical advantage of sensitivity over the boiling-point elevation method (see p. 250), it appears to have been little used for this molecular weight range. There is a great tendency for solid solutions to be formed, and extensive measurements in several solvents are required to show that this complication is absent.

Elevation of boiling-point. Recently, this method has been quite extensively applied, although the simple apparatus outlined earlier (p. 253) has to be modified to increase the sensitivity. At low concentrations, temperature rises of a few thousandths of 1°C have to be measured with an accuracy of (say) 1%. A very carefully designed differential ebulliometer is then essential. The temperature difference can be measured using a 20-junction thermocouple, or with thermistors incorporated in an amplified Wheatstone bridge circuit. Experimental details can be found elsewhere.[3,6]

Chemical or spectroscopic end-group assay. End-group assay is very useful for this molecular weight range, although it has certain limitations: (i) as the size of the molecule increases, incomplete reaction of the specific reagent with the functional group often occurs, (ii) the specific reaction may be accompanied by some degradation process which lowers the observed molecular weight, and (iii) side-reactions, which can completely invalidate the results, are not uncommon. Generally the upper limit for end-group assay is the detection of about one characteristic group per 100 monomers; below this amount of end-group, the accuracy decreases rapidly.

Condensation polymers have been extensively characterised by end-group assay, the classical example being the work of Haworth and Hirst on the natural polymers, starch, cellulose and the hemicelluloses. For these materials, chemical methods based on estimations of the reducing end-group are not as successful as those based on the non-reducing ends. The latter can be determined by methylation, hydrolysis and quantitative separation and estimation of the component sugars. A similar result can be obtained by oxidation with periodic acid. These estimations give the molecular weight only for linear molecules; for branched molecules the average length of unit-chain is obtained (see Fig. 6). Physical methods of end-group assay have not been used very much in the polysaccharide field.

In the case of synthetic condensation polymers, however, both chemical and physical methods have been used. Many of these polymers have

acidic or basic end-groups, e.g. the $-COOH$ group of polyesters or the $-NH_2$ group of polyamides. For linear polymers, the molecular weight can be found by direct titration. Details of these techniques and other functional-group analyses as applied to condensation polymers are given in ref. 6. Other techniques which have been used include the estimation of free amino-groups by the formation of a dinitrobenzene

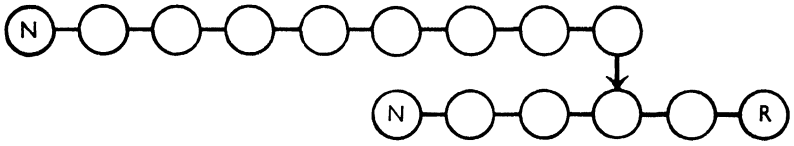


FIG. 6. Hypothetical polysaccharide structure

○ = sugar residue
Ⓝ = non-reducing end-group
Ⓡ = reducing end-group

Estimation of Ⓡ (one group per 15 units) gives the *molecular weight*.
Estimation of Ⓝ gives the *average length of unit-chain* (7·5 units).

derivative which can be measured colorimetrically (this technique can be used also for proteins), and the measurement of hydroxyl- and carboxyl-group concentrations (e.g. in polyethylene terephthalate) by infrared spectroscopy.

Generally, synthetic addition polymers are not amenable to such techniques as the end-group is not functional, but sometimes the incorporation of a chain transfer reagent during synthesis will give a detectable end-group, e.g. the use of mercaptans will incorporate sulphur which can be estimated by direct analysis.

The amount of sample required for end-group assay varies with the method used, but often estimations on the semi-micro scale are not possible.

Pseudo-equilibrium method of ultracentrifugation. The pseudo-equilibrium method of ultracentrifugation is ideally suited for this molecular weight range. The method outlined earlier (p. 257) is applicable, but corrections have to be applied for (i) complications arising from the molecular weight distribution and (ii) non-ideal behaviour. For samples with a molecular weight distribution, the average $\overline{M}_w$ value calculated by the Archibald procedure at both the meniscus and the bottom of the cell is a function of time. There is a preferential concentration of the larger molecules at the bottom of the cell during the experiment which leads to an increase in $\overline{M}_w$ with time; the corresponding depletion of large molecules at the meniscus leads to a decrease in $\overline{M}_w$. Extrapolation of the results to zero time is then necessary to

obtain the true weight-average molecular weight. Corrections for non-ideal behaviour can be made by carrying out measurements at several concentrations (c_0) and finding the apparent molecular weights (M_{app}). It has been suggested that the best method of extrapolation to infinite dilution is to plot M_{app}^{-1} against c_0.

Few measurements have been made so far by this method, but it has many advantages; only a small quantity of material is required and measurements should be possible in most solvents. Furthermore, as mentioned earlier, the agreement between molecular weights calculated from the meniscus and the bottom of the cell is a very sensitive test for heterogeneity.

Surface pressure. Surface pressure is the reduction in surface tension of a liquid—usually water or a buffer solution—produced by a *monolayer* of a compound. If the molecules have a large area of free movement, they may lie on the surface and behave as a two-dimensional gas. Under these conditions, the molecular weight can be found from measurements of the surface pressure exerted by a known weight of the substance at various surface areas using a two-dimensional equivalent of the osmotic pressure equation (p. 263). As the behaviour of the films is not ideal, the results must be extrapolated to zero surface pressure; this is sometimes difficult.

The method has the advantage of simplicity of apparatus and requires only a few mg of material (see refs. 7 and 8 for details). The molecular weights of proteins and some synthetic polymers have been measured in this way, but the method is limited to substances which form gaseous films.

Molecular Weights above 20,000

For this range of molecular weight, colligative properties—with the exception of osmotic pressure—become too small to be measured accurately, and other specialised techniques are required. This section is concerned, in fact, with the methods suitable for determining the molecular weight of high polymers. (Although many of these methods can give also an estimate of molecular *shape*, this aspect is not discussed.) In the majority of polymer solutions, the differences between the solute–solute, solute–solvent and solvent–solvent interactions are so large that considerable deviations from ideal behaviour result, and often this makes the measurement of molecular weight more difficult. However, the theory of Flory[9] predicts that for all polymers there is a solvent (or solvent-mixture) and temperature at which behaviour is pseudo-ideal. If such a *theta-solvent* and *theta-temperature* can be

found, molecular weight measurements are made considerably more easy.

The homogeneity of the polymer sample† should be established before measurements are made. However, it should be stressed that often this involves immense problems, particularly in the field of natural high polymers. These problems cannot be discussed here.

Typical compounds: Polysaccharides; nucleic acids; synthetic addition polymers; many proteins including viruses.

Methods: Osmotic pressure; light scattering; ultracentrifugation (sedimentation velocity, sedimentation equilibrium and pseudo-equilibrium); viscosity; electron microscopy.

Osmotic pressure. It can be shown that the osmotic pressure (Π) of an ideal solution is related to the mole-fraction of solute (x) by the relation $\Pi V_0 = -RT \ln (1-x)$, where R is the gas-constant, T is the absolute temperature, and V_0 is the molar volume of solvent. For dilute solutions, this approximates to the van't Hoff law, $\Pi/c = RT/M$, where c is the concentration (in g/l) of solute of molecular weight M.

Experimentally it is found that for polymer solutions, Π/c is usually dependent on c; an equation of the form

$$\Pi/c = RT/M + Bc + Cc^2 + \ldots$$

is found to hold, where B, C, etc. are termed the second, third, etc. virial coefficients. Molecular weights are calculated therefore from the empirical relation,

$$M = RT/\lim_{c \to 0} (\Pi/c).$$

Recent theoretical work on the properties of polymer solutions has been concerned with attempts to evaluate the virial coefficients. For example, Flory[9] has shown that

$$\Pi/c = (RT/M)\,[1 + \Gamma_2 c + \Gamma_3 c^2 + \ldots] \qquad (5)$$

where Γ_2, Γ_3, etc. are complex functions of molecular weight and polymer–solvent interaction which may be related to each other, i.e. it has been suggested that

$$\Gamma_3 = g\Gamma_2^2, \qquad (6)$$

where g is a constant approximately equal to 1/4 for good solvents.

† The term 'homogeneity' is used to indicate a polymer sample consisting of molecules having the same chemical composition, but not necessarily the same molecular weight. The problems entailed in the determination of the molecular weight distribution of a polymer are not covered in this section. Details can be found elsewhere.[6,9]

For many polymer-solvent systems, the third virial coefficient becomes important at relatively low concentrations (>0·3%), and hence osmotic pressure measurements always have to be made at very low dilutions to avoid errors in extrapolation. If equation (6) is assumed and substituted in equation (5), then

$$(\Pi/c)^{\frac{1}{2}} = (RT/M)^{\frac{1}{2}}(1+\Gamma_2 c+\tfrac{1}{4}\Gamma_2^2 c^2)^{\frac{1}{2}}$$
$$= (RT/M)^{\frac{1}{2}}(1+\tfrac{1}{2}\Gamma_2 c),$$

i.e. the graph of $(\Pi/c)^{\frac{1}{2}}$ against c should be linear. This relation is useful for extrapolations when good solvents are used. Difficulties in extrapolation can be avoided also if measurements are carried out in a theta-solvent at the theta-temperature.

For dilute solutions of high polymers, the osmotic pressure is equivalent to never more than a few cm head of solvent, and so can be measured directly with a travelling microscope. A typical osmometer is thus simply a device in which solvent is separated from solution by a semi-permeable membrane, and capillaries are provided for measurement of the pressure as shown in Fig. 7. The whole success of osmometry depends on the preparation of the semi-permeable membrane. Inorganic membranes of the classical ferrocyanide type, which can behave semi-permeably to sucrose, are extremely difficult to prepare and are not often used for polymer work. The most common membranes are based on cellulose: for aqueous solutions—collodion; for non-aqueous solutions—denitrated collodion, cellophane, gel-cellophane, or bacterial cellulose. Synthetic membranes of polyvinyl alcohol have been suggested but they do not appear to be reliable. For a sample with a very wide range

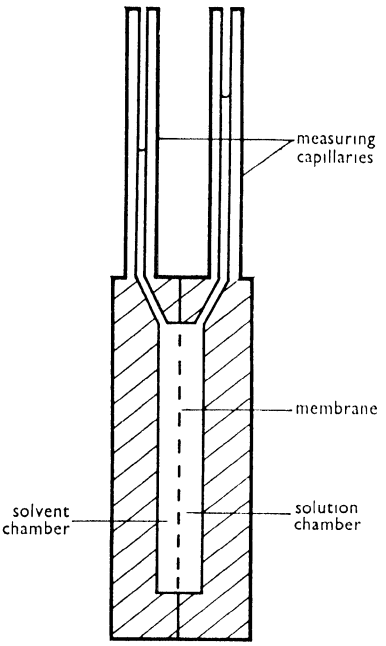


FIG. 7. Schematic diagram of an osmometer (device for filling and membrane support omitted)

of molecular weights, the use of imperfect semi-permeable membranes can give varying results, the value depending on the amount of sample diffusing through the membrane.

Two methods of measurement can be employed: (i) dynamic measurements in which a pressure is applied to the solution until there is zero movement of solvent, or (ii) static measurements in which the pressure-head is allowed to build up to a steady value (if the design of apparatus is such that there is no appreciable dilution of the solution, the pressure-head is then equivalent to the osmotic pressure). In general, static methods are the more accurate, particularly for very small pressures, although they are more time-consuming. The time for equilibrium can be reduced, however, by 'setting' the levels of liquid to near the expected values. The design of apparatus is important as a large area of taut membrane and a small solution volume will ensure more rapid equilibration. The static method also has the advantage that leakage of solute through the membrane can be detected very readily by the fact that the observed osmotic pressure will go through a maximum value and then fall off. It is very difficult to correct for such diffusion effects.

The type of apparatus used depends on whether aqueous or non-aqueous solutions are being studied. It should be noted that the study of aqueous solutions poses special problems in osmometry (e.g. Donnan effects). Full details of the experimental methods can be found in refs. 3, 6 and 7. Further an apparatus has been described in which the movement of the solvent is followed *by weight*. This 'osmotic balance' is theoretically very accurate, but the experimentation is very difficult and it has been little used.

The limits for osmotic pressure measurements are fixed by the sensitivity of the measuring device, for high molecular weights, and by the semi-permeability of the membrane, for low molecular weights. In practice, this means that the method is restricted to the molecular weight range of 20,000–500,000, although under certain circumstances this can be extended. A wide range of solvents can be used, and the amount of sample required is not excessive (*ca.* 0·5 g). It should be stressed that osmometry is an art; the method requires more time and manipulative skill than other molecular weight methods. However, the method is indispensable as it provides the only way of measuring the number-average molecular weight ($\overline{M}_n$) of a high polymer.

Light scattering. Although the phenomenon of light scattering has been known for many years and its theoretical foundations are well established, only in the last two decades has it been applied to polymers.

Polymer solutions are colloidal and hence a beam of light traversing a dilute solution is scattered. The magnitude of this scattering is considerably smaller than that from a sol, and a photoelectric cell is usually necessary to measure the intensity of the scattered light. Theoretical considerations have related the intensity and angular distribution of the scattered light to the size and shape of the scattering particles, i.e. the polymer molecules. The method can be applied to samples of low molecular weight (even the molecular weight of sucrose has been determined), but the experimental technique is then difficult; the accuracy of measurement increases with molecular weight as the intensities involved are correspondingly greater.

When light traverses a medium, two phenomena may occur: (i) part of the light may be absorbed, its energy reappearing as fluorescence or heat, or (ii) the light may be scattered. For (i) to occur, the incident radiation must correspond to a natural frequency of the molecules of the medium, and this effect is thus dependent on their chemical nature. Light scattering, on the other hand, is essentially an optical diffraction phenomenon in which the particles in the medium act as a secondary source of radiation of the same frequency (however, see p. 116). The method does not give any direct information about chemical structure unless this is reflected in the physical size and shape of the particles (see below).

The light scattered from small particles at an angle θ to the incident beam is conveniently measured in terms of the quantity R_θ, where if I_r is the intensity of scattered light at a distance r, and I_0 is the incident intensity, then

$$R_\theta = r^2 I_r / I_0 (1 + \cos^2 \theta).$$

This quantity is independent of the scattering angle. It can be shown that for an ideal solution of concentration c of small particles of molecular weight M,

$$R_\theta = KcM \tag{7}$$

where K is a constant. $K = [2\pi^2 n_0^2 (dn/dc)^2]/N\lambda_0^4$, where n_0 is the refractive index of the solvent at wavelength λ_0, (dn/dc) is the refractive index increment, and N is Avogadro's number.

An alternative approach to the problem is to define a conventional absorption coefficient (τ) as the measure of the reduction in intensity from I_0 to I of the transmitted beam, i.e.

$$\tau = \frac{1}{x} \ln (I_0/I),$$

where x is the path-length. τ is known as the turbidity. It can be shown that the turbidity is related to the quantity R_θ by $\tau = 16\pi R_\theta/3$. Hence substituting in equation (7), it follows that

$$\tau = 16\pi\, KcM/3 = HcM,$$

where H is a constant. Both methods can be used to evaluate M, but the former is preferred as the turbidity of many solutions is low and difficult to measure.

For non-ideal solutions of small particles it can be shown that

$$\frac{Hc}{\tau} = \frac{Kc}{R_\theta} = \frac{1}{\overline{M}_w} + \frac{2Bc}{RT} + \ldots,$$

where B is the second osmotic virial coefficient.

The light scattered by small particles is symmetrical about a position normal to the incident beam (this intensity distribution is known as *Rayleigh scattering*). However, when a dimension of the solute particle exceeds 1/20 of λ_0, the amount of light scattered is decreased, particularly in the backward direction, owing to destructive interference between light scattered from different parts of the particle. The light scattering from solutions of most high polymers is therefore relatively less than the Rayleigh scattering except when $\theta = 0$. The departure from Rayleigh scattering increases with size and is also dependent on shape. The particle scattering factor (P_θ) can be defined as R_θ/R_0, i.e. the ratio of the intensity at the angle θ to that at zero angle. This factor can be calculated for various model-forms, e.g. spheres, random coils, or rods, and it can be shown that the exact relation for all models is given by

$$\frac{Kc}{R_\theta} = \frac{1}{\overline{M}_w P_\theta} + \frac{2Bc}{RT} + \ldots \tag{8}$$

This relation is used to obtain $\overline{M}_w$ in two ways:

The dissymmetry method. For any model, the ratio of intensities at two angles, symmetrical about the normal, enables the particle scattering factor for any angle to be derived. Usually angles of 45° and 135° are taken, then I_{45}/I_{135} is known as the dissymmetry ratio (z). If z is measured, P_θ can be found from tables if the appropriate molecular model is known. This requirement limits the use of the method.

The extrapolation method. For all models, $P_0 = 1$, and hence equation (8) reduces to

$$\frac{Kc}{R_0} = \frac{1}{\overline{M}_w} + \frac{2Bc}{RT} + \ldots \tag{9}$$

Hence if R_0 can be found, $\overline{M}_w$ can be calculated without prior knowledge of the molecular shape. Obviously R_0 cannot be found by direct measurement, but it can be determined by extrapolation from values at sufficiently low angles. A graphical method suggested by Zimm is often used. In this, data obtained at various concentrations and angles are simultaneously extrapolated to zero values of θ and c to obtain $(Kc/R_\theta)_{\theta=0;\ c=0}$, which—from equation (9)—is the reciprocal of $\overline{M}_w$.

The general lay-out of a typical light-scattering photometer is shown in Fig. 8. Instrumentation is now no longer a problem, and a variety

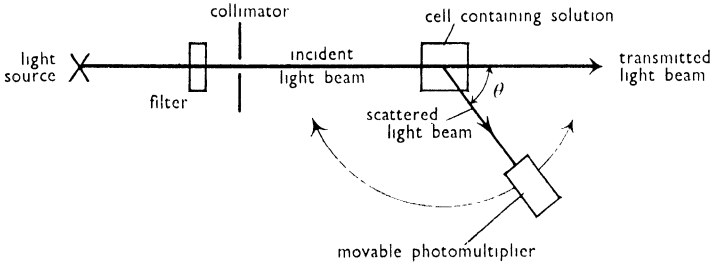


FIG. 8. Schematic diagram of light scattering apparatus

of commercial instruments are available. The main experimental difficulty is in the clarification of solutions free from dust and other extraneous large particles. The most general method is careful filtration through a very fine glass sinter followed by ultracentrifugation. Aqueous solutions are far more difficult to clarify than non-aqueous ones. Full experimental details can be found in refs. 10 and 11.

The light-scattering method is applicable to any size of molecule—in fact, the larger the value of the molecular weight, the easier is the measurement. Any solvent can be used, and the amount of sample required is not large. The method is the most satisfactory of all for determining the weight-average molecular weight.

Ultracentrifugation. All the ultracentrifugal techniques mentioned on p. 256 are applicable to high polymers.

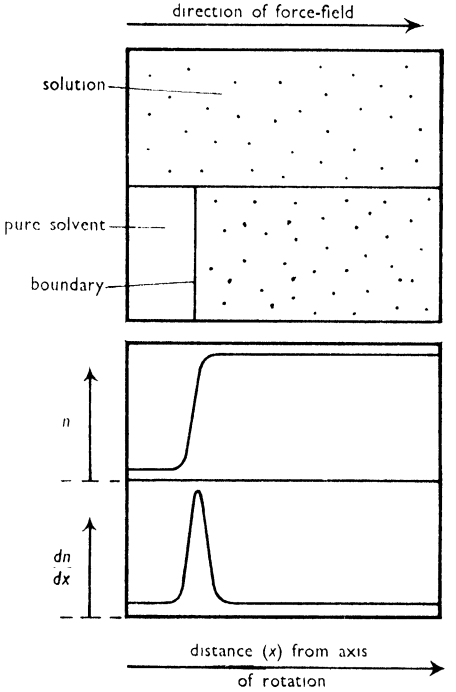
1. *Sedimentation velocity.* The rate of movement of solute is expressed in terms of the sedimentation coefficient (S), and for solute of molecular weight (M) it can be shown that:

$$M = RTS/(1-\overline{V}\rho)D \qquad (10)$$

where R is the gas constant, T is the absolute temperature, $\overline{V}$ is the partial specific volume of the solute, ρ is the density of the solution and D is the diffusion coefficient. Equation (10) is known as Svedberg's

equation. The sedimentation coefficient (S) is expressed as the rate of movement of solute per unit force-field, i.e. $S = (\mathrm{d}x/\mathrm{d}t)/\omega^2 x = (\mathrm{d} \ln x/\mathrm{d}t)/\omega^2$, where x is the distance moved in cm in t sec, and ω is the angular velocity in radians/sec (S can be evaluated conveniently from the gradient of the graph of $\ln x$ against t). 1 Svedberg unit (i.e. 1 S) $\equiv 1 \times 10^{-13}$ c.g.s. units. Sedimentation coefficients are usually corrected to standard conditions (i.e. water at 20°C).

As mentioned earlier on p. 256, optical methods are used for measuring concentration changes in the ultracentrifugal cell. The absorption



(*a*) initial solution before ultracentrifugation

(*b*) solution after ultracentrifugation at high force-fields showing formation of 'boundary'

(*c*) corresponding changes in refractive index (n) with distance (x) for conditions in diagram b

(*d*) corresponding changes in refractive index gradient ($\mathrm{d}n/\mathrm{d}x$) with distance (x) for conditions in diagram b, i.e. Schlieren optical pattern.

FIG. 9. Schematic diagrams of the effect of an ultracentrifugal field on solute particles in an ultracentrifuge cell (in practice, the sides of the cell are not parallel but meet at the axis of rotation)

method, which was used by Svedberg in his original work on proteins, is being used currently for studying nucleic acids. Concentrations as low as 0·002% can be examined and, at this dilution, the molecular behaviour is likely to be near-ideal. The most commonly used method is the cylindrical lens, or Schlieren method, developed by Philpot and

Svensson. Fig. 9 shows diagrammatically the changes in concentration and refractive index (n) during a sedimentation velocity measurement; Schlieren optics give the dn/dx pattern, or sedimentation 'peak', directly on a photographic plate† (see ref. 5 for details). It should be noted, however, that for dilute solutions ($<ca.$ 0·05%) the Schlieren method becomes insensitive, and absorption or interference optics are then necessary.

The measurement of molecular weight is not straightforward. The non-ideal behaviour of most polymers means that the sedimentation coefficient (S) increases with decrease in the concentration (c) as intermolecular entanglements then decrease and the molecules can move more freely. This effect can be very large. Unfortunately, extrapolation to infinite dilution is difficult. The empirical function $S^{-1} = f(c)$ appears to be the most satisfactory, but for highly concentration-dependent samples, extrapolation is still not easy.

Further—in all but carefully fractionated polymers—the distribution of molecular weight results in a corresponding distribution of sedimentation coefficient. Under ideal conditions, the two distributions would be identical, but the concentration effects discussed above influence the shape of the observed peak. As a result, it is difficult to obtain a simple average. Usually, the maximum ordinate of the curve is measured, and this only gives the sedimentation coefficient of the molecular species present in the highest concentration.

However, perhaps the main difficulty is that in order to obtain the molecular weight, the sedimentation coefficient has to be combined with a measured diffusion coefficient (D). The latter is a measure of the rate at which solute diffuses from high to low concentrations; its experimental determination is extremely difficult. Full details can be found in refs. 7 and 12.

The difficulty of requiring a separate measurement of the diffusion coefficient is often avoided by combining sedimentation measurements with viscosity measurements (see p. 272). Flory's theory[9] of polymer solutions has lead to the following equation

$$M = \left[\frac{N\eta_0}{\beta(1-\bar{V}\rho)}\right]^{\frac{3}{2}} S^{\frac{1}{2}}\,[\eta]^{\frac{1}{2}}$$

where N is Avogadro's number, η_0 is the solvent viscosity, β is a constant, $\bar{V}$ is the partial specific volume of the solute, ρ is the solvent

† The various types of Schlieren photographs shown in the literature arise from the use of different movable diaphragms in the optical system, e.g. a slit, bar, wire or phase-plate.

density, S is the sedimentation coefficient and $[\eta]$ is the limiting viscosity number. β depends on the molecular shape, i.e. for rigid spheres, $\beta = 2{\cdot}12 \times 10^6$; for rigid ellipsoids of axial ratio 1:6, $\beta = 2{\cdot}28 \times 10^6$; for random coils in good solvents, $\beta = 2{\cdot}5 \times 10^6$. Hence, if a value of $\beta = 2{\cdot}25 \times 10^6$ is used, the value of $M^{\frac{2}{3}}$ will have at least about 10% accuracy, and M will then be accurate to 15% without making any assumptions regarding molecular shape.

The most serious theoretical limitation of sedimentation velocity measurements—and one which is rarely stressed—is that the resultant molecular weight average is not simple. For example, the combination of a weight-average sedimentation coefficient and a weight-average diffusion coefficient does not give a weight-average molecular weight: it can be shown that *the average depends on the molecular shape.* For a free-draining coil, a number-average is obtained (!), whilst for a sphere, $\overline{M} = (\Sigma\, n_i M_i^{\frac{5}{3}})/(\Sigma\, n_i M_i^{\frac{2}{3}})$, which is not a simple average. Notwithstanding the above difficulties, sedimentation velocity measurements are invaluable as they can often show immediately that a polymer sample is not homogeneous; this is often extremely difficult to prove by any other method.

2. *Sedimentation equilibrium.* At sedimentation equilibrium it can be shown that if c_1 and c_2 are the equilibrium concentrations of solute of molecular weight M at distances x_1 and x_2 from the axis of rotation, then

$$M = \frac{2RT \ln (c_2/c_1)}{(1-\bar{V}\rho)\,\omega^2\,(x_2^2-x_1^2)}$$

where the other symbols have the significance given on p. 268. It is to be noted that this relation contains no diffusion coefficient.

However, the length of time required to attain equilibrium is inversely proportional to the molecular weight, e.g. a polymer may take 2–3 weeks! Special instruments have been designed to control the speed and temperature for this length of time (see ref. 13). Deviations from ideal behaviour also occur, and one theoretical method of correction leads to the relation

$$M = \frac{2RT\,(1+c\delta \ln \gamma/\delta c)}{(1-\bar{V}\rho)\,\omega^2\,(x_b^2-x_m^2)} \times \frac{(c_b-c_m)}{c_0}$$

where γ is the activity coefficient, and where b and m refer to conditions at the bottom and meniscus of the cell. The evaluation of results from this method is therefore not straightforward, and it has not been extensively used.

3. *Pseudo-equilibrium.* The method outlined on p. 257 is directly applicable to high polymers. Corrections for the distribution of molecular weight and non-ideal behaviour have to be applied. Although few measurements have been made so far by this method, it has great potentialities, as an independent measurement of the diffusion coefficient is not needed. Only small samples are required, but as stressed earlier the method is very sensitive to impurities.

Viscometry. Viscometry is the simplest method of characterising high polymers, and also gives the most reproducible results. Unfortunately, however, the method is not absolute and calibration is required.

The absolute value of the viscosity of a polymer solution is not of much importance in characterising the molecular weight. A more fundamental quantity is the specific viscosity (η_{sp}), which is a measure of the relative increase in viscosity due to the dissolved solute.

$$\eta_{sp} = \eta_{rel} - 1 = (\eta_c - \eta_0)/\eta_0$$

where η_{rel} is the relative viscosity, η_c is the absolute viscosity of a solution of concentration c and η_0 is the absolute viscosity of the solvent. The value of the specific viscosity divided by the concentration is known as the viscosity number. The viscosity number is dependent on concentration, and when measurements are made at low concentrations ($c < 1{\cdot}0$ g per 100 ml) it is found that

$$\eta_{sp}/c = A' + B'c$$

where A' and B' are constants. The value of η_{sp}/c at infinite dilution (i.e. A'), which can be obtained by extrapolation from the graph of η_{sp}/c against c, is known as the limiting viscosity number, $[\eta]$ (or 'intrinsic viscosity' in an earlier nomenclature), i.e.

$$[\eta] = \lim_{c \to 0} (\eta_{sp}/c).$$

$[\eta]$ has the dimensions of reciprocal concentration, and for a given polymer varies with the solvent and temperature.

Staudinger first proposed on an empirical basis that, for linear polymer homologues, there was a relation between the limiting viscosity number and the molecular weight (M) of the form,

$$[\eta] = KM$$

where K is a constant for the polymer–solvent system. Recently, the more general equation

$$[\eta] = KM^{\alpha} \qquad (11)$$

has been used, and hence any solute–solvent system can be characterised by two parameters K and α. α can have values between 0·5 and 1·5, depending on the *shape* of the polymer molecule. Obviously if $\alpha = 1$, then equation (11) reduces to the original Staudinger equation, and a weight-average molecular weight is obtained. If $\alpha \neq 1$, a more complex average is obtained (see p. 248).

Measurements of $[\eta]$ can be made simply in a capillary viscometer. Fig. 10 shows a design which enables dilutions of the solution to be made in the apparatus. For a capillary viscometer, it can be shown[6] that

$$\eta = A''\rho t - B''\rho/t,$$

where ρ is the density of the liquid, and t is the flow time. A'' and B'' are constants which can be determined by calibration. The term $B''\rho/t$ is a correction for energy losses which occur when the liquid changes flow characteristics on entering and leaving the capillary. This kinetic energy correction can be made negligible by careful viscometer design, otherwise corrections for it *must* be applied.

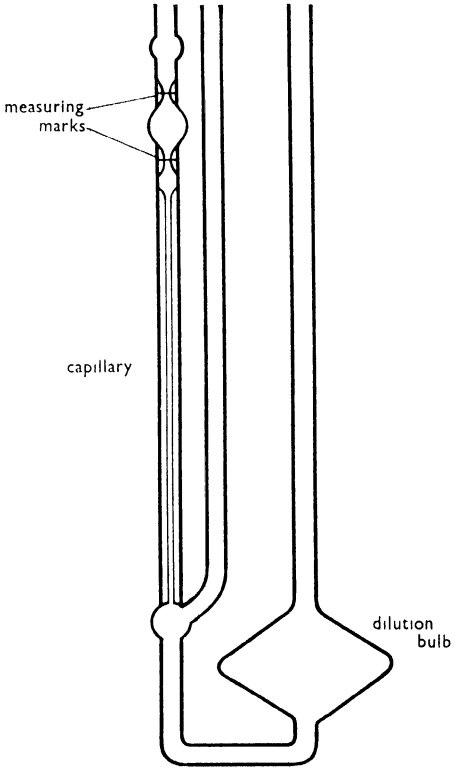


FIG. 10
Modified Ubbelohde viscometer

When $[\eta]$ is large (e.g. >500 ml g^{-1}) many polymer solutions show a variation in the apparent viscosity with the applied shear-force. The value of $[\eta]$ at zero shear can be found with either a rotating-cylinder viscometer, where the shear force is varied by altering the rate of angular rotation of the outer cylinder, or a modified capillary viscometer where variations in the pressure head give different shear forces.[6]

Values of K and α in equation (11) are obtained for a given polymer–

solvent system by separating the polymer into a series of fractions of narrow molecular weight distribution, and determining the molecular weight of each fraction *by an independent method*, preferably one—e.g. light scattering—which gives a weight-average value. K and α can then be evaluated from the graph of ln M against ln $[\eta]$. This procedure is difficult and tedious, but once satisfactory values of K and α are available, values of M are very readily obtained. Even if K and α are not known, changes in $[\eta]$ for a given solute–solvent system most likely represent changes in molecular weight, and hence viscosity measurements are widely used in studying polymer degradation or synthesis.

Electron microscopy.[7] In favourable circumstances, modern electron microscopes are capable of a resolution of ~ 5 Å and hence polymer molecules should be readily studied. However, for most organic polymers a limit of resolution of ~ 20 Å is more usual, as elements of low atomic number have low scattering power, resulting in lack of image contrast. It is obvious that, if individual polymer molecules can be 'seen', the molecular weight can be deduced from the observed volume and the density of the material. If the technical difficulties can be overcome, materials of molecular weight of $\sim 10^6$ (e.g. viruses) can be readily studied. Recently, improvements in experimental technique have enabled the molecular weight of the rod-like particles of poly-L-glutamic acid in the range 30,000–50,000 to be determined. There seems little doubt that the use of this method will be extended in the future.

In conclusion, it is pertinent to refer again to the general considerations on pp. 244–246. Each compound presents its own problems in a molecular weight determination. In some cases, only one or two of the above techniques may be applicable; in many others, the choice may be wide and the selection based on the available equipment. It should be recognised that some of the techniques, particularly the micromethods and many of the methods for polymers, require a considerable degree of manipulative skill; such requirements are not always apparent from printed descriptions. In all instances, the reliability of a molecular weight determination is best checked by the use of more than one method.

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Chapter 8

G. A. Sim

DIFFRACTION METHODS

Introduction

Diffraction methods differ from most physical techniques now available for the study of organic compounds in that they offer the possibility of a complete description of the position and nature of every atom in a molecule. In practice, however, a number of factors restrict the application of the various methods.

Atomic diameters are comparable with the wavelengths of X-rays (about 10^{-8} cm), and crystals, with their regular arrangement of atoms, can therefore behave towards X-rays as three-dimensional diffraction gratings. In fact, *X-ray diffraction* from single crystals can provide sufficient data to allow the complete description of the constitution and stereochemistry of even complex molecules, though the absence of a general solution to the 'Phase Problem' (see p. 295) usually means that the deduction of a structure from the diffraction pattern is by no means straightforward and, lacking favourable circumstances, may be impossible. Nevertheless, with the aid of modern electronic computing facilities, the structures of complex natural products (suitably modified, if necessary) can often be deduced more readily by X-ray diffraction than by a classical chemical approach. Furthermore, the additional information which X-ray diffraction can give about bond lengths, bond angles and the distances between non-bonded atoms is of great significance for structural theory and in the interpretation of reactivity. The structures (1), (3), (5), (6), (7), (12), (14), (15), (16), and (17), shown on the following pages, testify to the power and scope of the method. Even proteins are yielding to the attack.

A shortcoming of the X-ray method is that the accurate location of hydrogen atoms in a structure is difficult and is usually not even

attempted. *Neutron diffraction*,†[1] which is still in its infancy and whose principles are in a number of ways similar to those of the X-ray method, is of great potential importance since it can be used to provide an answer to this problem, particularly when the positions of the other atoms have already been determined by the X-ray method. Typical compounds which, because of interest in their hydrogen bonding, have been studied by the neutron method include α–resorcinol, sodium sesquicarbonate and potassium hydrogen bisphenylacetate. The last substance contains what is probably a truly symmetrical hydrogen bond with the proton located midway between two oxygen atoms.

Owing to the poor penetrating power of electron beams, *electron diffraction*[2–6] is of much less importance in the study of solids than X-ray diffraction. However, it has been widely used in work on gases and vapours. Since in the gas phase the molecules are far apart and randomly orientated, diffraction arises solely from interference between the rays scattered by different parts of the same molecule. The amount of information contained in the diffraction pattern is limited and the method is therefore restricted to the study of fairly small molecules. Nevertheless, it has proved valuable both in the determination of bond lengths and in the study of molecular conformation. For example, 1,2–dichloroethane has been shown to favour the *trans*-conformation (see p. 332) rather than the *gauche*-conformation at room temperature, and in butadiene, glyoxal and methyl glyoxal the *s–trans* form is preferred to the *s–cis* form. The planes of the two benzene rings in diphenyl are found to be inclined to one another in the vapour, although X-ray diffraction shows that they are coplanar in the solid state. In *trans*–decalin both rings are in the expected chair conformation with the hydrogen atoms at the bridge axial. In *cis*–decalin the rings are again in the chair form (contrary to earlier assumptions); one of the hydrogen atoms of the bridge is axial and the other equatorial.

The remainder of this section is devoted to X-ray diffraction as this method has been applied much more extensively than the others to problems of organic structure. Further information about the other two methods may be found in the references quoted above. Refs. 7–15 may be found useful as a basis for further reading on various aspects of X-ray diffraction.

† Neutron diffraction, and also electron diffraction, depend on the fact that a stream of particles has wave properties, the wavelength being given by $\lambda = \frac{h}{mv}$ where m is the mass of the particles, v is their velocity and h is Planck's constant.

In order to understand the problems and procedures involved in a detailed crystal-structure study, some description of the crystalline state is essential and the first few pages are concerned with this.

The Crystalline State

The unit cell. The essential distinction between crystalline (solid) matter and liquid or gaseous matter is the regular repetition in space of the molecules, ions or atoms, which pack together as closely as possible.

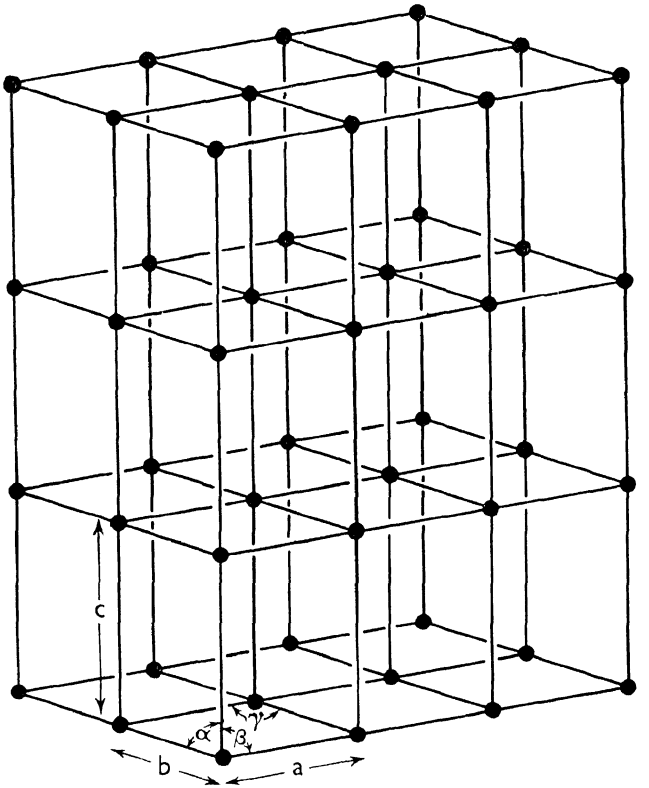


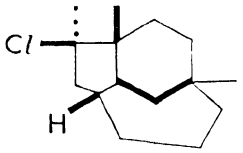
FIG. 1. A regular three-dimensional arrangement of points constituting a space lattice

It is convenient for some purposes to replace each identical repeat unit by a single point and so form a three-dimensional *space lattice* of points such as shown in Fig. 1. The lattice can be regarded in terms of the repetition of the *unit cell* defined by the three *primitive translations*

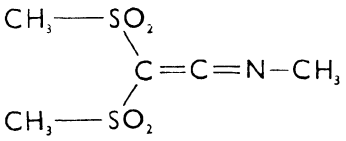
a, *b*, *c* and the angles α (between *b* and *c*), β (between *a* and *c*) and γ (between *a* and *b*).

Symmetry. A crystal is built up by the periodic repetition of the unit cell, the contents of which, furthermore, usually consist of a number of smaller equivalent units arranged in some systematic manner. Two of the possible two-dimensional repetition patterns are illustrated in Fig. 2.

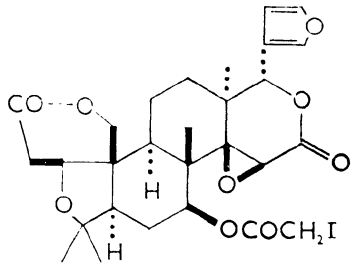
The basic unit from which the cell and thence the complete crystal is derived is referred to as the *asymmetric crystal unit* and may comprise a molecule, as in isoclovene hydrochloride (1); part of a molecule, as in *N*–methyl–2:2–di(methylsulphonyl)vinylideneamine (2); or even a number of molecules, as in epilimonol iodoacetate (3). The positions occupied by the asymmetric crystal unit and its repetitions within the unit cell are known as *equivalent positions* and are most suitably described in terms of *symmetry operations*.



(1)



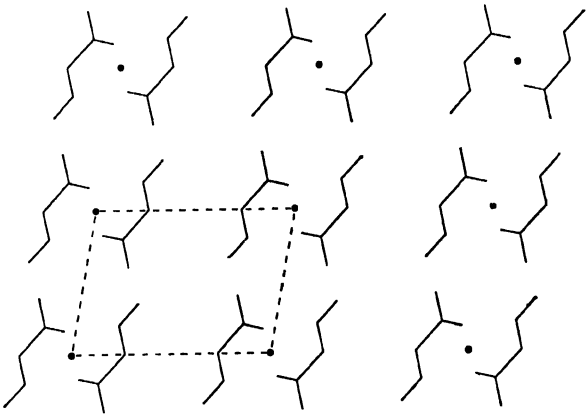
(2)



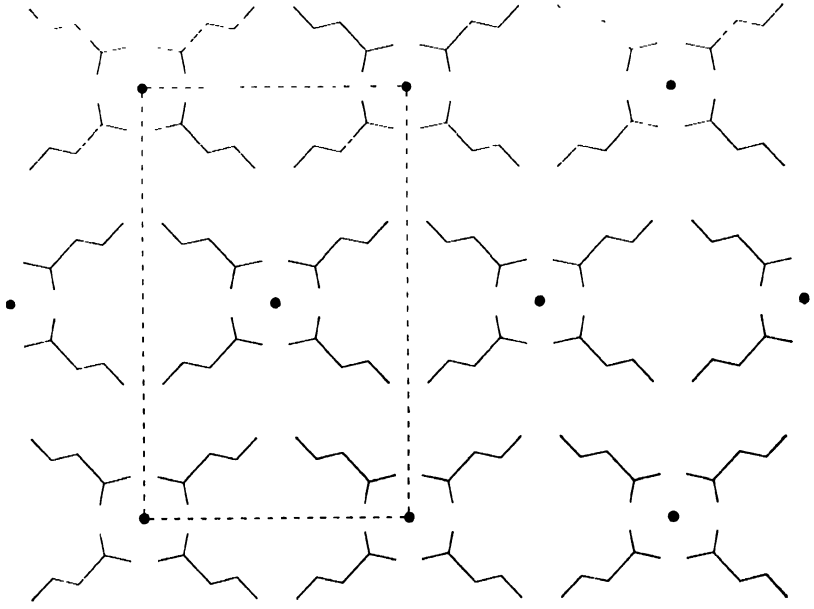
(3)

An object possesses symmetry when by suitable movements it can be made to assume an aspect indistinguishable from its initial one, and the movement which changes the initial aspect into an indistinguishable one is called a symmetry operation.

In the case of a molecule of naphthalene, for example, there are two *mirror planes* of symmetry at right angles to the molecular plane and one coincident with the molecular plane. The symbol employed to represent a mirror plane is *m* and the symmetry of naphthalene can be described as *mmm*. In the arrangement shown in Fig. 2(*b*) there are mirror lines parallel to the edges of the page and it is conventional to



(*a*)



(*b*)

FIG. 2. Two-dimensional repetition patterns. The unit cells are outlined by broken lines.

choose unit-cell edges coincident with these symmetry elements. This has the effect of specifying (with apparently a loss in simplicity) a *centred* unit cell in contrast to the *primitive* cell shown in Fig. 2(*a*), but is advantageous in emphasising the rectangular nature of the pattern.

In addition to the mirror plane of symmetry there are various other symmetry elements.

An *n-fold rotation axis* (symbol n) is such that rotation through $360°/n$ produces an equivalent configuration. The chloroform molecule, for example, possesses a three-fold axis along the carbon–hydrogen bond.

In the case of an *n-fold inversion axis* (symbol $\bar{n}$) a rotation through $360°/n$ is followed by inversion through a point on the axis—each point of the object is projected through the inversion point to an equal distance beyond. The methane molecule provides an example of a molecule with three four-fold inversion axes. These pass through the central carbon atom and the midpoints of the lines joining pairs of hydrogen atoms.

The only operation involved with a one-fold inversion axis ($\bar{1}$) is inversion through a point and this point is referred to as a *centre of symmetry*. Fumaric acid provides an example of a centrosymmetrical molecule. A two-fold inversion axis ($\bar{2}$) can readily be shown to be equivalent to a mirror plane.

Because of the repetition of the unit cell, symmetry elements involving translation can occur in crystals. There are two types, the *screw axis* and the *glide plane*. In the latter, reflexion takes place across a plane followed by translation through a simple fraction (usually one-half) of a unit-cell edge or diagonal. A glide plane with translation parallel to a cell edge is represented by *a*, *b* or *c* according to which edge is involved. A screw axis (n_p) involves rotation through $360°/n$ followed by translation through a fraction, p/n, of a unit-cell edge. Thus a two-fold screw axis 2_1 involves rotation through 180°, followed by translation through a distance equal to half a unit-cell edge.

Space groups. The necessity for repetition in space of the contents of the unit cell imposes limitations on the symmetry possible in a crystal, and only axes of order $n = 1, 2, 3, 4$ and 6 can occur. The possible elements of symmetry can be grouped together in various combinations to produce in all a total of 230 *space groups*. Detailed accounts of these are to be found in *International Tables for X-Ray Crystallography* (1952).[14]

The notation employed to represent a space group indicates the essential symmetry involved.

$P\bar{1}$ refers to a primitive, i.e. non-centred, lattice with a centre of symmetry as the only element of symmetry in the unit cell. It is convenient to choose the centre of symmetry as the origin of coordinates so that if an atom is situated at (x, y, z)† an identical atom must be located at $(-x, -y, -z)$. The cell is said to have two-fold multiplicity; there are two equivalent positions. The centre of symmetry imposes no restrictions on the relative magnitudes of the cell edges nor on the mutual orientation of these, the angles α, β, γ being free to assume any values. Such a cell is *triclinic*.

In the case of the space group $P2_12_12_1$ the lattice is primitive and the unit cell has three mutually perpendicular two-fold screw axes as symmetry elements. Since the unit-cell edges lie in the directions of the screw axes $\alpha = \beta = \gamma = 90°$ and the cell is *orthorhombic*. If an atom is situated at (x, y, z) the effect of the screw axes is to produce three further identical atoms with coordinates $(\frac{1}{2}-x, -y, \frac{1}{2}+z)$, $(\frac{1}{2}+x, \frac{1}{2}-y, -z)$, and $(-x, \frac{1}{2}+y, \frac{1}{2}-z)$.

TABLE 1. The seven crystal systems

System	*Unit cell*	*Number of space groups*
Triclinic	$a \neq b \neq c$ $\alpha \neq \beta \neq \gamma \neq 90°$	2
Monoclinic	$a \neq b \neq c$ $\alpha = \gamma = 90°$ $\beta \neq 90°$	13
Orthorhombic	$a \neq b \neq c$ $\alpha = \beta = \gamma = 90°$	59
Tetragonal	$a = b \neq c$ $\alpha = \beta = \gamma = 90°$	68
Hexagonal	$a = b \neq c$ $\alpha = \beta = 90°$ $\gamma = 120°$	27
Rhombohedral	$a = b = c$ $\alpha = \beta = \gamma \neq 90°$	25
Cubic	$a = b = c$ $\alpha = \beta = \gamma = 90°$	36

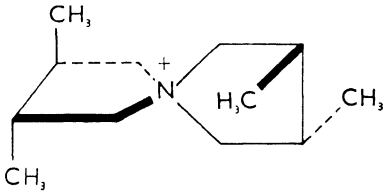
Crystal systems. The shape of the unit cell of a crystal is dependent on the symmetry elements present and seven distinct types of cell can

† x, y, z are fractional co-ordinates with respect to the unit-cell axes a, b, c.

be recognised. These are known as the seven *crystal systems* and are described in Table 1.

Most organic crystals belong to either the monoclinic or the orthorhombic system.

Implications of the space group. If a molecule is not to be superposable on its mirror image, it must not possess an inversion axis. Normally the absence of a centre ($\bar{1}$) and a mirror plane ($\bar{2}$) of symmetry suffices to produce a dissymmetric molecule, though a substance whose molecules lacked these elements of symmetry but possessed a four-fold inversion axis ($\bar{4}$), would not be resolvable. This actually happens in the case of the cation (4).



(4)

Space groups which include glide planes or inversion axes are forbidden to crystals of the D or L forms of dissymmetric molecules, and most optically active organic compounds crystallise in the space groups $P2_1$ and $P2_12_12_1$.

Space groups which include glide planes and inversion axes among their elements of symmetry are used by *meso* compounds and racemates. The most frequently occurring space group of this type is $P2_1/c$ which has four equivalent positions—(x, y, z); $(-x, -y, -z)$; $(x, \frac{1}{2}-y, \frac{1}{2}+z)$; $(-x, \frac{1}{2}+y, \frac{1}{2}-z)$. If there are four molecules in such a cell two of them, at (x, y, z) and $(-x, \frac{1}{2}+y, \frac{1}{2}-z)$, must be of, say, the D form while the other two, at $(-x, -y, -z)$ and $(x, \frac{1}{2}-y, \frac{1}{2}+z)$, must be of the enantiomorphic L form. If there are only two molecules present in a unit cell belonging to the space group $P2_1/c$, each molecule must necessarily be centrosymmetrical, the asymmetric crystal unit consisting of half a molecule. Such a determination of molecular symmetry can frequently be useful when allocating a structure to a compound.

For example, in the case of α, β-diethyldibenzyl $[(C_6H_5CHEt)_2]$ two isomeric forms are known, one solid at room temperature and the other liquid. One of these must be the centrosymmetrical internally compensated *meso* form and the other the racemate. A study of the solid form showed that it has two molecules in the unit cell and belongs to the space group $P2_1/c$. It must therefore be the *meso* compound.

Another example is provided by the vinylideneamine (2). The space group is such that the molecule must have a two-fold rotation axis along the chain, the asymmetric crystal unit being half a molecule.

An important consequence is that the C–C–N–C chain must be exactly linear.

Quite generally, apart from any question of deducing molecular symmetry, the determination of the space group of a substance prior to a complete structure analysis is necessary in order to reduce to a minimum the number of atoms to be located in the unit cell. One need be concerned only with the atoms in the asymmetric crystal unit, the remaining atoms in the unit cell being placed automatically by the symmetry operations of the space group.

Lattice planes. The orientation of a set of parallel planes in a crystal lattice can be described by the relative intercepts on the three axes *a*, *b*, and *c*, the reciprocals of these intercepts leading to the *Miller indices* (*hkl*). A set of planes with indices (213) is shown in Fig. 3. The intercept on *a* is *a*/2 so that *h* is 2, the intercept on *b* is *b* so that *k* is 1, and the intercept on *c* is *c*/3 so that *l* is 3. Indices involving negative *h*, *k* or *l* can occur. The symbol $\bar{x}$ rather than $-x$ is normally employed for a negative index.

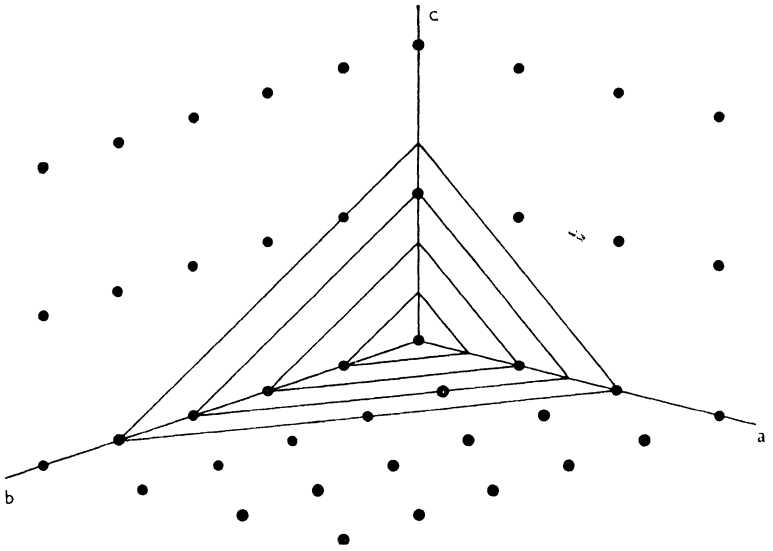


FIG. 3. A set of planes with Miller indices (213)

Diffraction of X-rays by crystals. When radiation is scattered from a row of points, the scattered rays interfere destructively with each other except for a few directions where the path lengths of rays scattered from successive points differ by a whole number of wavelengths. If the wave-

length of the radiation is λ and the angles which the incident and scattered rays make with the row of points of spacing a are ϕ_0 and ϕ respectively, the condition for reinforcement is

$$a \cos \phi_0 - a \cos \phi = h\lambda,$$

where h is an integer.

In a crystal there are parallel rows of points along the three lattice directions a, b, c, and the diffraction process is governed by three such equations (called Laue equations), involving three integers h, k, l, and the directions of the incident and scattered beams with respect to the axes. The direct application of these equations to the diffraction of X-radiation by crystals is rather difficult. Fortunately, however, it can be shown that the diffraction process, described by the three Laue equations, is equivalent to reflexion of the incident X-ray beam from the crystal plane with Miller indices (hkl), provided that the plane is oriented to the incident beam at an angle θ determined by the Bragg equation

$$\lambda = 2d \sin \theta,$$

where d is the interplanar spacing.

The Bragg equation implies that if a crystal is set in a random position in a monochromatic X-ray beam, there will in general be no diffraction, for it is unlikely that any of the planes in the crystal with suitable spacings will be correctly oriented. (Since $\sin \theta$ cannot exceed unity, only planes with spacings greater than $\lambda/2$ are accessible.) If the crystal is rotated, however, the Bragg equation will be satisfied at certain definite orientations of the crystal, and reflected beams will be observed.

The spacings of the crystal planes can be derived in terms of the Miller indices and the unit-cell constants. Substituting these into the Bragg equation, an expression for $\sin^2 \theta$ can be obtained. In the monoclinic case, for example,

$$\sin^2 \theta = \frac{\lambda^2}{4}\left(\frac{h^2}{a^2 \sin^2 \beta} + \frac{k^2}{b^2} + \frac{l^2}{c^2 \sin^2 \beta} - \frac{2hl \cos \beta}{ac \sin^2 \beta}\right)$$

X-Ray Diffraction: Experimental Procedure

In any diffraction experiment the measurable quantities are (i) the directions at which diffraction occurs from the specimen and (ii) the relative intensities of the diffracted beams. The deduction of molecular structure from such X-ray data is a lengthy process which is dis-

cussed in a later section (p. 292). Typical experimental procedures for recording the data and two important preliminaries, the determination of the unit-cell dimensions and of the space group, are considered first.

The sample. Single-crystal methods are almost invariably necessary for the detailed study of an organic compound. Individual crystals in the sample, therefore, should be large enough to be readily seen by eye, that is, they should have dimensions about 0·05 to 0·5 mm. If the crystals are larger than this, it is usually a simple matter to cut them to size with a razor blade.

To obtain well-formed single crystals, slow growth from dilute solution is advisable. The choice of solvent is a matter for experiment.

Provided that they are stable when irradiated by X-rays, a few good crystals suffice for the whole of the experimental side of a crystal structure investigation—one or two crystals from which to record the X-ray reflexions, and one or two for a density determination. All the work, if necessary, could be carried out with one crystal, though this would involve a good deal of experimental skill on the part of the operator. When, as is the case with proteins, the crystals decompose slowly on irradiation, a much larger quantity of material is required since only a limited number of reflexions can then be measured from any one crystal.

X-ray spectrometers designed for low-temperature studies are readily available so that the method is not confined to substances which happen to be solid at room temperature. A considerable number of structure analyses employing data collected at about the temperature of liquid air have in fact been reported: cyclo-octatetraene and the boron hydrides are typical compounds studied in this way.

Types of photograph. A number of different X-ray techniques are available for the measurement of unit-cell dimensions. Some of the methods also give essential information about the symmetry of the cell, and provide a convenient record of the relative intensities of the various reflexions.

In order to be able to manipulate the small crystal, it is customary to attach it by an adhesive to a thin glass fibre, the other end of the fibre being embedded in a lump of plasticine. The plasticine is then placed on top of a *goniometer head* which consists of two arcs, which can be adjusted to alter the tilt of the crystal, and two horizontal movements which serve to centre the crystal in the X-ray beam.

The rotation photograph. For this type of photograph the crystal has to be mounted with one axis, say *b*, vertical. The rows of lattice points with spacing *b* perpendicular to the X-ray beam are then responsible for

diffraction, the condition for reinforcement of the scattered rays (see Fig. 4) being

$$n\lambda = b \sin \phi_n.$$

When $n = 1, 2, 3$, etc., a set of cones with semi-vertical angles $(90 - \phi)°$

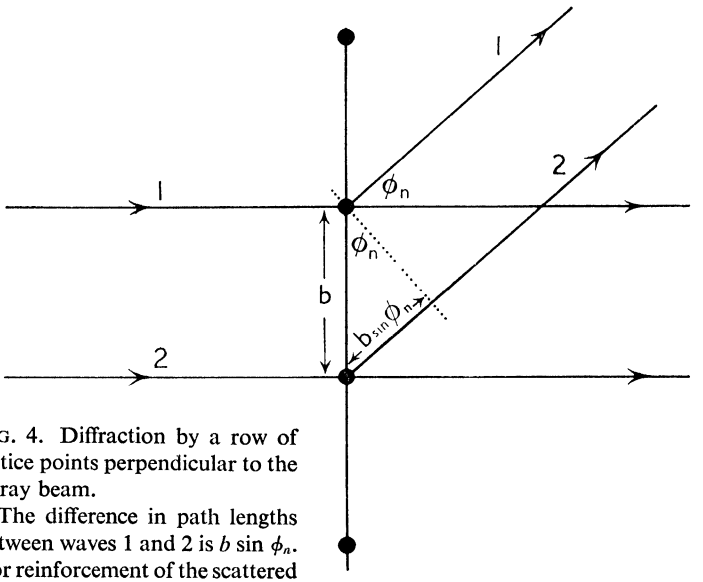


FIG. 4. Diffraction by a row of lattice points perpendicular to the X-ray beam.

The difference in path lengths between waves 1 and 2 is $b \sin \phi_n$. For reinforcement of the scattered waves this difference must be a whole number of wavelengths.

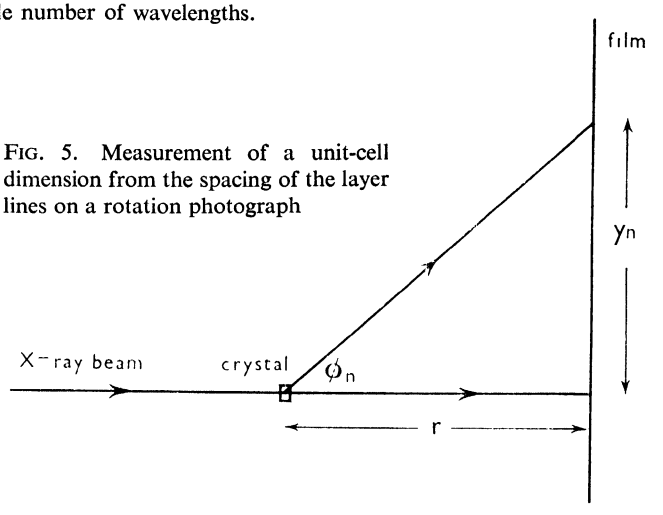


FIG. 5. Measurement of a unit-cell dimension from the spacing of the layer lines on a rotation photograph

is defined. These cones intersect a cylindrical film surrounding the crystal at a distance r in a series of lines known as *layer lines*. If the distance between the equatorial layer and the nth layer is y_n, then (Fig. 5),

$$\tan \phi_n = y_n/r,$$

and consequently,

$$b = n\lambda \operatorname{cosec} (\tan^{-1} y_n/r).$$

For diffraction to occur, the Bragg equation has also to be satisfied. The crystal, therefore, is rotated about its vertical axis in order to bring each crystal plane into the appropriate orientation for reflexion. As only a limited number of planes is accessible, the layer lines are not continuous but are broken up into discrete spots: this can be seen clearly on the typical rotation photograph shown in Fig. 6.

The oscillation photograph. Because of the large number of spots crowded together along each layer line, the assignment of Miller indices to the various reflexions on a rotation photograph can be very difficult. The photograph, moreover, gives no direct indication of the symmetry in the crystal. These difficulties can be overcome by limiting the angular movement about the vertical axis to 15° (or some other small angle), the resultant photograph being termed an oscillation photograph.

If corresponding layer lines above and below the equatorial layer are identical in appearance in an oscillation photograph, the implication is that there is either a symmetry axis parallel to, or a plane of symmetry perpendicular to, the axis of oscillation. A triclinic crystal yields no symmetrical oscillation photograph. When oscillated about the unique axis (b), a monoclinic crystal yields a symmetrical photograph, whereas when oscillated about any other axis the photograph is unsymmetrical. An orthorhombic crystal yields symmetrical photographs when oscillated about any of the cell edges.

The Weissenberg photograph. The most satisfactory way of separating the various reflexions is by a moving-film technique, of which the most widely used is that due to Weissenberg. In this method the rotation of the crystal is coupled to a to-and-fro movement of the film, parallel to the axis of rotation of the crystal, and a pair of metal screens is used to isolate one particular layer of reflexions. If b is chosen as axis of rotation the ($h0l$) reflexions are recorded on the equatorial layer photograph and the ($h1l$), ($h2l$), etc., on the upper layer photographs. A typical Weissenberg photograph is shown in Fig. 7.

The fibre photograph. Fibrous materials such as cellulose, wool

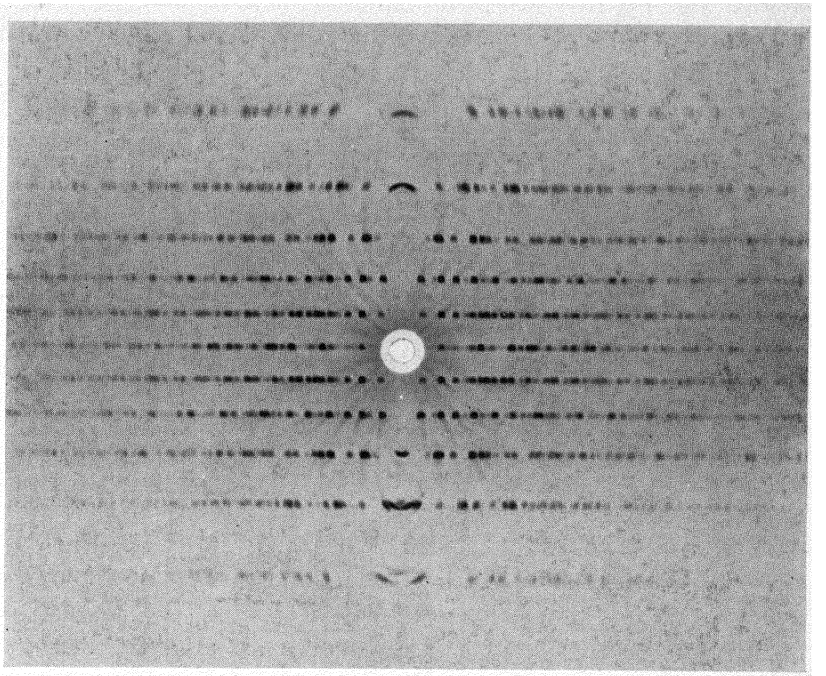


FIG. 6. A rotation photograph (methyl bromogibberellate)

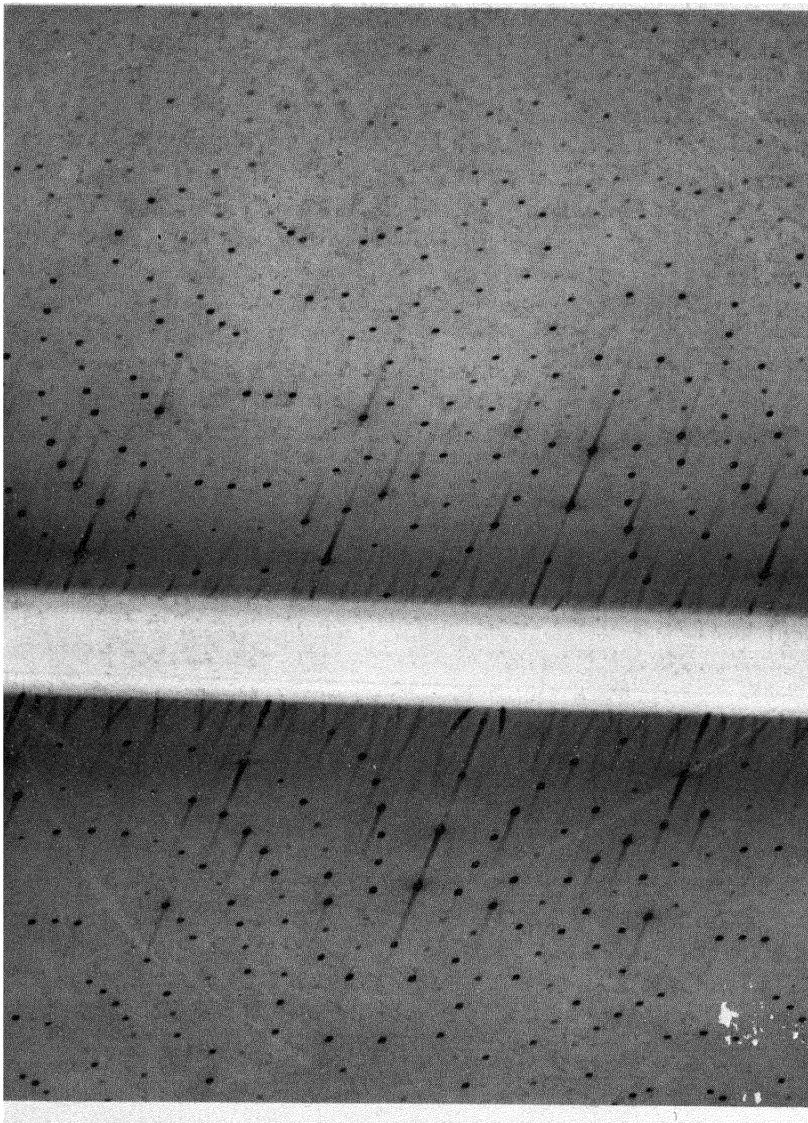


FIG. 7. A Weissenberg photograph (methyl bromogibberellate)

and silk consist of chainlike polymer molecules. In general, small bundles of molecules, in more or less good alignment, are present in the fibre, and these bundles are referred to as *micelles*. The micelles pack together with their chain directions in common but with random lateral orientation. If a monochromatic beam of X-radiation is passed through the fibre at right angles to its length, the effect of the random lateral orientation of the micelles is that the diffraction pattern obtained is similar to the rotation photograph given by a single crystal, though the spots are usually somewhat diffuse because of the small size of the micelles and the deviations of the chains from the common direction.

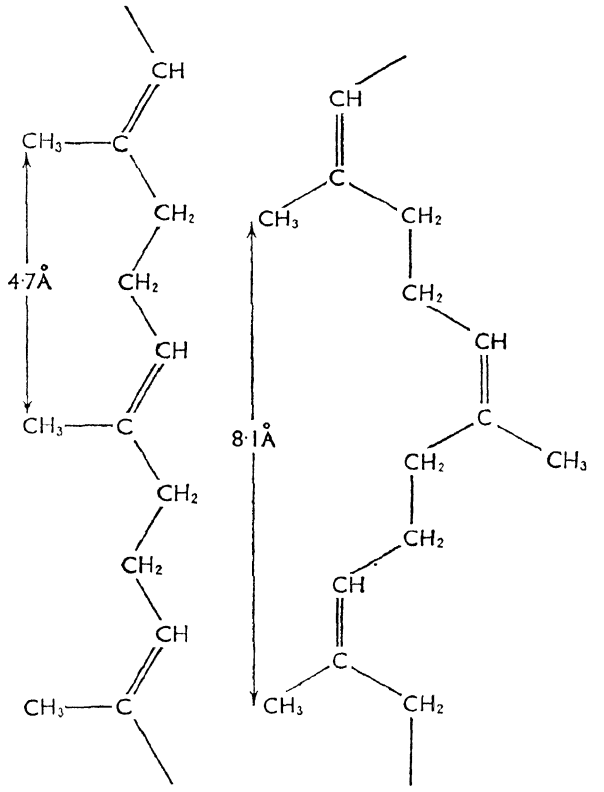


FIG. 8. Configuration of the polyisoprene chains in gutta-percha (*trans*) and rubber (*cis*)

The repetition distance along the chain can be determined from the separation of the layer lines and this information combined with

chemical knowledge often suffices for the molecular structure to be deduced. For instance, in natural rubber the chain repeat distance is 8·1 Å, corresponding to two isoprene units, while in gutta-percha, another isoprene polymer, the repeat distance of 4·7 Å corresponds to one isoprene unit. These observations can be explained if the configuration about the double bonds is *cis* in rubber and *trans* in gutta-percha (see Fig. 8).

The powder photograph. If a powdered specimen (contained in a thin-walled glass tube) is irradiated with monochromatic X-radiation, those of the crystals which are correctly oriented will give reflexions. As the component crystals occupy all possible orientations in the sample, each reflexion occurs as a cone of rays with semi-vertical angle 2θ, where θ is the Bragg angle. If a cylindrical strip of photographic film surrounds the sample, a pair of arcs will be formed where each cone cuts the film, the separation of the two arcs giving a measure of 4θ and hence of d, the interplanar spacing.

Since the spacings d are related in a fairly complicated way to the unit-cell parameters, the indexing of a powder photograph and the subsequent deduction of the unit-cell parameters is in general very difficult, and one of the main applications of the powder method is simply identification, each crystalline substance yielding a completely characteristic pattern of lines of varying intensity.

Determination of the space group. Translational symmetry elements cause a number of X-ray reflexions to be unobserved, the absences being referred to as *systematic absences.*

The systematic absences corresponding to the various space groups are listed in ref. 14 and serve to identify uniquely a number of space groups. If, for example, the only absences are (h00) when h is odd, (0k0) when k is odd, and (00l) when l is odd, the space group must be the orthorhombic $P2_12_12_1$. Similarly, if the only absences are (0k0) when k is odd and (h0l) when l is odd, the space group must be the monoclinic $P2_1/c$.

Translation-free elements of symmetry, such as centres of symmetry or mirror-planes, do not give rise to systematic absences, and in consequence space groups which differ among themselves only by the presence of these symmetry elements give exactly the same systematic absences and so cannot be distinguished on this basis. If the substance is optically active, space groups with mirror planes or centres of symmetry can be excluded. If the substance is not optically active, the ambiguity can usually be overcome by examining the distribution of intensities among the reflexions, a centrosymmetrical structure giving

rise to a greater proportion of weak reflexions than a non-centrosymmetrical structure.

The number of molecules in the unit cell. The volume of the unit cell can be derived from the cell dimensions. In the case of an orthorhombic crystal, for example,

$$V = abc,$$

while in the case of a monoclinic crystal

$$V = abc \sin \beta.$$

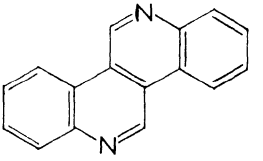
The product of the volume of the unit cell and the density (ρ) of the crystalline material yields the total weight of the contents of the cell. The contents must comprise an integral number (Z) of molecules of the compound under examination and possibly also a number of molecules of solvent. If the crystals are free of solvent,

$$ZM = \rho VN,$$

where M is the molecular weight of the compound and N is Avogadro's number. If solvent molecules are present, their weight must be subtracted from the right-hand side of the above equation.

An accurate value for the molecular weight can be obtained in this way provided that the number of molecules in the cell is known. If a rough estimate of the molecular weight is available this serves for the deduction of Z. If nothing is known about the magnitude of the molecular weight, the space group multiplicity may limit the number of possibilities: in the space group $P2_12_12_1$, for example, the number of molecules in the cell must be a multiple of four.

In the case of calycanine, a degradation product of the alkaloid calycanthine, two molecular formulae, $C_{16}H_{10}N_2$ and $C_{21}H_{15}N_3$, were under consideration when the unit-cell dimensions were determined by Hargreaves. His measurements indicated that the C_{21} formula would require the presence of $1\frac{1}{2}$ molecules in the cell and so cannot be correct. The C_{16} formula requires two molecules in the cell. The space group, moreover, is $P2_1/c$ which has four-fold multiplicity and as the unit cell contains only two molecules these must be centrosymmetrical (cf. p. 283). Chemical evidence established the structure (5) at a later date.



(5)

Measurement of intensities. The various X-ray reflexions are usually recorded on photographic film (e.g. the Weissenberg method) and the relative intensities of the spots estimated visually by comparison with a set of standard spots obtained by exposure of a film to a diffracted beam for known periods of time. In order to accommodate a sufficient range of intensities, the *multiple-film technique* is employed, a pack of four or five films, placed one behind the other, being exposed simultaneously. Since the intensity falls off from one film to the next by a factor of about three, spots which are too black to estimate on the uppermost film can be estimated on the lower films.

With visual estimates, the random errors in the intensities amount to about $\pm 20\%$. Much more accurate results ($\pm 5\%$) can readily be obtained with counter spectrometers and these are gradually coming into widespread use.

X-Ray Diffraction: Structure Analysis

The structure amplitude. The intensity of an X-ray reflexion from a crystal depends on the atomic arrangement in the crystal and on the experimental procedure used to record the reflexion. It is possible to write

$$I(hkl) = |F(hkl)|^2 \times Lp,$$

where $I(hkl)$ is the intensity of the reflexion and Lp is a function of the angle θ, the precise form of the function depending on the experimental procedure. For further details *International Tables for X-Ray Crystallography*, Vol. 2 (1959)[15] should be consulted.

The quantity $|F(hkl)|$, known as the *structure amplitude*, is the amplitude of the wave resulting from the combination of the waves scattered by all the atoms in the unit cell in the direction required for the reflexion, and is therefore a function of the number, positions and *scattering factors* of the atoms. (The scattering of X-rays is due to the electrons of the atoms and the atomic scattering factor, f, is defined as the ratio of the amplitude of the wave scattered by all the electrons of an atom to the amplitude which would be scattered by a single electron. For very small scattering angles f approaches the number of electrons associated with the atom, but at higher angles its value is reduced by interference between the waves scattered by the various electrons.)

When the positions of the various atoms in the unit cell are *known*, it is possible to calculate all the structure amplitudes* and compare the

* The equation used for calculating the structure amplitudes is $|F(hkl)| = \sqrt{A^2 + B^2}$,

results with the structure amplitudes derived from the intensities. A measure of the disagreement is provided by the average discrepancy

$$R = \frac{\Sigma \, | \, | F_o | - | F_c | \, |}{\Sigma \, | F_o |}$$

that is, the sum of the differences, irrespective of sign, between the observed ($| F_o |$) and calculated ($| F_c |$) structure amplitudes expressed as a fraction of the sum of all the observed amplitudes. For the postulated structure to be acceptable, the average discrepancy should be small, say less than 0·2. If it is greater than this, some or all of the atomic coordinates need adjustment.

Before the above test can be applied the positions of the atoms must be known. Unfortunately, although the structure amplitudes can be calculated if the atomic arrangement is known, the converse calculation cannot be accomplished directly. The determination of the atomic arrangement requires a knowledge not only of the amplitudes of the scattered waves, but also of their phases relative to an origin in the unit cell. Unlike the structure amplitudes the phase constants, α, cannot be measured directly, although they can be calculated if the atomic arrangement is known.† The determination of the positions of the atoms therefore involves a vicious circle referred to as the *Phase Problem.*

The closely related problems of determining the atomic arrangement and of calculating the phase constants will now be considered in turn.

Fourier synthesis. An important consequence of the periodicity of a crystal structure is the possibility of representing the distribution of scattering matter, that is, the electrons, by means of a Fourier series involving the structure amplitudes and phase constants. The series is of the form

$$\rho(xyz) = \frac{1}{V} \sum_h \sum_k \sum_l | \, F(hkl) \, | \cos \{2\pi \, (hx+ky+lz) - \alpha(hkl)\},$$

where $\rho(xyz)$ is the electron density at the point with fractional coordinates x, y, z, and V is the volume of the unit cell. The summations

---

where

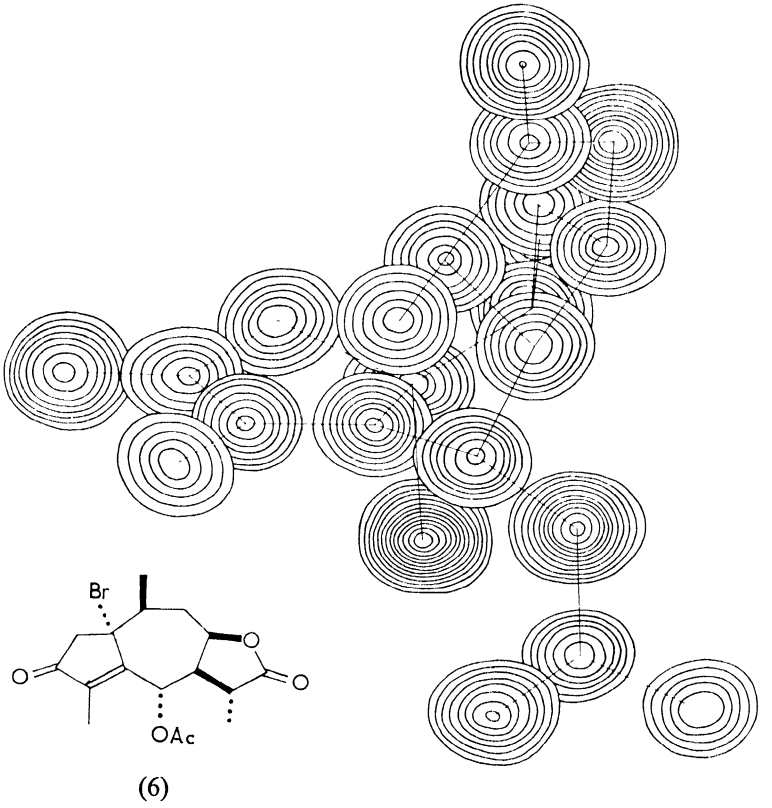
$$A = \Sigma f_i \cos 2\pi \, (hx_i + ky_i + lz_i),$$
$$B = \Sigma f_i \sin 2\pi \, (hx_i + ky_i + lz_i).$$

Here x_i, y_i, z_i are fractional coordinates of the ith atom with respect to the unit-cell axes and f_i is the corresponding scattering factor. The summations are taken over all the atoms in the unit cell. When the space group is known the expressions can be modified so that the summations are over only the atoms of the asymmetric unit. (See ref. 14.)

† The relevant equation is $\alpha(hkl) = \tan^{-1} B/A$.

are taken over all possible values of h, k, and l. The zero term of the series, $F(000)$, is the total number of electrons in the unit cell.

In the derivation of the electron-density distribution it is convenient to consider a set of equally spaced sections through the cell. On each of the sections the electron density is evaluated at a regular set of points, for example at every $\frac{1}{60}$th of the cell sides, and contours of equal electron density are drawn by interpolation in the results. When the various contoured sections are placed on top of each other a three-dimensional representation of the electron-density distribution is obtained, the maxima corresponding to atomic positions. In Fig. 9 are shown superimposed contour sections illustrating the three-dimensional electron-density distribution in bromogeigerin acetate (6).



(6)

FIG. 9. Contour sections through the atoms, superimposed to show the three-dimensional electron-density distribution over a molecule of bromogeigerin acetate

The computation of a triple Fourier series involves a vast amount of work, for upwards of a thousand structure amplitudes and 60^3 values of the variables x, y, and z require to be considered. Before the advent of punched-card machines such calculations were rare. In more recent times electronic computers have brought the method into widespread use.

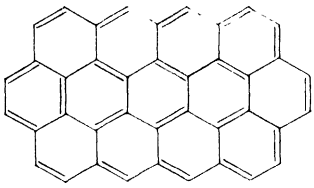
In many cases where it is desired to ease the computational burden, it is possible to employ a double Fourier series,

$$\rho(xy) = \frac{1}{A}\sum_h \sum_k | F(hk0) | \cos \{2\pi (hx+ky) - \alpha(hk0)\},$$

to yield a projection of the electron density, A being the projected area of the unit cell. The summations can readily be carried out on a desk calculating machine since only reflexions of a single zone, such as ($hk0$), and two variable coordinates, x and y, are involved. However, unless one axis of the unit cell is short, say less than 6 Å, the atoms will not all be resolved in projection and the accurate location of the atoms will be very difficult. Even if good resolution is obtained in one projection, it is almost certain that there will be considerable overlap in projections along the other axes. In unfavourable cases, in fact, it is possible to obtain electron-density projections which are not readily interpretable unless the atomic positions are known beforehand. In such cases the method cannot usefully be used for the assignment of a constitution and stereochemistry to a molecule and resort must be made to three-dimensional methods.

The Phase Problem. In the calculation of an electron-density distribution by means of a Fourier series both the structure amplitudes $| F |$ and the phase constants α are involved. The structure amplitudes can be derived in a straightforward manner from the intensities of the X-ray reflexions, but unfortunately there is no way of measuring the phase constants. Since the electron density has to correspond to the correct number of atoms arranged in a chemically acceptable way and without any regions of negative density, it has been claimed that the *Phase Problem* ought to have a general solution based solely on the measured structure amplitudes. However, so far, in spite of a great deal of theoretical work, no such method, capable of solving any unknown structure of considerable complexity, has come into general use.

In the absence of a direct solution of the *Phase Problem*, it is usually necessary to derive approximate values of the phase constants by calculation based on approximate positions for at least some of the atoms in the structure. A Fourier synthesis using these *calculated* phases and



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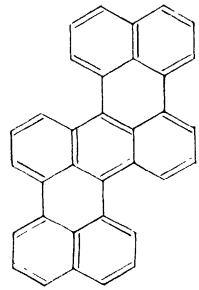

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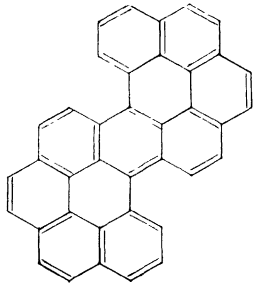

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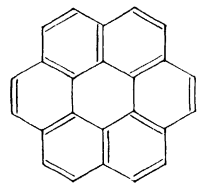

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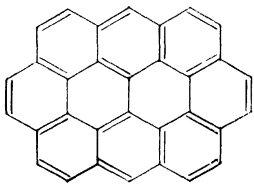


(11)

the *observed* structure amplitudes will then yield a rather better representation of the structure. This allows more exact atomic locations to be derived and these, in turn, lead to more accurate phases. The cycle of operations is repeated until satisfactory agreement between the observed and calculated structure amplitudes is obtained.

In order to obtain the initial approximate set of phase constants a number of procedures can be used.

1. *Trial and error.* When the chemical structure is known, or when there are only a few alternatives, it is possible on the basis of past experience of interatomic distances and interbond angles to try various sets of likely positions in the cell until some agreement between observed and calculated structure amplitudes is obtained.

In the case of aromatic hydrocarbons, this method has been widely used since, to a good approximation, the benzene rings involved can be treated as regular hexagons of side 1·4 Å. The case of circumanthracene (7) provides an interesting example. When di*peri*naphthyleneanthra-

cene (8) is condensed with maleic anhydride and then decarboxylated with soda-lime the expected product (9) is accompanied by another hydrocarbon. The space group of this is $P2_1/a$ and the unit-cell dimensions and crystal density indicate that, if there are two centrosymmetric molecules in the cell, their formula must be $C_{40}H_{16}$. A comparison of the cell dimensions with those of coronene (10) and ovalene (11) suggested the structure (7). A trial set of atomic positions based on the analogy with coronene and ovalene appeared promising and further refinement by two-dimensional methods led to satisfactory agreement between observed and calculated structure amplitudes and so confirmed (7).

2. *The heavy-atom method.* When dealing with a molecule of unknown chemical structure the method of trial and error is obviously impracticable. In such circumstances the solution of the *Phase Problem* must usually depend on having present in the unit cell a few atoms whose atomic numbers are considerably greater than those of the remaining atoms.

The introduction of a heavy atom can be accomplished in a number of ways. If, for example, the molecule contains a hydroxyl group, an iodoacetate may be formed. This was the derivative chosen in the case of limonin, the bitter principle of citrus fruits. Borohydride reduction of the ketone group yielded epilimonol, which was then converted into its chloroacetate and thence into its iodoacetate. Structure (3) was then established for this derivative by means of a crystal-structure determination and independent chemical studies.

Other procedures involve the conversion of an acid into a potassium or rubidium salt, and of a basic substance such as an alkaloid into a hydrobromide or methobromide. Crystallisation from a solvent such as methylene bromide can, on occasion, yield a solvate suitable for detailed X-ray study.

For the heavy-atom method to work effectively it is desirable (though not essential) that the sum of the squares of the atomic numbers of the heavy atoms should equal or exceed the sum of the squares of those of the light atoms. Thus for a molecule containing a total of about thirty carbon, nitrogen and oxygen atoms, one bromine atom or four chlorine atoms should suffice.

When the heavy atoms in the unit cell have been located (usually by method 4) phase constants based on these atoms alone are calculated and are used with the experimental structure amplitudes to derive an electron-density distribution. This is then examined for peaks representing the light atoms, normally carbon, nitrogen and oxygen. The

situation at this stage is generally confused by the presence in the Fourier synthesis of false maxima due to the phase-constant errors, and the crystallographer requires to make use of past experience of interatomic distances and interbond angles to identify chemically sensible fragments

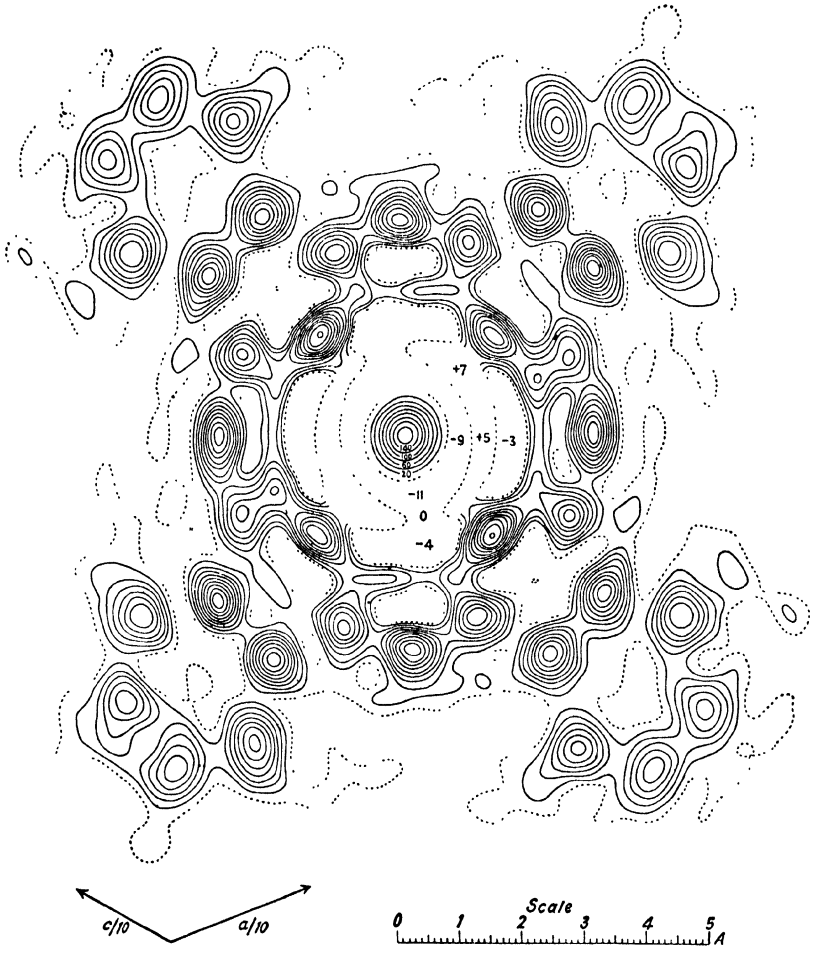
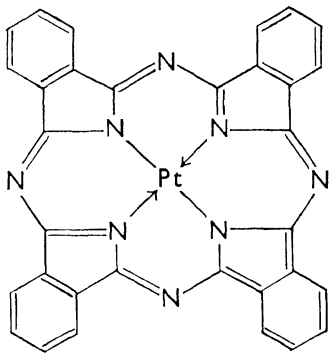


FIG. 10. Electron-density projection of platinum phthalocyanine (from Robertson and Woodward, *J. Chem. Soc.*, 1940, 38)

of the structure. The problem is naturally considerably easier in the three-dimensional case, where correct distances and angles are readily observable, than in the two-dimensional case where overlapping atoms can nevertheless have a large separation along the direction of projection.

When a number of atoms have been positioned they are included in a second calculation of phase constants and the iterative process of Fourier synthesis and phase-constant calculation is continued until the structure has been established. Although an atom may be assigned with confidence to a peak in the electron-density distribution at an early stage of the refinement procedure, it is usually only at a considerably later stage that it is possible to decide from a consideration of the height of the peak and the lengths and directions of the bonds radiating from the peak what type of atom is involved. If there are doubts about the assignment, a knowledge of the number and kinds of oxygen and nitrogen functions in the molecule can be of considerable help.

The method is particularly direct for a centrosymmetrical structure with the heavy atom situated at a centre of symmetry which can be taken as the origin. Platinum phthalocyanine (12) provides such an example and Fig. 10 shows the electron-density map Robertson and Woodward obtained for this molecule.



(12)

The arrangement of the heavy atoms in the unit cell can exhibit on occasion a higher symmetry than the true space-group symmetry and this can complicate the selection of genuine atomic sites in the first approximate electron-density distribution. The troublesome pseudo-symmetry is gradually destroyed in succeeding syntheses as additional atoms are included in the calculation of the phase constants.

In view of possible symmetry troubles it is worthwhile when planning an X-ray study of a natural product by way of the heavy-atom method to try to prepare more than one derivative. A preliminary study of the unit-cell dimensions and space groups often indicates that one substance is more suitable than the rest for further detailed study.

3. *Isomorphous replacement.* In this method evidence is obtained about the phase constants by comparing the X-ray intensities from a pair (or a series) of compounds whose molecular and crystal structures differ only in respect of one or more replaceable atoms. Examples of such isomorphous compounds are frequently provided by chloro- and bromo-compounds and by potassium and rubidium salts.

The method is most easily applied to centrosymmetrical isomorphous

crystals differing at one atomic site. A notable example is provided by Robertson's determination of the crystal and molecular structure of phthalocyanine with the aid of the isomorphous nickel derivative.

In the non-centrosymmetrical case, the isomorphous replacement method is rather more difficult to apply, but nevertheless it is playing an important role in the elucidation of protein structures. In this case ambiguities arise when only two isomorphous derivatives are examined, but can be overcome by the study of one or more further derivatives involving isomorphous substitution at a different site.

4. *The Patterson function.* It was shown by Patterson in 1934 that a Fourier synthesis based on $|F|^2$ rather than $|F|$ and α, that is,

$$P(uvw) = \frac{1}{V^2}\sum_h\sum_k\sum_l |F(hkl)|^2 \cos 2\pi(hu+kv+lw),$$

provides information about the vector distances between pairs of atoms. A peak at (uvw) in the Patterson function implies that there are two atoms in the unit cell with

$$\begin{aligned} x_1 - x_2 &= u \\ y_1 - y_2 &= v \\ z_1 - z_2 &= w \end{aligned}$$

When there are N atoms in the unit cell, there are approximately N^2 Patterson peaks, and the resultant considerable overlapping of individual maxima makes the correct location and assignment of peaks to pairs of atoms extremely difficult unless there are favourable circumstances.

Since the height of a peak depends on the product of the atomic numbers of the atoms involved, interpretation of the Patterson function is greatly facilitated when a small number of heavy atoms is present in the cell. The high peaks due to pairs of heavy atoms can then readily be identified and coordinates assigned to these atoms.

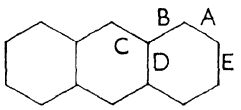
X-Ray Diffraction: Applications

Useful results can often be obtained from a preliminary study of unit-cell dimensions and space groups: some examples have already been given.

A number of the more usual applications of detailed crystal-structure studies are described below.

Bond length measurements. The determination of bond lengths and angles in organic molecules (aromatic compounds in particular) is frequently undertaken to provide experimental criteria by which theo-

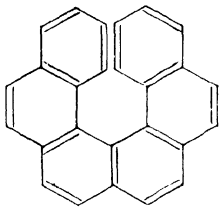
retical models for molecules can be judged. As a typical example the experimental bond lengths for anthracene are compared below with theoretical values obtained by the molecular-orbital method.



Bond	*X-ray meas.*	*M.O. calcs.*
A	1·370 Å	1·382 Å
B	1·423	1·420
C	1·396	1·406
D	1·436	1·430
E	1·408	1·410

Intramolecular steric effects. A consequence of the balance between the strongly repulsive forces arising between non-bonded atoms at small interatomic distances and the weakly attractive forces predominant at large distances is that fairly definite minimum separations occur between non-bonded atoms. These separations have been tabulated in the form of van der Waals radii.

For many substances, undistorted molecular structures would involve smaller approaches between non-bonded atoms of the same molecule than the usual van der Waals gaps and molecular distortion therefore occurs. A striking example of the degree of distortion possible in an aromatic compound is provided by phenanthrophenanthrene (13) which must deviate considerably from planarity. Molecular dissymmetry arises



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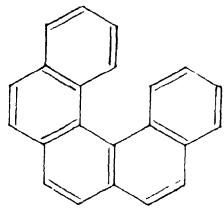


(14)

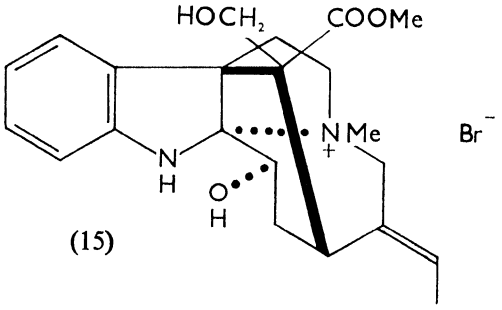
as a consequence of the overcrowding and the hydrocarbon has been resolved. Detailed X-ray studies can yield quantitative measurements of the molecular distortions in such compounds. In 3,4: 5,6 dibenzophenanthrene (14) the closest approach of non-bonded carbon atoms would be 1·4 Å if the molecule were planar. The molecule is in fact helical and the separation of the interfering carbon atoms is increased to 3·0 Å.

Benzene derivatives containing *ortho* groups also provide examples of molecules exhibiting distortion. In *o*-chlorobenzoic acid, the exocyclic C – C and C – Cl bonds are displaced slightly away from the ideal radial positions and the carboxyl group is rotated 14° out of the plane of the benzene ring. In 2-chloro-5-nitrobenzoic acid, the larger angle

of twist of the carboxyl group (23°) can be attributed to the buttressing effect of the nitro group in the 5 position on the hydrogen at position 6. In salicylic acid, on the other hand, the smaller size of the hydroxyl group and the strong intramolecular hydrogen bonding between that group and the carbonyl oxygen of the carboxyl group result in a completely coplanar structure.

Elucidation of incompletely known chemical structures. With the ready availability of modern electronic computing facilities, the evaluation of a three-dimensional electron-density distribution is now a routine matter. In these circumstances the heavy-atom and isomorphous replacement methods can attain their full power and offer attractive alternatives to the usual chemical procedures for the elucidation of molecular structure. If the molecule is complex the X-ray method can often yield a definitive solution more rapidly than can the chemical approach.

A number of substances characterised by the X-ray method have been mentioned before (formulae 1, 3, 6, 7 and 12). Further examples are provided by the quaternary alkaloid salt echitamine bromide (15), by benzylpenicillin (16), and by vitamin B_{12} (17).



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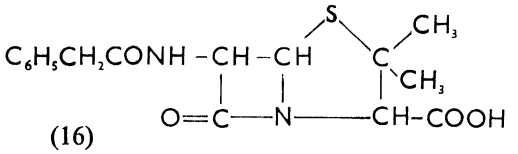
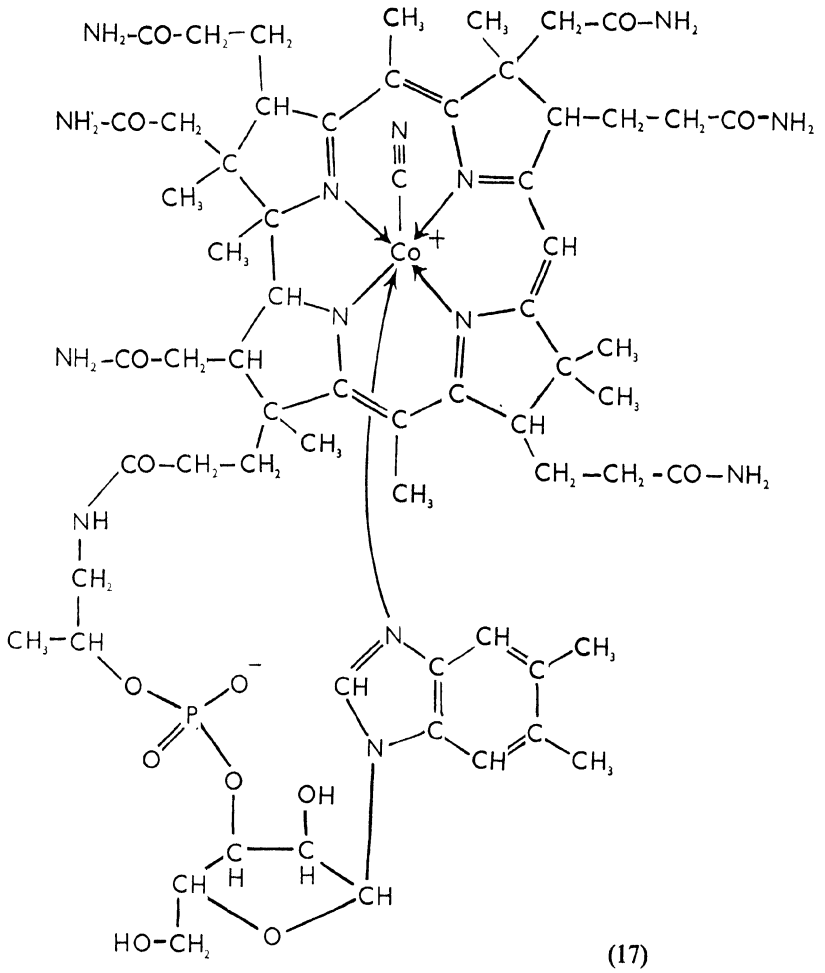


(16)

Numerous globular proteins (insulin, ribonuclease, lysozyme, haemoglobin and myoglobin) are being actively studied at present, generally by the isomorphous replacement method, and the analysis of myoglobin by Kendrew and his colleagues is at an advanced stage. In the unit cell, the myoglobin polypeptide chain is disposed in the form of a helix folded in an intricate manner. The sequence of amino acids along



(17)

portions of the chain has been deduced and it is clear that in the near future the complete sequence will be established.

Assignment of absolute configuration. In normal circumstances the X-ray reflexion (*hkl*) from a crystal has exactly the same intensity as the reflexion ($\bar{h}\bar{k}\bar{l}$). Consequently, if, after completion of the crystal analysis, the directions of the unit-cell axes are reversed, leading to the enantiomorphic structure, this solution equally well satisfies the X-ray intensity data, and the absolute configuration of the molecule remains in doubt.

The equality $|F(hkl)| = |F(\bar{h}\bar{k}\bar{l})|$ breaks down when the X-ray

wavelength is such that the inner electrons of one type of atom are excited. Though the effect is small, it is then possible to distinguish between reflexions (*hkl*) and ($\bar{h}\bar{k}\bar{l}$), and, therefore, also possible to assign an absolute configuration to the molecule concerned.

The first such determination of the absolute configuration of an organic molecule was by Bijvoet and his collaborators who irradiated sodium rubidium D–tartrate with X-rays from a zirconium target in order to excite the rubidium atom. For eleven pairs of reflexions (*hkl*) and ($\bar{h}\bar{k}\bar{l}$), the intensity differences calculated on the basis of the Fischer convention for tartaric acid were consistently of the same sign as the observed differences. The Fischer convention therefore happens to be correct.

As the absolute configurations are now known for a large number of molecules, a simple expedient for obtaining the absolute configuration of a molecule of unknown configuration is to substitute into the molecule an asymmetric group of known absolute configuration. A normal crystal-structure analysis then serves to determine the absolute configuration of the whole molecule, for of the two possible enantiomorphic solutions one can be rejected as incompatible with the known stereochemistry of the substituent group.

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Chapter 9

R. I. Reed

MASS SPECTROMETRY

The functions of a mass spectrometer are to produce positive ions from the substance under investigation, to resolve these ions into a series of ion beams which are homogeneous with respect to their mass/charge ratio, and to measure the relative abundances of the ions in these beams. Most of the early applications of mass spectrometry were in the fields of physics and physical chemistry, but recently the mass spectrometer has become an important tool in organic chemistry, both for a variety of analytical purposes and in the determination of structure. This chapter is confined to a brief survey of those applications which are of the greatest interest to organic chemists. Attention is drawn to recent monographs[1,11-13] and reviews[8,14] which give full details of these applications.

Theory and Operation

Since their introduction, the design of mass spectrometers has been greatly developed, principally by Nier. The various types of instruments have recently been reviewed,[1,11,12] The main commercially available instruments are those in which the ions traverse an arc of 180° under the influence of a magnetic field, and sector instruments in which the arc is 60° or 90°. The principles of operation of a 90° sector instrument are as follows.

The material under examination is introduced into the ionisation chamber of the apparatus (see Fig. 1) in vapour form. There it is bombarded with an electron beam of variable energy [usually energies of 50–70 eV (electron volts) are employed]. The material is ionised by electron impact forming positive ions in accordance with the equation

$$e + XY \rightarrow XY^+ + 2e.$$

These ions are formed in the neighbourhood of a positively charged

plate, the ion-accelerating plate, whose potential is also variable but is customarily at 2 kV. The positive ions are repelled from this plate in accordance with the equation governing electrostatic repulsion

$$\tfrac{1}{2} Mv^2 = eV, \tag{1}$$

where M is the mass of the ion, e the electronic charge, V the potential of the ion-accelerating plate, and v the velocity of the particle. In its

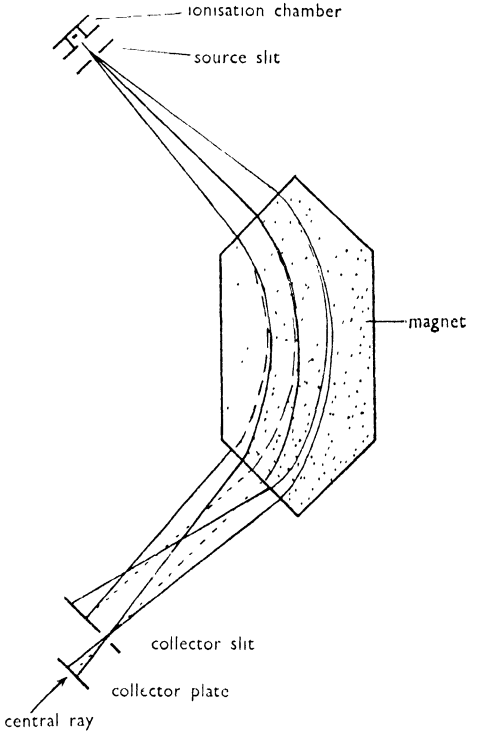


FIG. 1. Sector type mass spectrometer (by courtesy of Associated Electrical Industries Ltd.)

passage through the source the beam of ions is focused on an exit slit by means of subsidiary, charged plates. The ions now pass between the poles of an electromagnet where they are deflected along a circular path according to the relation

$$\frac{Mv^2}{r} = Hev, \tag{2}$$

where r is the radius of the path and H is the field strength of the magnet.

Combining equations (1) and (2) yields

$$\frac{M}{e} = \frac{H^2 r^2}{2V}, \tag{3}$$

from which it follows that, for given values of H and V, ions having different values of $\frac{M}{e}$ follow different paths in the instrument. The magnetic deflection also has a focusing effect upon the ion beams, and beams that are homogeneous with respect to their mass/charge ratio converge to the same focus.

The values of the ion-accelerating voltage and the magnetic field are so adjusted that a selected beam of ions passes through a slit, which is placed at its focus, and falls on the collector—a Faraday cage—placed behind the slit. Other beams which do not, of course, follow the same path in the instrument will not pass through the slit and will not, therefore, arrive on the collector.

It is clear from equation (3) that the values of the ion-accelerating voltage and the field strength of the magnet will determine the mass/charge ratio of the ion beam received at the collector. The usual practice in machines having a 60° or 90° arc path is to maintain the accelerating voltage at a fixed value and to vary the magnetic field. In 180° instruments the magnetic field is held constant and the accelerating voltage is varied.

The magnitude of the ion current arriving on the collector is usually rather small; an abundance of particles will give rise to a current of about 10^{-12} amp. Modern electron multipliers allow a lower limit of detection of *ca.* 10^{-18} amp or about six ions per second. Such currents are usually amplified greatly by means of a suitable electronic device before measurement. The measured current is directly related to the abundance of the ionic species examined.

Machines of this type in which the ions, once formed, are focused only by the action of the magnetic field are known as single-focusing instruments.

Double-focusing instruments. Recently designs have become available in which the ion beam is brought to a focus following electrostatic repulsion and before the ions are magnetically deflected[2] (see Fig. 2). These devices possess exceedingly high resolving power, the applications of which are discussed later. Single-focusing instruments cannot possess such resolution, because some of the ions possess appreciable kinetic energy before acceleration. In single-focusing instruments such ions travel a slightly different path from the bulk of the ions of the same

mass/charge ratio. Consequently a rather broadened ion beam is obtained. With double-focusing machines, these ions follow a divergent path in the electrostatic analyser and do not pass through the focusing

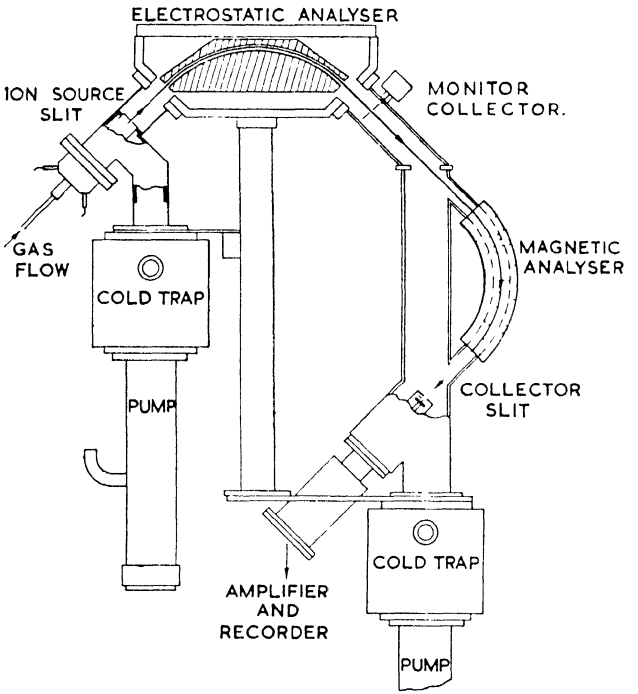


FIG. 2. Double-focusing mass spectrometer (by courtesy of Associated Electrical Industries Ltd.)

slits at its end. Only a well-defined ion beam is therefore admitted to the magnetic analyser.

'Time-of-flight' instruments. A mass spectrometer which operates upon quite different principles has also been introduced recently. This is the 'time-of-flight' instrument.[3] A greatly simplified outline of its mode of operation is as follows.

The ions are formed by electron bombardment of the material. They are then admitted to the analyser by means of an electronic device which allows a sample of all the ions present to enter at the same instant. The analyser consists of a tube, some 40 cm or more in length, at the far end of which is the collector. In front of the collector is a form of electronic shutter, the opening and closing of which is controlled by the device for admitting the ions to the analyser. The ions entering

the tube, having different masses, will travel down the tube with different velocities by reason of the law of electrostatic repulsion (equation 1). The time taken for an ion of any given mass/charge ratio to arrive at the shutter in front of the collector can be determined, and the time delay between admitting the ions and the opening of this shutter can be adjusted to allow ions of selected mass/charge ratio to reach the collector. By varying this time lag all the ions present are allowed in turn to arrive at the collector. As with other instruments, the number of ions of each $\frac{M}{e}$ arriving at the collector may be computed.

Sample requirements. One of the great advantages of mass spectrometric techniques, both of analysis and structure determination, is that the sample requirements are very small. Quantities of the order of 1 mg or even less are sufficient for many purposes.

In nearly all instruments the sample is introduced in vapour form; the provision of heated sample systems allows the volatilisation of relatively involatile material. However, compounds which have vapour pressures of less than 0·1 mm of mercury at 350°, or which vaporise with decomposition, may be unsuitable for routine examination. Such compounds may sometimes be examined as more volatile and more stable derivatives e.g. acids may be converted to esters.

Applications of Isotopic Abundance Measurements

Historically important, and still widely applied, is the ability of mass spectrometers to determine the relative abundance of the various isotopic species in a given material. Thus if the analysis of carbon dioxide shows that the ratio of the ion current of mass 45 ($^{13}C^{16}O_2$) to the ion current of mass 44 ($^{12}C^{16}O_2$) is 1·132%, it follows that the natural abundance of the carbon–13 isotope (i.e. the number of atoms of ^{13}C per 100 carbon atoms) is 1·12%. (The species $^{12}C^{16}O^{17}O$ will also make a contribution to the ion current at mass 45. However, the natural abundance of ^{17}O = 0·041% is so small that this can be neglected except in studies of the highest accuracy.)

It should be noted that mass spectrometers do not, in general, measure isotopic abundance ratios exactly. They do, however, measure variations in abundance with great precision, and the usual procedure is to compare the abundance ratio of an unknown with that of a standard sample. The variation in abundance is then determined with high accuracy.

Small variations in isotopic abundances may be encountered in materials derived from different natural sources. This is of particular

interest in geochemistry, but one application to organic chemistry may be mentioned; it is possible to distinguish fermentation ethyl alcohol from ethyl alcohol produced from ethylene on the basis of deuterium–hydrogen ratios.

Tracer studies. The artificial modification of isotopic abundances provides a valuable method of 'labelling' a particular atom. For example, the esterification of benzoic acid with methanol enriched in ^{18}O gives methyl benzoate enriched in ^{18}O and water with the normal isotopic abundance ratio:

$$C_6H_5COOH + CH_3{}^{18}OH = C_6H_5CO{}^{18}OCH_3 + H_2O$$

This demonstrates clearly that the oxygen of the water is derived from the acid and not from the alcohol. Such tracer studies are of great importance in investigations of reaction mechanisms (see ref. 4). Tracer studies can also reveal whether a certain reaction takes place. For example, nitrogen enriched in ^{15}N has been widely and successfully used in an examination of plants to determine whether or not they, or certain symbiotic organisms associated with them, have the ability to fix atmospheric nitrogen. Essentially the plant grows in an atmosphere containing nitrogen enriched with respect to nitrogen-15 and eventually parts of the plant are removed, the nitrogenous material is reconverted to molecular nitrogen and this is assayed against a sample of ordinary atmospheric nitrogen.

Tracer studies can, of course, be undertaken using radioactive tracers (e.g. ^{14}C); the assay of these does not require a mass spectrometer and is therefore more convenient than that of stable isotopes. There are, however, two common elements for which no satisfactory radioactive isotope has emerged, namely oxygen and nitrogen.

Isotope dilution analysis. The determination of isotopic abundances also forms the basis of a powerful and versatile method for determining the amount of a particular element or compound in a given sample. The estimation of the growth stimulant gibberellic acid in fermentation broths provides an example. A known weight of gibberellic acid in which hydrogen has been replaced by deuterium to a known extent is added to the sample and a specimen of gibberellic acid is then isolated from it (the isolation need not be quantitative!). The deuterium–hydrogen ratio of the isolated material depends on the extent to which the label in the added acid has been 'diluted' by the unlabelled acid in the original sample, and the concentration of the latter can therefore be calculated.

Element identification. Isotopic abundances often provide informa-

tion regarding the presence of a particular element in a compound. This is further discussed below.

Thermochemistry and Cracking Patterns

Thermochemistry. In the production of ions by electron bombardment, the electron must strike the molecule with sufficient energy to expel another electron. As mentioned earlier, the energy of the electron beam can be varied. It is therefore possible to determine the minimum energy necessary to produce ions from a given material (i.e. its *ionisation potential*) by observing the ion current at various electron energies. In practice the application of this simple principle is more difficult. For a description of the problems and of their solution more comprehensive texts[5,11] should be consulted.

As the electron energy is increased above the ionisation potential the abundance of the molecular ion also increases. Eventually the energy is sufficient to cause dissociation of the molecular ion, a dissociation which begins at a definite energy value (the *appearance potential* of the fragment ion) to yield an ion and a neutral radical. This fragmentation may be represented as follows

$$\begin{aligned} e + XY &\rightarrow XY^+ + 2e \\ XY^+ &\rightarrow X^+ + Y\cdot \quad \text{or} \quad X\cdot + Y^+. \end{aligned}$$

Given sufficient electron energy both possibilities occur, but usually one of these dissociations will have a lower energy requirement (appearance potential) than the other. Generally dissociations requiring lower energy will be favoured and the resulting ion will therefore be more abundant.

Ionisation and appearance potentials are of considerable interest in thermochemical studies, the latter being related to bond dissociation energies. Full details may be found elsewhere.[5,6,11,13]

Cracking patterns. As the electron energy is still further increased, the fragmentation of the molecule becomes more and more extensive until the energy imparted to the molecule is sufficient to allow the fragmentation of any bond. Experimentally this occurs at around 25 eV. Beyond this, the pattern of fragmentation does not greatly change, but because of greater stability of the operating conditions, and consequently of reproducibility, it is usual to work with energies of 50–70 eV. Under these conditions most organic molecules fragment in very many ways to give a great variety of fragment ions of different abundance. This is called the *cracking pattern* of the molecule. In general, individual compounds have distinctive cracking patterns, if not in the mass/charge

ratios of the ions at least in their relative intensities. This is true of molecules which so closely resemble each other as the *cis* and *trans* decalins, although in exceptional cases the mass spectra of related substances may be so similar as to be indistinguishable. Cracking patterns are of great significance both in analysis and in structural work; some of their uses are described below.

In the literature, cracking patterns are generally recorded by giving the intensities of the various ion beams as a percentage of the intensity of the strongest peak (the *base peak*). They are often presented graphically as shown in Fig. 3. It is customary to state the sensitivity of detection relative to a standard, e.g. *n*–butane. It should be noted that cracking patterns show some dependence on the operating conditions and, especially, on the type of instrument used.

Analytical applications of cracking patterns. Catalogues giving the cracking patterns of molecules (especially of hydrocarbons) have been prepared[7] and these can be used for identification purposes. Very small samples (e.g. fractions emerging from a gas-liquid chromatography column) can be examined in this way.

Cracking patterns also provide a method of quantitative analysis which is of particular importance in the petroleum industry. To a first approximation, the mass spectrum of a mixture depends only on the relative amounts of the components and on their mass spectra (the latter must be weighted to take the relative sensitivities of detection of the pure components into account). If these mass spectra and the relative sensitivities of detection are known, the quantitative composition of a mixture can be determined from its mass spectrum. Details of this procedure and its limitations may be found elsewhere.[1,8,9] In practice, such analyses involve the solution of a large number of simultaneous equations. The computational problems have been much simplified by the advent of electronic computers, and mixtures of 25 or more components can now be analysed without great difficulty. The quantity of sample required is very small.

A related analytical application in which the high sensitivity of the mass spectrometer is valuable is the detection and estimation of trace impurities.

Structural Applications of Mass Spectrometry

From the organic chemist's point of view, the most interesting application of mass spectrometry is its use in the determination of molecular structure. The extensive application of mass spectrometry to structural problems is a comparatively new development (since *ca.* 1953) and it has

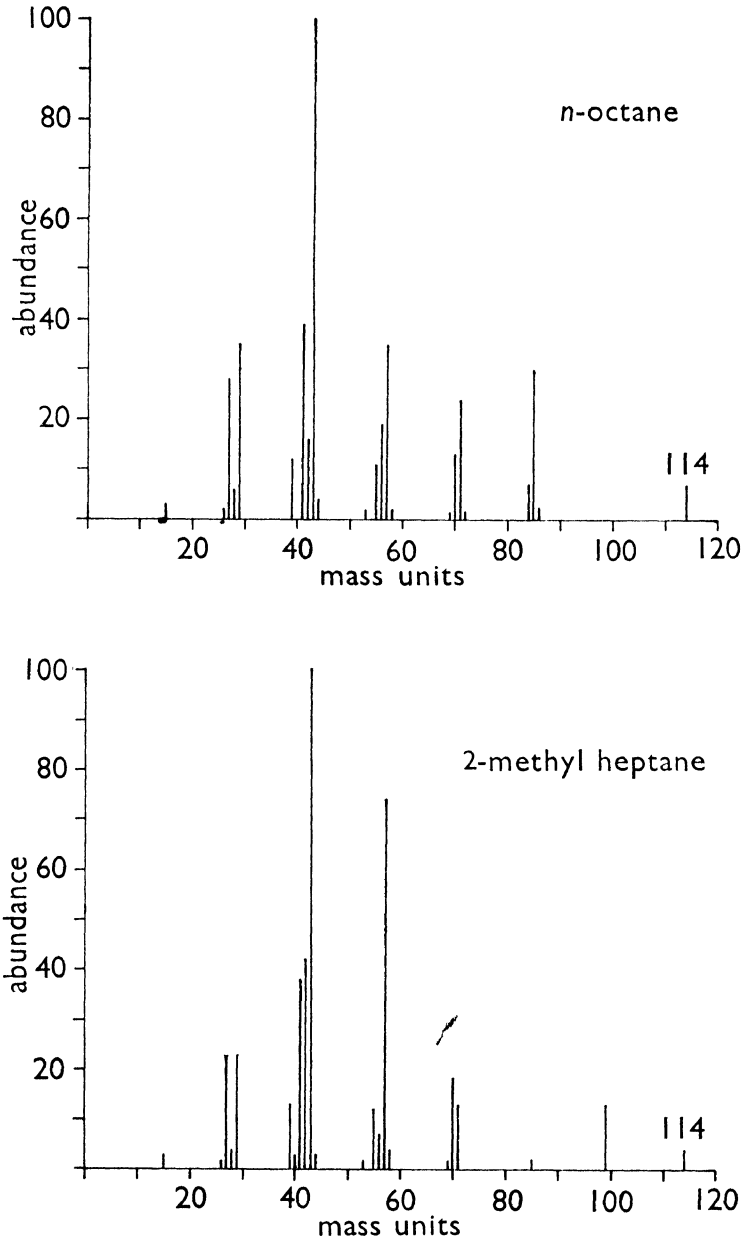


FIG. 3. The mass spectra of *n*–octane and 2–methyl heptane

a promising future. Mass spectrometry can be used not only to determine molecular weights, but also to deduce molecular formulae, to elucidate structural details (such as the presence of certain functional groups) and to decide between alternative structures. In suitable cases it may be possible to derive the complete molecular structure of a compound from mass spectrometric data alone. These applications will now be considered in turn.

Molecular weight determination. In most cases the parent molecular ion is present in the cracking pattern, and it is therefore relatively easy to obtain the molecular weight of a compound by mass spectrometry, provided that it can be volatilised without decomposition. This method of determining molecular weights is capable of greater precision than any of the other available methods (see Chapter 7). The high accuracy is particularly valuable in distinguishing between alternative formulae, such as $C_{28}H_{50}$ (C, 87·0; H, 13·0%) and $C_{29}H_{52}$ (C, 86·9; H, 13·1%), for which the variations in the proportions of the elements and in the molecular weight are very small. Such situations are often encountered in steroid and triterpenoid chemistry.

In some cases the peak of the parent molecular ion may be so weak that it is easily overlooked, and a fragment ion may then be mistaken for the parent ion. Erroneous conclusions may also arise from the presence of an impurity. Both errors can often be avoided by examination of the whole of the cracking pattern; a number of other methods for distinguishing molecular ions and fragment ions are described elsewhere (see ref. 1, p. 307).

Reduction of the energy of the bombarding electrons below the value required for the production of the complete cracking pattern sometimes increases the relative intensity of the parent ion as compared with fragment ions. However, in most routine work, it is customary to use higher electron energies, both because of the additional information obtained and because the general efficiency of ionisation is greater.

The determination of molecular formulae. Molecular weight determination to the nearest mass unit restricts the molecular formula of an unknown to a limited number of possibilities. Some of these can often be excluded on chemical grounds; for example the presence or absence of a particular element (e.g. nitrogen) may be evident from the method of formation or the reactions of the compound. In other cases, the presence of, say, oxygen might be inferred from the infrared spectrum. Similar information can sometimes be obtained from the presence of certain fragment ions in the cracking pattern (see below). Apart from this, there are two important mass spectrometric methods, namely iso-

topic abundance measurements and accurate mass determinations, which can be used to elucidate molecular formulae. These methods are briefly described below (see ref. 1, chapter 8 for further details).

Isotopic abundance measurement in the determination of molecular formulae. This method can be illustrated by considering ethylene dichloride as an unknown. Examination of the mass spectrum reveals that (neglecting ^{13}C isotopes) there are three parent molecular ions of masses 98, 100 and 102, and of relative abundance 9:6:1. This result could be explained by the presence in the formula of a single atom of an element (E) which had isotopes at E+2 and E+4 with the above abundance ratio, but no such element exists. Accordingly the compound may contain two atoms of an element, such as chlorine, which possesses two isotopes differing by two mass units. In fact the natural abundance ratio of ^{35}Cl and ^{37}Cl is 3:1 and it can be readily calculated that the abundance ratio of the three species $X^{35}Cl_2$, $X^{35}Cl^{37}Cl$ and $X^{37}Cl_2$ (where X is the residue of the molecule) is 9:6:1. Bromine and certain other elements could also have given the above separation of masses, but the abundance ratios would have been different.

Accordingly the molecular formula of the unknown contains two chlorine atoms and the molecular weight of the residue X is 28 mass units. Now, in addition to the ion of mass 98 there is also one of mass 99 which has *ca.* 2% of the abundance of the former. The same is true for the ion systems 100, 101 and 102, 103. Carbon–13 has a natural abundance of *ca.* 1% and accordingly it may be deduced that the molecule contains two carbon atoms, whence, knowing the mass of the molecule, the molecular formula will be $C_2H_4Cl_2$.

This is an exceedingly simple example. In more complex cases, the results may sometimes be difficult to interpret unambiguously, although the determination of the number of chlorine and bromine atoms is generally relatively simple. The ease with which particular alternatives can be distinguished can readily be assessed from tables constructed by Beynon,[1,16] which list the abundances of the isotopic peaks at M+1 and M+2 for compounds of carbon, hydrogen, oxygen and nitrogen up to a molecular weight (M) of 250 (500 in the case of ref. 16).

Fragmentation involving the loss of hydrogen atoms may sometimes cause large errors in isotopic abundance measurements. Complications from this source can often be reduced by lowering the energy of the bombarding electrons or by careful examination of the relative intensities of all the ions in the relevant mass region.

Accurate mass measurement. The introduction of double-focusing instruments has greatly increased the accuracy of mass measurement.

Consequently differences in molecular weight arising from differences in the atomic packing fractions can be used to distinguish between alternative formulae. For example, the molecular weights† of the most abundant isotopes of C_6H_{12} (84·0938), C_5H_8O (84·0574) and $C_4H_8N_2$‡ (84·0686) can readily be distinguished. Accurate mass data for alternative formulae are listed in the tables mentioned above. In the high mass regions, distinction between alternatives becomes more difficult, but, for compounds of moderate molecular weight, the combined use of isotopic abundances and accurate mass determination generally suffices to select a unique molecular formula.

A double-focusing instrument can also be used to determine the composition of fragment ions by accurate mass measurement. For example, the loss of CO which occurs in the fragmentation of many organic compounds containing oxygen can be distinguished from the loss of C_2H_4. This is often of great value in structural work.

The correlation of mass spectra and structure. As mentioned above, the mass spectrometric cracking patterns of molecules depend in a characteristic way on their molecular structures. This dependence has been the subject of much investigation, both for the purpose of understanding the mechanism of dissociation of the molecular ion, and also with a view to attempting the inverse operation of using the cracking pattern to determine molecular structure. Attempts have been made to interpret and predict the fragmentation patterns in terms of the electron displacements of the classical electronic theory of organic chemistry, and also on more fundamental considerations. However, although some progress has been made, the use of mass spectra in the investigation of structural details depends largely on the purely empirical information provided by *correlation studies*. These are studies in which series of compounds are examined with a view to correlating certain fissions in the various molecules with the presence of particular structural features. The empirical rules which arise out of such investigations are of obvious value in structural work.[1,8,11–14]

Hydrocarbons. Correlation studies have shown, for example, that branched hydrocarbons tend to break at the branch points. Thus 2–methyl heptane (Fig. 3; p. 314) loses a methyl group to give an ion of mass 99, while this ion is absent from the spectrum of the isomeric *n*–octane. Another generalisation which is also illustrated by these compounds is that the intensity of the parent ion decreases as the degree

† Calculated upon the new recommended standard $^{12}C = 12{\cdot}00000$.

‡ It may be noted that covalent organic molecules which have an even molecular weight cannot contain an odd number of nitrogen atoms.

of branching increases. In cycloalkanes the intensity of the parent is greater than for a corresponding paraffin; differences in fragmentation patterns can be used to distinguish cycloalkanes from the corresponding olefines. The latter tend to rupture preferentially at an allylic bond. In fused-ring alicyclic compounds the intensity of the parent tends to be still greater; the mass spectra of steroids have proved useful not only in determining molecular weights but also in deducing the length of the side chain.

The cracking patterns of aromatic compounds and of many heterocyclics reflect the stability of aromatic rings. The parent ions are very intense, especially in unsubstituted compounds, and the fragmentations of greatest abundance tend to retain the stable ring system.

The interpretation of mass spectra is greatly complicated by the occurrence of rearrangements which give rise to ions which would not be expected from simple bond cleavage; for example, the mass spectrum of isobutane, $(CH_3)_3CH$, contains a small peak corresponding to $(C_2H_5)^+$. Rearrangements have been classified by McLafferty[10] into (i) 'random' rearrangements, which give a number of different rearrangement ions of relatively low abundance, and (ii) 'specific' rearrangements, which give an ion of high abundance by a single favoured path. The latter are usually promoted by the presence of functional groups. Rearrangements involving migration of hydrogen are especially common; some examples are given below.

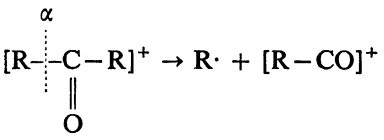
Effect of functional groups. In general, hydrocarbons do not give strong peaks in the mass regions 16–24 and 30–36. Abundant ions in these and certain other regions generally indicate the presence of hetero-elements. Thus many oxygen compounds give an abundant ion at mass 31 (see below).

The presence of a functional group tends to promote cleavage of the molecular ion at bonds α and/or β with respect to this group, although, as might be expected, the spectra of higher homologues which contain only one functional group tend to become more and more like those of the corresponding hydrocarbons. β–Cleavage is common with alcohols, ethers and amines, and may be exemplified by the reaction

$$[R-CH_2-\overset{\beta}{\vdots}-CH_2-OH]^+ \rightarrow R-CH_2\cdot + [CH_2-OH]^+$$

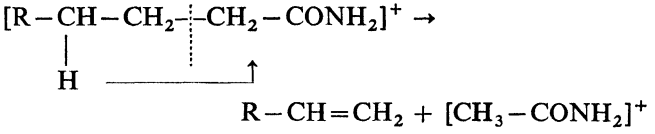
This process gives rise to an ion at mass 31 which is strong in the spectra of primary alcohols; this ion can also be formed as a rearrangement

peak in the spectra of other alcohols and oxygen compounds. α-Fission may be illustrated by the reaction



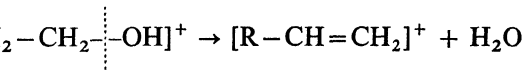
which is common with ketones and carboxylic acids.

Both processes may be accompanied by rearrangements, β-fissions with hydrogen transfer being particularly common. For example, the process typified by



occurs with amides, carboxylic acids, nitriles and aldehydes (and to a lesser extent with ketones). The source of the migrating hydrogen can be investigated by appropriate deuterium labelling.

α-Fission may also be accompanied by hydrogen transfer. Thus many alcohols give a prominent peak at (parent *minus* 18), probably resulting from the reaction



although dehydration of the alcohol prior to ionisation could give the same result.

General. The elucidation of structure by classical chemical methods depends on the large body of knowledge which has been derived from the study of the reactions of countless organic compounds. In the same way, the determination of structure from cracking patterns depends on an understanding of the 'chemistry' of the fragmentations. This new 'chemistry' is inherently complex and, as we have seen, it is further complicated by rearrangements, many of which are exceedingly involved. For example, the formation of the ion $[B(CH_3)_2]^+$ (the most abundant in the spectrum) from the cyclic trimer $[-P(CH_3)_2-BH_2-]_3$ requires that all four of the original bonds of the boron atom are broken and that both methyl groups migrate. One important consequence of rearrangements is that they tend to blur the differences between the spectra of isomers. Such complications may make the reconstruction of a molecule from its cracking pattern a difficult or even impossible task.

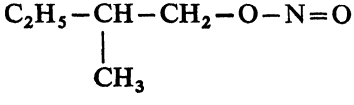
Whether a given structural problem can be solved correctly depends on several factors, including the complexity of the compound, the resolving power of the instrument and (as in most scientific pursuits) the intuition, persistence and critical faculty of the operator. Complete structure determination by mass spectrometry is often possible, especially if the molecular weight is below about 150. Even when this is not feasible, the information which mass spectrometry can provide about molecular weights, molecular formulae and structural characteristics may be of decisive value in conjunction with evidence from other physical and chemical sources.

Cracking patterns—an application. The following is a simple example of the analysis of a cracking pattern which shows both the strength and weakness of the method.

An unknown organic compound, containing the elements carbon, hydrogen, oxygen and nitrogen, gives rise to a very weak parent molecular ion at mass 117, corresponding to the molecular formula $C_5H_{11}NO_2$. The next lower ion of any abundance is at mass 71, that is, at (parent *minus* 46). Below this there are further abundant ions of masses 27, 29, 41, 43 and 57, all of odd mass. The only prominent ions of even mass are at mass 60 and 30.

The loss of forty-six mass units suggests that this is the mass of the functional group. In the absence of confirmatory evidence from a double-focusing instrument, it may be assumed that this corresponds to an NO_2 grouping. The presence of an abundant ion at mass 30 (NO^+) suggests that this is probably a nitrite group rather than a nitro group, although it must be noted that nitro-compounds can provide NO^+ by rearrangement.

The presence of a nitrite group favours fission of a β-bond and the fragment arising from this may be identified as that with mass 60; hence its structure can only be $-CH_2NO_2$. The typical progression of masses listed above suggests that the remaining part of the molecule is purely hydrocarbon in character and therefore of the formula C_4H_9. Now, the ion at mass 29 is the most abundant in the spectrum. Since an ion of this mass is seldom obtained in great quantity unless there is an ethyl group at a branch, one can deduce that there must be an ethyl branch in the aliphatic chain. Accordingly, the unknown compound has the formula



In fact, the unknown was isoamyl nitrite, $(CH_3)_2CHCH_2CH_2ONO$, and the deduced structure is wrong. It is possible that the abundant ion at mass 29 is a rearrangement product and corresponds to the formula HCO or CH_3N. If this is the case, the above error might have been avoided, as accurate mass measurement would have revealed the composition of the ion.

The present example is a very simple one and mass spectrometry can in fact be applied successfully to molecules of very much greater complexity. Details may be found in recent reviews.[1,8,11–14] For example, the method has played a decisive part in the elucidation of the structures of a number of complex alkaloids.[15] One particularly successful type of application is the determination of the structures of minor alkaloids. These often differ from a major alkaloid of the same plant or plant species only by the presence or absence of a methyl or methoxyl group and such similarities in structure can readily be inferred from the cracking patterns.

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(References 1, 8 and 11–15 include detailed analyses of a number of mass spectra).

Chapter 10

B. L. Shaw

DIPOLE MOMENTS

Introduction

A molecule is said to have a dipole moment if its centres of positive and negative charge do not coincide. If the positive and negative charges ($+\delta e$ and $-\delta e$ respectively) are separated by a distance l then the dipole moment (μ) is equal to $\delta e.l$ and is a vector quantity. Dipole moments are usually measured in Debye units; one Debye unit (1 D) = 10^{-18} e.s.u. The electronic charge is $4{\cdot}8 \times 10^{-10}$ e.s.u., so that two such charges of opposite sign separated by 1 Å would have a moment of $4{\cdot}8 \times 10^{-18}$ e.s.u. or 4·8 D.

The interpretation of the electric dipole moments of organic molecules has been a very useful physical method with important applications in structural work and in the study of reactivity. Before discussing some of these applications (pp. 327–336) a brief outline of the theory and measurement of electric dipole moments is given.

Dipole moment and dielectric constant. The determination of dipole moments usually involves measurements of dielectric constants and it is therefore of interest to consider the behaviour of molecules in an electric field. If a molecule is subjected to a uniform electric field it will become polarised. This polarisation is made up of three independent contributions as follows: (1) The field will pull the electrons and nuclei of the molecule in opposite directions giving rise to an induced dipole moment. This is called *electron polarisation* and the induced moment is proportional to the applied field and is temperature independent. (2) If the molecule contains polar bonds, its constituent atoms will carry different effective charges, and in an electric field the nuclei will be displaced relative to one another. This distortion is known as *atom polarisation* and is a much smaller effect than electron polarisation. Like

the latter, it is proportional to the applied field and is temperature independent. Molecules both with and without permanent dipole moments can undergo electron and atom polarisations. (3) Molecules with a permanent dipole moment will tend to orientate themselves along the field but this tendency will be opposed by thermal motions. This is called *orientation polarisation* and is temperature dependent.

The relationship between the dielectric constant of a compound and the dipole moment of its individual molecules was worked out by Debye (1912). It may be expressed by the equation

$$\frac{\varepsilon-1}{\varepsilon+2}\cdot\frac{M}{d}=\frac{4}{3}\pi N\left(\alpha_E+\alpha_A+\frac{\mu^2}{3kT}\right)$$

where ε, M and d are the dielectric constant, molecular weight and density of the compound, α_E, α_A the electron and atom polarisabilities (i.e. the moments induced by unit field) and μ the dipole moment of the molecule. The Debye equation holds well for gases and surprisingly well for dilute solutions of substances in non-polar solvents. The quantity on the left-hand side of the equation is known as the total molar polarisation ($_TP$) and the three terms on the right are the electron polarisation ($_EP$), atom polarisation ($_AP$), and orientation polarisation ($_OP$). It follows that

$$\frac{\varepsilon-1}{\varepsilon+2}\cdot\frac{M}{d}={}_TP={}_EP+{}_AP+{}_OP$$

Determination of dipole moments. Although several methods have been used to measure dipole moments (molecular beams, Stark effect, etc.—see refs. 7–9), only those based on measurement of dielectric constants are important for routine use in organic chemistry. From the Debye equation it can be seen that a series of density and dielectric constant measurements at different temperatures could be used to determine a dipole moment. Since electron and atom polarisations are temperature independent, μ could be determined from the slope of a plot of total molar polarisation ($_TP$) against $1/T$. This method is used for gases and vapours but it requires great accuracy and is tedious.

A method which is commonly used to determine dipole moments in solution is to calculate the total molar polarisation ($_TP$) of the solute from measurements of (1) the specific volume (the reciprocal of the density) and (2) the dielectric constant, of dilute solutions of the compound in a non-polar solvent at some standard temperature (see following paragraph for details). The electron polarisation ($_EP$) and the atom polarisation ($_AP$) are then determined or estimated as discussed below.

The dipole moment is then calculated from the formula

$$\mu = \sqrt{\frac{9kT_{O}P}{4\pi N}} = 0{\cdot}01281\sqrt{{}_{O}P \,.\, T}$$

where ${}_{O}P = {}_{T}P - {}_{E}P - {}_{A}P$.

The specific volume may conveniently be determined in a pyknometer. The dielectric constant is measured by using the solution as dielectric in a calibrated cylindrical condenser. Of the various methods which may be used to measure the capacity of the condenser the heterodyne beat frequency technique is perhaps the best. A density measurement can be made on as little as a few milligrams of material and, unless the dipole moment is small, a dielectric constant measurement on a similar quantity. For very accurate work, the limiting values for zero solute concentration of the expressions $\Delta\varepsilon/w_2$ and $\Delta v/w_2$ are determined (w_2 is the weight fraction of solute, and $\Delta\varepsilon$ and Δv are the increases in dielectric constant and specific volume respectively of the solution over the solvent). The total molar polarisation of the solute may then be evaluated using the Halverstadt-Kumler equation[1]

$${}_{T}P_2 = M_2\left\{3\left(\frac{\Delta\varepsilon}{w_2}\right)\frac{v_1}{(\varepsilon_1+2)^2}+\left(v_1+\frac{\Delta v}{w_2}\right)\left(\frac{\varepsilon_1-1}{\varepsilon_1+2}\right)\right\}$$

(subscripts 1 and 2 refer to solvent and solute respectively). This equation is derived from the Debye equation and is in a form convenient for calculating ${}_{T}P_2$ from experimental data on solutions. A critical review of the method of evaluation of electric dipole moments from experimental data on dilute solutions may be found in a paper by Everard, Hill and Sutton[1b] (see also refs. 7–9).

The molar electron polarisation (${}_{E}P$) of a compound can be taken as equal to its molar refractivity for sodium light. This is because, owing to inertia and friction (in liquids), polar molecules cannot follow an alternating electric field if the frequency is high enough. Thus at the frequency of visible light (*ca.* 10^{15} cycles/sec) the orientation polarisation and most of the atom polarisation will be cut out, leaving only the electron polarisation to contribute to the dielectric constant. From the Maxwell relation, $n^2 = \varepsilon$ (where n is the refractive index at the same frequency of measurement as ε), it follows therefore that the electron polarisation of a compound may be taken as equal to its molar refractivity, as given by the Lorenz-Lorentz formula

$$\frac{n^2-1}{n^2+2}\cdot\frac{M}{d}$$

The molar refractivity of a compound can be calculated from refractive index measurements on its solutions in a Pulfrich divided cell refractometer (the necessary density measurements will have been made in determining ${}_{T}P$). Alternatively, it can be estimated from a knowledge of the structure of the molecule and the appropriate atomic or bond refractions, tables of which are available.

Atom polarisation is very difficult to measure but fortunately it is usually small. With an accuracy sufficient for most purposes, atom polarisation may be assumed to be equal to a small percentage of the electron polarisation, e.g. 5%. For molecules containing highly polar bonds (e.g. coordinate linkages), however, it is usually taken to be much higher, e.g. 15%.

Since μ is a function of $\sqrt{{}_{O}P}$, errors either in measurement or due to uncertainty in atom and electron polarisations become increasingly important as the dipole moment becomes small, especially as ${}_{O}P$ is then a small difference between two large numbers. Sutton considers that in solution work a precision of 1% can be attained with $\mu \geqslant 1{\cdot}5$ D but the errors may be of the same order as the moment for $\mu = 0{\cdot}4$ D. In good work on vapours a result even as small as 0·1 D can be considered significant.

Effect of solvent. The Debye equation when applied to solutions ignores solvent effects. It is not surprising therefore that the apparent moment of a molecule varies with the solvent. Some measurements on aniline illustrate this (Table 1).

TABLE 1. The dipole moment of aniline

Solvent	*Observed moment* (D)
Heptane	1·477
Dioxan	1·750
Carbon tetrachloride	1·458
Benzene	1·505
Carbon disulphide	1·420
(Vapour)	1·48

Various empirical equations have been proposed to correct for this solvent effect. When the dipole moments of a series of similar compounds are being compared, however, it is usually sufficient if they have been determined in the same solvent and corrections for solvent effects are not usually made.

I GENERAL RESULTS

Moments of simple organic molecules. Simple organic molecules have dipole moments generally of the following approximate order of mag-

nitude: saturated hydrocarbons—0 D; olefins, acetylenes, some aromatic hydrocarbons and heterocyclic compounds, e.g. toluene, thiophen, furan—0 to 1 D; ethers, primary amines—1 to 1·6 D; alcohols, phenols, esters, alkyl and aryl monohalides—1·5 to 2 D; aldehydes and ketones —2·4 to 3 D; nitriles and nitro-compounds—3 to 4 D; amine oxides, sulphoxides and sulphones—4 to 5·5 D. Many organic molecules containing polar bonds have zero dipole moments. This provides important structural evidence since it shows the molecule to be symmetrical. Examples of such molecules are carbon tetrachloride, *trans*-dichloroethylene (distinction from the *cis*-compound!), *p*-dichlorobenzene, *p*-dicyanobenzene, *p*-dinitrobenzene, etc. The zero dipole moments of the 4,4′-dihalodiphenyls are of historical importance since they disproved the non-axial structure proposed by Kaufler for diphenyls.

Bond moments. Chemical bonds can be considered to have an electric dipole moment associated with them (for bonds joining atoms of the same element it may, of course, have zero value). The moment of a molecule would thus be made up of the vector sum of its individual bond moments. For many aliphatic molecules the moment of a given bond (e.g. the C–Cl bond) remains *approximately* constant in different molecules so that it is possible to draw up a list of bond moments[2] (see Table 2) and use them to make rough estimates of dipole moments. The values given in Table 2 have been calculated assuming the value 0·4 D for the moment of the H–C bond, with the hydrogen at the positive end of the dipole.† The above method of estimating dipole moments breaks down when mesomeric effects become important.

TABLE 2. Bond moments (D)

(The positive atom is on the left)

H–C	0·40	N–O	0·5	C–Br	1·38
H–N	1·31	C–N	0·22	C–I	1·19
H–O	1·51	C–O	0·74	C=O	2·3
H–P	0·36	C–F	1·41	C≡N	3·5
H–S	0·68	C–Cl	1·46		

CONTRIBUTIONS TO THE ELECTRONIC THEORY OF ORGANIC CHEMISTRY

Dipole moment measurements are an important source of information about electron distributions, and in this connexion they have

† Although there is fairly general agreement on the absolute value of the H–C bond moment (viz. *ca.* 0·4 D) opinions differ as to the sense of the polarity of the H–C bond. Thus molecular orbital calculations indicate that the hydrogen is at the negative end of the dipole whereas infrared studies and the relative electronegativities of carbon and hydrogen indicate the moment to be in the opposite sense. If the C–H bond moment is taken with the opposite sign to that in Table 2, different values must be given to some of the other bond moments to compensate.

played a major part in the development of the electronic theory of structure and reactivity. The determination of dipole moments was the first physical technique which confirmed the reality of the electron displacements commonly known as the inductive and mesomeric effects. More recently, evidence from dipole moments has proved useful in the elucidation of the fine structure of molecules which cannot be adequately represented by a single classical structure (e.g. meso-ionic compounds).

Aromatic substitution and mesomeric moments. Sutton[3] has compared the electric dipole moments of corresponding aliphatic (Alk–X) and aromatic (Ar–X) compounds to show that with *ortho-para*-directing substituents (X) the electron drift brought about through the mesomeric effect was towards the aromatic ring, and for *meta*-directing substituents, away from the ring. Thus if we call positive those moments of which the negatively-charged end is away from the substituent X (i.e. where the group X is an electron donor) and call those in the opposite direction negative, then if $\mu_{(Ar-X)} - \mu_{(Alk-X)}$ is positive, X is *ortho-para*-directing; if it is negative, X directs *meta*. This rule, which holds for a large number of different groups (X) is illustrated by the examples given in Table 3.

TABLE 3. Mesomeric moments

Group X	μ(Ar–X)	μ(Alk–X)	μ(Ar–X) − μ(Alk–X)	*Directive influence*
CH_3	+0·35	0	+0·35	*op*
NH_2	+1·52	+1·32	+0·20	*op*
Cl	+1·60	−2·01	+0·41	*op*
CCl_3	−2·07	−1·57	−0·50	*m*
C≡N	−4·05	−3·60	−0·45	*m*
NO_2	−4·01	−3·25	−0·76	*m*

The quantity $\mu_{(Ar-X)} - \mu_{(Alk-X)}$ gives an indication of the direction and magnitude of the mesomeric drift of electrons across the aromatic ring and has been called the *mesomeric moment*. The above discussion illustrates that differences in electron distribution, determined by dipole moment measurements, can be correlated with differences in reactivity. However, it should be noted that this approach omits one important factor, namely the polarisation of the molecule by the attacking reagent (the electromeric effect).

Unsaturated aliphatic compounds. Table 4 gives a comparison between moments of unsaturated aliphatic halides and the moments of corresponding saturated compounds. The moments suggest that there is a mesomeric drift of electrons from the halogens across the unsaturated linkages opposing the inductive effect. The mesomeric drift of electrons

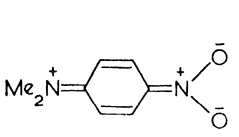
is greater in acetylenic compounds than in ethylenic compounds. Indeed, in aryliodoacetylenes, e.g. PhC≡CI, the iodine is at the positive end of the dipole and the mesomeric moment is about 2 D.

TABLE 4. Moments of halides (measured on the vapour)

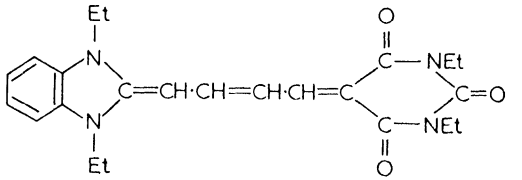
Alkyl radical	Cl	Br	I
CH_3-CH_2-	2·05	2·01	1·87
$CH_2=CH-$	1·44	1·41	1·26
$HC\equiv C-$	0·44	0	—

It should be noted that the concept of mesomeric moments is not entirely satisfactory; part of the differences mentioned above may be due to other factors, such as the effect of changes in hybridisation on the individual bond moments (including that of the C–H bond).

Large mesomeric moments. These have been observed in compounds where electron-acceptor and electron-donor groups are separated by a conjugated system. Thus the dipole moment of *p*-dimethylaminonitrobenzene (6·87 D) is greater than the sum of the dipole moments of nitrobenzene (4·00 D) and dimethylaniline (1·58 D), indicating a large contribution by polar structures such as (1) to the resonant state.



(1)



(2)

The compound (2) which is one of a series studied by Smyth and coworkers has a dipole moment of 17·7 D (in dioxan) demonstrating the large displacement of charge permitted by the long conjugated system.

Steric inhibition of resonance. It is well known that full conjugation between conjugating groups and an aromatic ring can be prevented by steric interaction with *ortho*-substituents such as methyl groups. In such cases, one would expect the mesomeric moments of the hindered

TABLE 5. Steric effects on dipole moments

Conjugating substituent	*Moments* (D)		
	Phenyl	*Mesitylenyl*	*Durenyl*
Br	1·55	1·52	1·55
Cl	1·58	1·55	—
NH_2	1·53	1·40	1·39
NO_2	4·00	3·67	3·62
CO – Me	2·88	2·71	2·68

compounds to be less than those of the unhindered ones. This is demonstrated in Table 5, where the moments of corresponding phenyl, mesitylenyl and durenyl derivatives are compared. Only with bromo and chloro as the conjugating substituents are there no significant reductions in mesomeric effects.

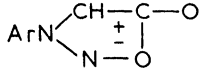
Cyanides and isocyanides. Since nitrogen is more electronegative than carbon, one would expect that organic cyanides would have the carbon atom at the positive end of the $-CN$ dipole. Dipole moment studies have confirmed this. Thus the introduction of the electron-donating methyl group into the *para*-position of benzonitrile increases the moment by 0·43 D whereas the electron-attracting chlorine atom decreases the moment by 1·89 D (see Table 6). On the other hand, in organic isocyanides the carbon atom is the negative end of the $-NC$ dipole since the moment of *p*-tolylisocyanide is greater than the moment of phenylisocyanide and that of *p*-chlorophenylisocyanide is less. This suggests that the structure $R-\overset{+}{N}\equiv\overset{-}{C}$ makes an important contribution to the resonant state of isocyanides.

TABLE 6. Dipole moments (D) of cyanides and isocyanides

p-Substituent	*Phenylcyanides*	*Phenylisocyanides*
CH_3	4·40	3·98
H	3·97	3·49
Cl	2·08	2·07

Pseudo-aromatic systems. Many ring systems have a strong tendency to lose or gain an electron and thus attain an aromatic sextet of electrons. Generally the compounds containing these ring systems have unusually large dipole moments. A variety of these compounds is described below.

Meso-ionic compounds. The most widely studied of these are the sydnones, formed by treating *N*-aryl-*N*-nitrosoglycines with acetic anhydride. Sydnones cannot be represented by any single covalent structure, but have a mesomeric structure derived from a number of ionic forms: hence the term meso-ionic. They can be represented by formula (3) or, to emphasise their aromaticity (Baker and Ollis[4]), by the formula (4): the five-membered ring has a strong tendency to lose an electron to the exocyclic oxygen atom, thereby acquiring an aromatic



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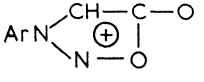


(4)

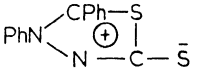
sextet. The charges are rather less than the unit charges indicated by the formula (4). The dipole moments of the substituted sydnones shown

in Table 7 demonstrate the substantial transfer of negative charge from the ring.

TABLE 7. Dipole moments (D) of sydnones

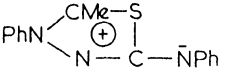
N–Phenylsydnone	6·48
N–*p*–Chlorophenylsydnone	5·01
N–*p*–Tolylsydnone	6·89

Other meso-ionic compounds and their dipole moments are given below (formulae (5), (6) and (7)). Compound (7) is 'nitron', used in analysis as a nitric acid precipitant.



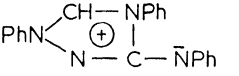
(5)

$\mu = 8{\cdot}8$ D



(6)

$\mu = 8{\cdot}4$ D

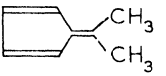


(7)

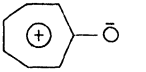
$\mu = 7{\cdot}2$ D

Azulenes and fulvenes. Azulene has a dipole moment of 1 D due to a drift of electrons from the seven- to the five-membered ring. The moments of 6,6–dimethylfulvene (8; 1·48 D), 6,6–diphenylfulvene (1·34 D) and 6,6–di–*p*–chlorophenylfulvene (0·68 D) also indicate a drift of electrons towards the five-membered ring.

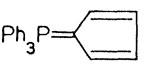
Tropone. Cycloheptatrienone or tropone has a dipole moment of 4·3 D, suggesting that the polar structure (9) contributes substantially to the state of the molecule.



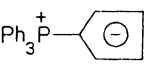
(8)



(9)



(10)



(11)

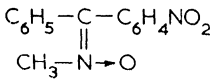
Cyclopentadienylides. The dipole moment (7·0 D) of triphenylphosphonium cyclopentadienylide indicates roughly equal contributions from the double-bonded and dipolar structures (10) and (11) respectively.

PROBLEMS IN STEREOCHEMISTRY

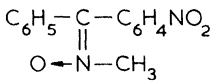
Many problems in stereochemistry have been studied by dipole moment measurements. Some of these are now of historical importance only—thus attempts have been made to estimate valency angles in simple molecules by comparing their dipole moments with the dipole moments of derivatives. This method gives implausible values in some cases; thus a comparison between the dipole moments of diphenyl ether and di(*p*–bromophenyl)ether leads to a high value (140°) for the oxygen

valency angle. Valency angles can now, of course, be measured with great accuracy by X-ray and electron diffraction, and are seldom estimated from dipole moment measurements. However, in many classical problems of stereochemistry (e.g. the configuration of oximes, *cis-trans* isomerism about carbon–carbon double bonds, etc.) dipole moments have proved of great value. They have also been used frequently in investigating conformation.

Configuration of oximes. Taylor and Sutton measured the dipole moments of the two isomeric *N*–methyl ethers of *p*–nitrobenzophenone oxime, and found values of 6·60 D and 1·09 D, clearly indicating configurations (12) and (13). This work confirmed that the Beckmann



(12)
$\mu = 6{\cdot}60$ D

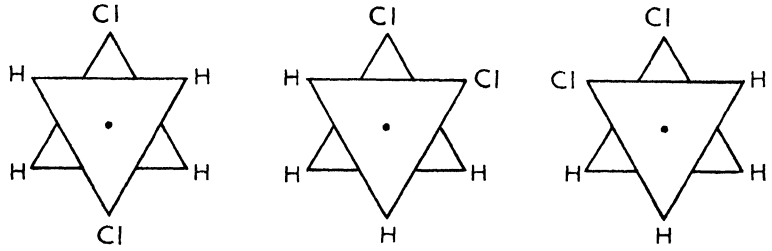


(13)
$\mu = 1{\cdot}09$ D

transformation of oximes proceeds by an exchange of *trans*-groups, since the oxime corresponding to the *N*–methyl ether (12) yields *p*–nitrobenzanilide on Beckmann rearrangement while its isomer yields benzo–*p*–nitranilide.

Rotational effects. Dipole moments have been used to study rotational (or conformational) effects in both aliphatic, alicyclic, and aromatic compounds.

It can be shown, for example, that if the rotation about the carbon–carbon bond in ethylene dichloride is not restricted, a dipole moment

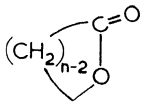


(14) The *trans*- and *gauche*-forms of ethylene dichloride looking along the C–C bond

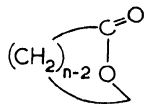
of 2·40 D would be expected. However, the observed moment is less than this value and is found to vary with temperature, being 1·07 D at −70° and 1·41 D at +30°C. These low moments arise because the

bulky and negatively charged chlorine atoms repel each other and the molecule tends to assume a *trans*-conformation; this tendency will be opposed by thermal effects. Ethylene dichloride has three stable conformations—one *trans*- and two *gauche*-forms (14)—and from dipole moment studies the energy difference between the *trans*- and *gauche*-forms has been found to be 1·1 kcal/mole. For ethylene dibromide, the energy difference is 1·99 kcal/mole. Halogen compounds, the conformations of which have been investigated by dipole moment measurements, include dihalocyclohexanes, halocyclohexanones and steroid haloketones.

Conformation of lactones. The dipole moments of lactones of the type $O(CH_2)_{n-2}CO$ show that for $n = 4$–7 the lactone has the polar *cis*-conformation (15) but for $n > 10$ the lactone has the less polar *trans*-



cis-form
(15)



trans-form
(16)

form (16). At $n = 8$ or 9 there is a transition from *cis*- to *trans*-forms. The dipole moments and conformations of the lactones $O(CH_2)_{n-2}CO$ are given in Table 8.

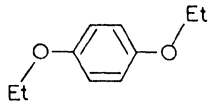
TABLE 8. Dipole moments and conformations of lactones

n	μ (D)	*Conformation*
4	3·8	*cis*
5	4·09	*cis*
6	4·22	*cis*
7	4·45	*cis*
8	3·70	*cis* + a little *trans*
9	2·25	*trans* + a little *cis*
10	2·01	*trans*
11–16	1·86–1·88	*trans*

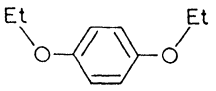
The dipole moments of the *trans*-lactones are of the same order as the dipole moments of straight chain saturated aliphatic esters. The rates of alkaline hydrolysis of these lactones can be correlated with their dipole moments. Thus the highly polar lactones with *cis*-conformations hydrolyse very much faster than the less polar *trans*-lactones.

Aromatic compounds. Although *p*–dimethyl–, *p*–dihalo– and *p*–dinitro–benzene have zero moments, many *para*-disubstituted benzenes

$C_6H_4X_2$ have positive moments. In these the group X is a 'bent' substituent; examples are (with the moment of $C_6H_4X_2$ shown in parenthesis) OEt (1·7 D), $OCOCH_3$ (2·2 D), NMe_2 (1·23 D) and $COOCH_3$ (2·2 D). These 'bent' groups can rotate about the axis of the C–O, C–N, or C–C bond, and one would expect, for example, that the moment of *p*-diethoxybenzene would be intermediate between the value for conformation (17) (i.e. zero) and the value for conformation (18). It is difficult to estimate the dipole moment of conformation (18) owing to mesomeric effects.

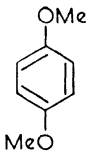


(17)

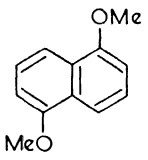


(18)

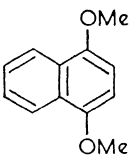
Everard and Sutton have used dipole moments to determine the preferred conformations of several methoxyaryl compounds. Some of these compounds are shown below with their dipole moments. The



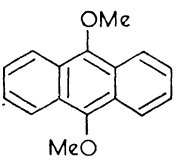
(19)
μ = 1·73 D



(20)
μ = 0·67 D



(21)
μ = 2·09 D



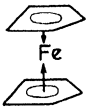
(22)
μ = 1·73 D

dipole moments indicate that in the two dimethoxynaphthalenes shown, the methoxyl groups are bent away from the *peri*–hydrogen atoms; this was attributed to steric interaction. In 9,10–dimethoxyanthracene (22) each methoxyl group has *peri*–hydrogen atoms on either side and therefore *cis*- and *trans*-conformations are equally likely, as in 1,4–dimethoxybenzene. Hence these two compounds have the same moment (1·73 D).

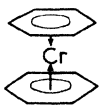
IV. Organometallics

Dipole moment measurements are particularly useful in the investigation of the stereochemistry and electronic structures of organometallic compounds. Thus the symmetrical structures of dicyclopentadienyliron(II) (23), dibenzenechromium(0) (24), diphenylmercury(II) and analogous compounds follow immediately from their zero dipole moments.

On the other hand the moments of dicyclopentadienyltin(II) and -lead(II) are 0·96 D and 1·29 D respectively, indicating that they are not symmetrical.

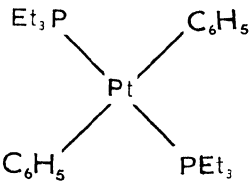


(23)

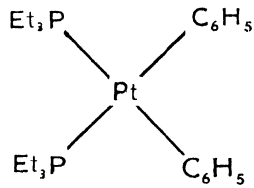


(24)

Dipole moments are especially valuable in determining the configurations of alkyl and aryl derivatives of transition metals complexed with other ligands. Thus two isomers are known with the constitution $[Pt(C_6H_5)_2(PEt_3)_2]$ and, rather surprisingly, the higher melting form (m.p. 180°) is found to have a zero dipole moment and therefore has the *trans*-configuration (25); the other form (m.p. 154°) has a high dipole moment (7·2 D) and therefore has the *cis*-configuration (26).



(25)

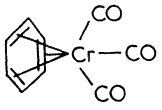


(26)

Similarly, the pentachlorophenyl derivatives of cobalt and iron of the type $[M(C_6Cl_5)_2(PEt_2Ph)_2]$ (M = Co, Fe) have very small or zero dipole moments and must therefore have the *trans*-planar arrangement of ligands.

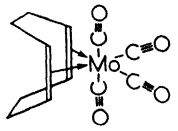
The dipole moments of transition metal complexes derived from aromatic rings, olefins and acetylenes are useful in assigning structure, and give information on the type of bonding. Thus the dipole moments of the three complexes shown below, viz. benzenetricarbonylchromium(0) (27), cyclo-octa-1,5-dienetetracarbonylmolybdenum(0) (28) and di(*p*-nitrophenyl)acetylenebis(triphenylphosphine)platinum(0) (29; $R = R' = NO_2$) are in agreement with the assigned structures.

The moments of the chromium complex (27), and of its derivatives with various substituents in the benzene ring, have been used to study the 'sandwich' bonding between the metal and the aromatic ring. In general, groups which make the ring more negative (e.g. methyl) increase the moment and strengthen the bond, whereas those which make it positive (e.g. −COOMe) lower the moment and weaken the bond.



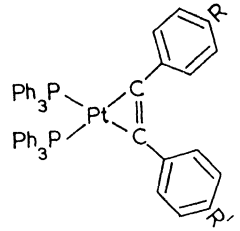
(27)

$\mu = 4{\cdot}92$ D



(28)

$\mu = 4{\cdot}88$ D



(29)

$\mu = 10{\cdot}2$ D

With the platinum complexes of the type (29), the stabilities and dipole moments increase with increasing electronegativity of the groups R and R′. In fact, di(*p*–nitrophenyl)acetylene readily displaces other atceylenes from complexes of this type to give the very stable and highly polar complex (29; $R = R' = NO_2$). E. O. Fischer's excellent review[5] of coordination compounds of unsaturated hydrocarbons with metals includes some discussion of dipole moments.

General. As Le Fèvre points out (ref. 7, p. 109), the most successful applications of dipole moments in structural work have been those for which an exact quantitative analysis of results has not been necessary, e.g. those involving a choice between alternatives which have widely different moments. This criterion is satisfied for only relatively few problems in 'pure' organic chemistry. The complex conformations of many organic molecules would make an interpretation of their dipole moments impracticable. In the field of organometallics, organometalloids and coordination compounds of organic ligands with metals and non-metals, however, conformational effects are often of minor importance and differences in dipole moments between isomers are very large. In these fields, therefore, dipole moments will continue to be used extensively.

The writer is very grateful to Dr J. Chatt, F.R.S., for reading and commenting on this chapter.

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Refs. 6-10 are general reviews which are recommended for further study.

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INDEX OF COMMON GREEK SYMBOLS

$\Delta\nu_{\frac{1}{2}}$ (half-band width) 41
δ (in-plane bending vibration) . 50 (e.g. 82)
γ (out-of-plane bending vibration) . 50 (e.g. 37)
ε (molar extinction coefficient) . . . 25
ε^a (apparent molar extinction coefficient) . 26
λ (wavelength) 26
μ (micron) 6
ν (frequency) 6
ν (stretching vibration) . . 50 (e.g. 37, 39, 82)
ν (wavenumber) 6, 26, 50
π (pi orbital), see general index
ρ (rocking vibration) . . . 50 (e.g. 82)
σ (sigma orbital), see general index
τ (twisting vibration) . . . 50 (e.g. 82)
τ–values 173–178
ω (wagging vibration) . . . 50 (e.g. 82)

INDEX

Note. In general, compounds are indexed under the appropriate class (e.g. carboxylic acids, ketones, olefins, etc., also proteins, steroids, etc.). The names of individual compounds are not included unless they are discussed at length or are of special interest.

Abbreviations used in Index

esr = electron spin resonance
ir = infrared
mol. wt. = molecular weight
nmr = nuclear magnetic resonance
ord = optical rotatory dispersion
uv = ultraviolet

A_n B_m spin systems, 188, 189, 193, 194
Absolute configuration, 17, 220, 226, 231, 234, 236, 237, 239, 241, 303
Absorbance, 24
Absorption bands:
  asymmetric, 218, 230–232
  of electronic spectra, 24, 129
  of ir spectra, 24, 36
  optically active, 218, 230–232
Absorptivity, 24
Acetaldehyde, nmr spectrum, 182
Acetylacetone, ir and uv spectra, 104, 142
Acetylenes:
  dipole moments, 327, 329, 335
  ir spectra, see also C≡C and C – H vibrations, 52, 102–104
  nmr spectra, 174–177, 179, 192
  Raman spectra, 121
  uv spectra, 137, 144, 160, 164
Acids, see carboxylic acids, amino acids, hydroxy acids and acids, unsaturated
Acids, unsaturated:
  ir spectra, 98–101
  nmr spectra, 175, 201
  uv spectra, 144
Alcohols:
  dipole moments, 327
  ir spectra, see also O – H and C – O vibrations, 52, 72, 76, 80–89, 106–108
  mass spectra, 318, 319
  nmr spectra, 174–178
  optical rotation and ord, 232, 236, 240, 241
  uv spectra, 145
Aldehydes:
  dipole moments, 327
  ir spectra, 52, 75
  mass spectra, 319
  nmr spectra, 174–177, 180, 183, 189, 192, 193
  uv spectra, 138, 147–150
Aldehydes, unsaturated, uv spectra, 139
Alkaloids:
  ir spectra, 66, 108–110
  mass spectra, 321
  nmr spectra, 193–195
  optical rotation and ord, 211
  uv spectra, 157, 162, 164
  X-ray structure analysis, 291, 297, 302
Alkyl substitution:
  and ir spectra, 71, 95
  and uv spectra, 133–135, 139–141, 144, 146, 151
Allenes:
  ir spectra, 40, 52
  nmr spectra, 177
  Raman spectra, 119
Allowed transition, 129, 159, see also selection rules
Amides:
  ir spectra, 53, 64, 69, 75
  nmr spectra, 193
  optical rotation and ord, 232, 234, 235, 239
  uv spectra, 143
Amide rule, 239
Amines:
  dipole moments, 326, 327, 334
  ir spectra, 53, 108–113
  ir spectra of salts of, 53, 108–113

Amines (*cont.*):
mass spectra, 318
nmr spectra, 174–178, 193
optical rotation and ord, 232
uv spectra, 133, 145–150
Amino acids:
ir spectra, 110–113
optical rotation and ord, 231, 239
Amplitude of Cotton effect curves, 213, 220, 223, 226
Ångström unit, 6
Anhydrides, ir spectra, 52, 61, 64
Anthracene, ir and uv spectra and bond-lengths, 95, 148, 301
Antibiotics:
ir spectrum of griseofulvin, 116
optical rotations of sugars from, 239
polymeric, mol. wt. determination, 259
X-ray structure analysis of benzyl-penicillin, 302
Anti-Stokes lines, 119
Appearance potential, 312
Area of bands:
in ir spectra, 41, 65, 84
in uv spectra, 129
Aromatic compounds, see also aromatic hydrocarbons
dipole moments, 328–336
esr of, 205–207
ir spectra, 52, 93–95, 104, 110–116
mass spectra, 318
nmr spectra, 174–177, 181, 192–200
non-benzenoid, 151, 330
optical rotation and ord, 213
uv spectra, 132, 146–164
X-ray structure analysis, 299, 301
Aromatic hydrocarbons:
dipole moments, 327
esr of, 205
ir spectra, 52, 93–95
nmr spectra, 181, 182
optical rotation, 222
Raman spectra, 121
uv spectra, 146–151, 164
X-ray structure analysis, 296, 301
Asymmetric absorption band, 218, 230–232
Asymmetric crystal unit, 279
Atom polarisation, 323–326
Auxochrome, 128
$A_n X_m$ spin systems, 184–189, 193, 194
Axis:
inversion, 281
rotation, 281
Axis (*cont.*):
screw, 281
Azides:
ord of, 232
uv spectra, 145
Azo-compounds, uv spectra, 145, 147, 160
Azulenes:
dipole moments, 331
uv spectra, 151, 164

Band width in ir spectra, 41 (e.g. 72)
Barrelene, uv spectrum, 154
Base peak, 313
Bathochromic shift, 128
Beckmann apparatus, 251
Beer's law, 24, 47
Bending vibrations, 37–39, see also under individual bonds
in-plane, 39, 82
out-of-plane, 39, 82
Benzene, uv spectrum, 149
Benzene derivatives, see aromatic compounds
Benzoic acid, ir and uv spectra, 111, 149
Benzosemiquinone anion, esr spectrum, 207
Benzylamine and its hydrochloride, ir spectra, 108
Biradicals, 205
Bixin, uv and nmr spectra, 160, 200
Boiling-point elevation, 252–254, 260
molal, 251–254
Bond angles, 37, 276, 300, 331
Bond dissociation energy, 312
Bond lengths, 37, 42, 276, 277, 300
Bond moments, 327, 329
Bragg equation, 285, 288
Brewster's calculations of optical rotations, 237
Bromogeigerin acetate, electron-density distribution, 294
Bullatenone, ir spectrum, 101
Butyraldehyde, nmr spectrum, 189

Carbohydrates:
ir spectra, 106–108
mol. wt. determination, 248, 256, 259, 260, 263
optical rotation, 211, 216, 217, 221, 222, 231, 237–239, 241
Carbon disulphide as solvent for ir spectra, 44, 54, 80

Carbon tetrachloride as solvent:
for ir spectra, 44, 54, 58, 59, 79–81
for nmr spectra, 173
for Raman spectra, 120
for uv spectra, 131
Carbonates, ir spectra, 113
Carbonyl group, see ketones, esters, etc., also C=O stretching vibration
estimation by ir, 55
Carboxylic acids, see also hydroxy and amino acids and acids, unsaturated
ir spectra, see also C=O and O–H vibrations, 52, 76, 98–101, 110–113
ir spectra of salts of, 52, 110–113
mass spectra, 310, 319
mol. wt. determination, difficulties in, 245
nmr spectra, 174, 175, 178
optical rotation and ord, 213, 232, 234
Raman spectra, 122
uv spectra, 143
X-ray structure analysis, 297, 301
Carotenoids:
nmr spectra, 200–203
uv spectra, 134, 160, 164, 200
C–C stretching vibrations, 39, 51, 72
in Raman spectrum, 121
C=C stretching vibrations, 39, 41, 51, 52, 56, 85–92
conjugation and, 65, 69, 76, 77, 88, 96, 98, 102
electronegative substituents and, 69
factors affecting, 55–78
physical state and, 58–61
prediction of frequencies of, 76, 77
in Raman spectrum, 121
ring size and, 64, 92
structural effects on, 63–66, 69, 71–73, 76, 77, 89, 92
C≡C stretching vibrations, 39, 41, 51, 52
conjugation and, 104
in excited electronic state, 138
in Raman spectrum, 121
Cells:
for ir spectroscopy, 42–44
for uv spectroscopy, 131
C–H bending vibrations, 50
in aromatic compounds, 46, 52, 93, 110–112
in ketones, 52, 84, 85
in olefins, 37, 52, 56, 63–66, 69, 72, 76, 77, 85–92, 101
in Raman spectra, 122
C–H bending vibrations (*cont.*):
in saturated hydrocarbons, 51, 80–93, 101
conjugation and, 65, 69, 76, 89
physical state and, 58–61, 89, 101
prediction of frequencies of, 76
ring size and, 64, 92
structural effects on, 63–66, 69, 72, 76, 77, 80–93, 110–112
C–H stretching vibrations, 39, 51, 52
in acetylenes, 52, 59, 104
in aromatic compounds, 52, 93, 104
in olefins, 52, 56, 58–61, 62–64, 66, 85–92
in saturated hydrocarbons, 51, 80–92
physical state and, 58–61
ring size and, 64
structural effects on, 62–64, 66, 80–93, 104
Chain, average length of unit-, 260
Charge resonance spectra, 154
Charge transfer absorption, 140, 150, 163
Chelates:
ir spectra, 67, 104–106, 114
nmr spectra, 178, 196
uv spectra, 142
X-ray diffraction study, 302
Chemical shifts, 172–182
of common types of protons, 173–178
Chlorobenzene, uv spectrum, 149
Chloroform as solvent:
for ir spectra, 44, 54, 58, 59, 79–81
for nmr spectra, 173
for Raman spectra, 120
for uv spectra, 131
5-α-Cholestane derivatives:
ir spectra, 45, 84, 92
ord curves, 224, 233
uv spectra, 137, 139
Cholesterol, ir spectrum, 85
Chromophore, 127
asymmetric, 218, 230–232
optically active, 218, 230–232
Chrysanthenone, ir spectrum and ord, 96, 214
Circular birefringence, 217
Circular dichroism, 12, 211, 218–220, 232–234
structural and stereochemical applications, 220, 232–234
Circularly polarised light, 217
Circumanthracene, X-ray structure analysis, 296

Cisoid:
dienes, 135
unsaturated ketones, 65
C – N stretching vibrations, 39, 51, 69, 108
C=N stretching vibrations, 39, 51, 53
C≡N stretching vibrations, 39, 51, 53
C – O stretching vibrations, 36, 39, 51, 52, 62, 80–89, 98, 106, 107
alkyl substitution in alcohols and, 72
conformation and, 89
electronic changes in esters and, 69
C=O stretching vibrations, 39, 41, 42, 51, 52, 80–88, 95–106, 110–116
alkyl substitution and, 71, 72
conjugation and, 65, 69, 74, 77, 78, 88, 95–98, 113–116
dipole interactions and, 70
electronegative substituents and, 68–71
factors affecting, 55–78
hydrogen bonding and, 58–62, 67, 71, 74, 100, 106, 113
physical state and, 58–62
prediction of frequencies of, 73–78
ring size and, 63, 84
structural effects on, 55, 63–78
transannular effects on, 71
Cocaine, ir spectrum, 108
Combination bands, 39, 93
weakness in Raman spectra, 121
Configuration:
absolute, 17, 220, 226, 231, 234, 236, 237, 239, 241, 303
of olefins, 17, 72, 101, 160, 177, 191–193, 196, 200–203, 327
of organometallic compounds, 335
of oximes, 332
relative, 17, 68, 107, 210, 220–242, 283
Conformation, 18
circular dichroism and, 234
dipole moments and, 332–334
electron diffraction and, 277
ir spectra and, 40, 57, 68, 70, 89
nmr spectra and, 178, 179, 191
optical rotation, ord and, 216, 220, 221, 225, 229, 231, 239
uv spectra and, 135, 138
Conjugation:
cross-, 141
and dipole moments, 329
and ir spectra, 65, 69, 74, 76–78, 88, 95–98, 113–116
and uv spectra, 19, 127, 128, 132–156, 159
Conjugation (*cont.*):
π-π, 132, 150
p-π, 128, 133, 150
Correlation studies, 317
Cotton effect, 219, 232
Cotton effect curves, 212
amplitude of, 213, 220, 223, 226
multiple, 213
sign of, 212, 220, 223
single, 212
structural and stereochemical applications, 220, 223–232
Coupling:
constant, 184–193
spin-spin, see spin-spin coupling
vibrational, 40, 69, 72, 74, 92, 102, 107
Cracking pattern, 312
analytical applications, 313
structural applications, 317–321
Cross-conjugation, 141
Cryoscopic method, 250–252, 260
Crystal systems, 282
Crystalline state, 278–285
Cubic crystal, 282
Cyanides:
dipole moments, 327, 330
ir spectra, 51, 53
mass spectra, 319
nmr spectra, 176, 177
uv spectra, 144
Cyclohexane, ir spectrum, 80
Cyclohexanol, ir spectrum, 61, 80
Cyclohexanone, ir spectrum, 64, 82
Cyclopentanone, ir spectrum, 64, 82
Cyclopropanes:
ir spectra, 52, 93
nmr spectra, 174, 175

Debye equation, 324
Debye unit, 323
Depolarisation factor, 120
Deuteration:
ir spectra and, 49, 51, 62, 84, 106, 108
mass spectra and, 311, 319
nmr spectra and, 178, 196
Dielectric constant and dipole moments, 323–325
Dienes:
electron diffraction, 277
ir spectra, 40
ord of, 231, 232
uv spectra, 133–137
Dienones:
ir spectra, 116

Dienones (*cont.*):
uv spectra, 142
Diethyl ether, nmr spectrum, 189
Difference method for detection of impurities, 45
Diffraction methods, 4, 12–15, 276–304, see also electron, neutron and X-ray diffraction
Diffusion coefficient, 268, 270, 271, 272
Dihedral angle and coupling constants, 190
Diketones:
ir spectra, 71, 77, 104–106
uv spectra, 142, 143
Dinitrophenylhydrazones:
nmr spectra, 177
uv spectra, 131, 143
Diphenyls:
dipole moments, 327
electron and X-ray diffraction, 277
optical rotation and ord, 222, 232
uv spectra, 147, 159
Dipole moments, 14, 323–337
conformation and, 332–334
determination of, 324–326
electronic theory and, 327–331
solvent effects on, 326
stereochemistry and, 331–334
steric inhibition of resonance and, 329
Dissymmetry, 210, 283, 301
Dissymmetry method, 267
Distance rule, 236
Disulphides:
ord of, 232
uv spectra, 145
Dithiocarbonates:
ord of, 232
uv spectra, 145
Drude equation, 219, 234
Dyes, electronic spectra, 154, 164

Ebulliometry, 252–254, 260
Elaidic acid, ir spectrum, 98
Electromagnetic spectrum, 4
Electron affinity, 163
Electron-density distribution, 294–299, 302
Electron diffraction, 12, 277
Electron impact, 306, 312
Electron microscopy, 274
Electron paramagnetic resonance, see electron spin resonance
Electron polarisation, 323–326
Electron spin resonance, 10, 168, 169, 203–207
absorption and derivative spectra, 204
applications of, 203–207
spin-spin coupling (hyperfine structure) in, 205–207
Electronic spectra, see ultraviolet and visible spectra
Electronic transitions, types of, 127
End-group assay, 255, 260
Ene-diones, uv spectra, 143
Equivalent positions, 279
Esters:
dipole moments, 327, 333, 334
ir spectra, see also C–O and C=O vibrations, 52, 69–72, 95–101, 110, 113
nmr spectra, 174–176
optical rotation and ord, 228, 232, 240
uv spectra, 138, 143
Esters, unsaturated:
ir spectra, 95–101
nmr spectra, 175, 200–203
uv spectra, 144
Ethers:
dipole moments, 327
ir spectra, 52, 106, 107
mass spectra, 318
nmr spectra, 174–177, 189, 193, 195
uv spectra, 145
Ethyl acetoacetate, ir spectrum, 104
Ethyl geranate and isomer, ir spectra, 96
Extinction coefficient, 24
apparent, 26, 41
molar, 25, 129
Extrema, 212

Faraday effect, 211, 215
Fermi resonance, 40, 61, 74, 78, 100, 111, 123
Fibre photograph, 288
Fibres, ir spectra, 58
Fine structure:
rotational, 24
vibrational, 24, 36, 128, 129, 134, 138, 146
Fingerprint region, 33, 45
Flavones:
ir spectra, 101
nmr spectra, 195–200
uv spectra, 164
Florube-3, 43, 80
Flory's theory of polymer solutions, 263, 270

Forbidden transitions, 38, 41, 129, 146, 160
Force constant, 38
Fourier synthesis, 293–300
Fragment ion, 312, 317–321
Freezing-point depression, 250–252, 260
  molal, 250–252
Frequency, 6
Functional group assay, 255
Fundamental modes, 37–39

Gem-dimethyl group, ir spectra, 90–92, 104
Geranate, ethyl, ir spectrum, 96
Glide plane, 281
Glucopyranoside, methyl α-D-, ir spectrum, 106
Glycosides:
  ir spectra, 106–108
  optical rotation, 237, 238, 239
Golay cell, 27
Goniometer, 286
Griseofulvin, ir spectrum, 113
Group frequencies, 37–39, 48–78, see also C=O stretching vibrations, etc.
  factors affecting, 55–78
  intramolecular factors and, 62–78
  physical state and, 57–62
  prediction of, 73–78
  Raman spectroscopic, 121
  relation with other properties, 41, 58
  structure and, 62–78

Half-band width in ir spectra, 41 (e.g. 72)
Halides:
  dipole moments, 327–329, 332, 333
  electron diffraction, 277
  ir spectra, 54, 80, 113
  mass spectra, 316
  nmr spectra, 174–177
  uv spectra, 127, 145–149
Halide discs, ir spectra of, 43
Haloketones:
  dipole moments, 333
  ir spectra, 70, 78
  ord of, 229–231
  uv spectra, 138
Halverstadt-Kumler equation, 325
Heavy-atom method, 297–300, 302
Heteroannular diene, 135–137
Heterocyclic compounds:
  dipole moments, 327
  ir spectra, 59, 101, 102, 110–116
  nmr spectra, 175, 177
Heterocyclic compounds (*cont.*):
  uv spectra, 151, 152, 164
  X-ray structure analysis, 291
Hexagonal crystal, 282
Hexahelicene, 218, 222, 301
Homoannular diene, 135–137
Homogeneity, polymer, 263, 271
Hudson's rules, 237–239, 241
Hydrocarbons, see also olefins, acetylenes and aromatic hydrocarbons
  mass spectra, 317
  Raman spectra, 121
  saturated, ir spectra, 51, 80–90
Hydrogen bonding, 18
  ir spectra and, 56–62, 66–68, 74, 76, 85, 100, 105, 106, 108, 114
  mol. wt. determination and, 245
  neutron diffraction studies, 277
  nmr spectra and, 178, 196
  X-ray diffraction study, 302
Hydroxy acids, optical rotation and ord, 231, 237, 239, 241
Hydroxyl group, see also alcohols, phenols, hydrogen bonding and O–H vibrations
  estimation by ir, 55
Hyperconjugation and uv spectra, 133
Hypsochromic shift, 128

I (nuclear spin number), 168
Impurities, detection of:
  ir spectra and, 45, 79
  mass spectra and, 313
  uv spectra and, 160
Inductive effect, 18, 42, 68, 179, 317, 328
Infrared spectroscopy, 8, 22–125, see also group frequencies
  applications of, 44–55, 78–116
  correlation charts, 50–54
  intensities in, 40, 50, 55, 62, 65, 84, 88, 92–93, 98, 101, 111
  interpretation of spectra, 48–78
  quantitative analysis by, 46, 55
  sampling methods, 42–44
  solid state effects, 43, 57, 89, 101
  solvent effects, 57–62, 73, 74, 76
  solvents for, 44, 54, 79–81
Inorganic ions, uv spectra, 154
Intensities:
  in ir spectra, 40, 50, 55, 62, 65, 84, 88, 92–93, 98, 101, 111
  in nmr spectra, 173
  in Raman spectra, 121
  in uv spectra, 129, 132, 154, 159, 160

Intensities (*cont.*):
in X-ray diffraction, 292
Inversion axis, 281
Ionisation constants, from uv spectra, 152, 161–163
Ionisation potential, 163, 312
Isomorphous replacement, 299, 302
Isopiestic method, 249
Isosbestic point, 163
Isothermal distillation, 249, 259
Isotope dilution analysis, 311
Isotopic abundance measurement, 310, 316

J (coupling constant), 184–193

K-bands, 127
Karplus curve, 190, 191
Ketal formation, ord and, 226
Ketals, ir spectra, 107
Ketones:
circular dichroism, 233
dipole moments, 327
halogenated, see haloketones
ir spectra, see also C=O vibrations, 45, 52, 63–78, 80–88, 95–98, 104, 113–116
mass spectra, 319
nmr spectra, 174–177, 193, 195
optical rotation and ord, 223–231, 240
uv spectra, 138–143, 147–150, 154, 160
Ketones, unsaturated:
ir spectra, 65, 74, 77, 84–88, 95–98, 104, 113–116
nmr spectra, 175–177
ord of, 230, 231
uv spectra, 139–142
Kinetics, 18, 161, 178, 191, 217

Lactones:
dipole moments, 333
ir spectra, 52, 64, 71, 78, 123
optical rotation and ord, 228, 241
Raman spectra, 123
uv spectra, 138
Lactone rule, 241
Lambert's law, 24
Landé spectroscopic splitting factor, 204
Lapachenole, uv spectrum, 156
Lattice, 278
plane, 284
Laue equations, 285
Light scattering, 247, 265–268
Lunacrine and lunine, nmr spectra, 193

Magnetic resonance spectroscopy, 10, 168–207, see also nuclear magnetic resonance and electron spin resonance
Magnetic shielding, see shielding
Magneto-optical rotation, 211
Mass spectrometer, 306–310
double-focusing, 255, 308, 316
single-focusing, 255, 308
time-of-flight, 309
Mass spectrometry, 14, 306–321
analysis by, 311, 313
molecular formulae from, 315–317
molecular weights by, 195, 255, 315
sample requirements, 310
structure determination by, 317–321
Mc/s, 6
Mercaptans:
ir spectra, 54
Raman spectra, 121
uv spectra, 145
Meso-ionic compounds, 330
Mesomeric effect, 18, 42, 69, 106, 179, 317, 328
Mesomeric moments, 328
Methyl groups:
analysis for, by ir, 90
ir frequencies, 51, 85–93, 104
nmr spectra, 174, 188, 192, 193
Methylene groups:
adjacent to carbonyl group, analysis for, 84
ir frequencies, 51, 80–93
nmr spectra, 175, 176, 192
splitting of vibrational modes in solids, 57, 89, 101
Methyl oleate, ir spectrum, 98
Micron, 6
Microwave spectroscopy, 5, 6, 203–207
Miller indices, 284
Millimicron, 6
Molar absorptivity, 24
Molar extinction coefficient, 25, 129
apparent, 26, 41
Molar polarisation, total, 324
Molar refractivity, 325
Molecular amplitude, 213, 223, 226
Molecular formulae:
determination by mass spectrometry, 315–317
molecular weight determination and, 245
Molecular ion, 312, 315

Molecular rotation, 212
differences, method of, 240
Molecular weight averages, 246–248, 271, 273
Molecular weight determination, 14, 244–274
applications of, 244, 245
association, dissociation and, 245, 252, 254
mass spectrometric, 195, 255, 315
methods for, 244–274, see also under individual methods
non-ideal behaviour and, 244, 251, 254, 262, 263, 267, 270, 272
by uv spectroscopy, 163, 256
by X-ray diffraction, 254, 291
Monochromator, 27
Monoclinic crystal, 282, 285, 288, 290

N → Q transition, 127
N → R transition, 127
N → T transition, 127
N → V transition, 127
$n \rightarrow \pi^*$ transitions, 127–129, 138, 139, 143, 144, 150, 152, 160
$n \rightarrow \sigma^*$ transitions, 127, 145
Neutron diffraction, 14, 277
N – H bending vibrations, 51, 108–111
N – H stretching vibrations, 39, 42, 51, 53, 108–111
hydrogen bonding and, 59, 108
physical state and, 57–60
Nitrates, ir spectra, 53, 101
Nitriles, see cyanides
Nitrites:
ir spectra, 53, 101
mass spectra, 320
ord of, 232
uv spectra, 145
Nitrobenzene, uv spectrum, 147, 149
Nitro-compounds:
dipole moments, 327
esr of, 207
ir spectra, 53, 100, 101
mass spectra, 320
ord of, 232
uv spectra, 144, 147, 150
Nitroso-compounds:
ir spectra, 53, 101
ord of, 232
uv spectra, 145
Normal modes, 37
Nuclear magnetic resonance, 10, 168–203
applications of, 173–182, 188–203
Nuclear magnetic resonance (*cont.*):
chemical shifts in, 172–182
exchange phenomena in, 178, 191
experimental procedure, 170–173
intensities, 173
solvents for, 173
spin-spin coupling in, 183–193
Nujol, 42, 80
Number-average mol. wt., 246, 259, 265
Numismine, 162

Octane, mass spectrum, 314
Octant rule, 228–231
O – H bending vibrations, 51, 56, 62, 100, 111
O – H stretching vibrations, 39, 42, 51, 56, 80–89, 100, 105
alkyl substitution and, 72
electronegative substituents and, 70
factors affecting, 55–78
hydrogen bonding and, 56–62, 66–68, 76, 85, 100, 105, 106, 114
physical state and, 57–62, 85, 101
prediction of frequencies of, 76
structural effects on, 62, 66–68, 70–77
Oleate, methyl, ir spectrum, 98
Olefins:
configuration of, 17, 72, 101, 160, 177, 191–193, 196, 200–203, 327
dipole moments, 327, 329, 335, 336
ir spectra, see also C=C and C – H vibrations, 52, 56, 64, 72, 76, 85–93, 96–98, 101
mass spectra, 318
nmr spectra, 174–177, 180, 187, 191–193, 196–203
optical rotation and ord, 232, 240
Raman spectra, 119, 121
uv spectra, 133–137
Optical activity, 210
presence of, and structure, 222
Optical density, 24
Optical rotation, 10–13, 210–242
analytical applications of, 216
effect of temperature, solvent and concentration on, 216
measurement of, 212–216
rules, 221, 228, 236–242
sample requirements, 215
single wavelength, 211, 220, 235–242
structural and stereochemical applications, 220–242
theories of, 217–221, 228, 231, 237, 242

Optical rotatory dispersion, 12, 211–235, 242
  analytical applications of, 216
  curves, types of, 212
  measurement of, 212–216
  sample requirements, 215
  structural and stereochemical applications, 220, 223–235
Optically active absorption band, 218, 230–232
Organometallic compounds:
  dipole moments, 334–336
  esr of, 203
Orientation polarisation, 324–326
Orthorhombic crystal, 282, 288, 290
Oscillation photograph, 288
Osmotic pressure, 246, 263–265
Overtones, 37, 39, 82, 84, 89, 93
  weakness in Raman spectra, 121
Oximes, dipole moments, 332

$\pi \rightarrow \pi^*$ transitions, 127, 133–160
Paracyclophanes, uv spectra, 154
Parent ion, 315
Partial rotation, 218–220, 232, 236
Patterson function, 300
Pentacene, ir and uv spectra, 95, 148
Phase constants in X-ray diffraction, 293–300
Phase problem, 276, 293–300
Phenanthrene, ir and uv spectra, 95, 148
Phenols:
  dipole moments, 327
  ir spectra, 59, 67, 70, 76, 106, 114
  nmr spectra, 178, 196
  uv spectra, 147–148, 152, 153, 162
Phosphorus compounds, ir spectra, 54, 56
Photometric accuracy, 31, 132
Phyllocladene derivatives, ir spectra, 90
Plain rotatory dispersion curve, 212, 220, 234–237
Platinum phthalocyanine, electron-density distribution, 298
Polarimeter, 213–215
Polarised light:
  circularly, 217
  ir spectra with, 58
  plane, 217
Polyacetylenes, uv spectra, 138, 160, 164
Polyenes:
  nmr spectra, 200–203
  uv spectra, 132, 134, 160
Polyethylene glycol–400, ir spectrum, 106
Polymers:
  esr of, 207
  ir spectra, 57, 58, 90, 106–108
  molecular shape of, 262, 267, 271, 273
  mol. wt. determination, 244–274
  X-ray diffraction by fibrous, 289
Polymorphism and ir spectra, 43, 57, 101, 108
Polystyrene, ir spectrum, 28, 31
Powder photograph, 13, 290
Primitive translations, 278
Proline, ir spectrum, 111
Proteins:
  ir spectra, 69
  mol. wt. determination, 259, 261–263, 269, 275
  optical rotation and ord, 211, 235
  X-ray structure analysis of, 286, 300, 302
Proton magnetic resonance, see nuclear magnetic resonance
Pyridoxine, uv spectrum, 152
Pyrones, ir spectra, 101, 102

Quantitative analysis, 18, 34
  by infrared spectroscopy, 46, 55, 84, 90, 96
  by mass spectrometry, 311, 313
  by nmr, 173
  by optical rotation, 216
  by Raman spectroscopy, 121
  by uv spectroscopy, 160, 161

R-bands, 127
Radicals, free, investigation by esr, 203–207
Radiofrequency spectroscopy, 5, 6, 169 *et seq.*
Raman frequency, 119
Raman shift, 119
Raman spectroscopy, 8, 116–123
  apparatus and sampling, 120
  applications, 121–123
  comparison with ir spectroscopy, 121–122
  group frequencies in, 121
  intensities in, 121
  origin of spectra, 116–120
  quantitative analysis by, 121
  selection rules in, 120
  solvents for, 120
Rast method, 252

Rayleigh scattering, 116, 267
Rearrangements in mass spectrometry, 318
Reserpine, uv spectrum, 157
Resolving power, 31, 42
Resonance frequency, 169, 171
Rhombohedral crystal, 282
Rotation, see also optical rotation
  axis, 281
  magneto-optical, 211
  molecular, 212
  partial, 218–220, 232, 236
  photograph, 286
  specific, 212
Rotational spectra, 7, 23, 119
Rotational strength, 218
Rotatory dispersion, see optical rotatory dispersion
Rule of optical superposition, 237
Rule of shift, 238

S (electron spin number), 168
$\sigma \rightarrow \sigma^*$ transitions, 127
Santonin, uv spectrum, 141
Scattering factor, 292
Schlieren method, 256–259, 269
Screw axis, 281
Sedimentation coefficient, 269, 270
Selection rules, 38, 120, 129
Semicarbazones:
  nmr spectra, 177
  uv spectra, 143
Sericetin, 195
Shielding:
  diamagnetic, 179, 181
  long range, 182, 195, 200
  magnetic, 170–172, 179–182
  paramagnetic, 179
Shoolery's shielding contributions, 175, 176
Side band technique, 171–172
Skeletal vibrations, 39, 51, 80–82, 93, 122
Sodium benzoate, ir spectrum, 111
Solvents:
  for ir spectra, 44, 54, 79–81
  for nmr spectra, 173
  for optical rotation measurements, 216
  for Raman spectra, 120
  for uv spectra, 130, 131, 140, 145
Solvent-compensated ir spectra, 44, 79–81
Solvent effects:
  on ir spectra, 57–62, 73, 74, 76
Solvent effects (*cont.*):
  on nmr spectra, 173
  on optical rotation, 216
  on uv spectra, 129, 130, 133, 140, 153
'Solvent shifts' in ir spectra, 49, 58–61
Space group, 281–284, 290
Specific rotation, 212
Spectrograph, 27
Spectrometer, 27
Spectrophotometer, 27–31
  double-beam, 29
  grating, 42, 79, 133
  'Infracord', 78
  single-beam, 29
  Unicam SP.130, 36
  Unicam SP.200, 28
Spectropolarimeter, 211, 213–215
Spin:
  electron, 168, 203
  nuclear, 168
Spin number, 168
Spin-spin coupling:
  $A_n B_m$ systems, 188, 189, 193, 194
  $A_n X_m$ systems, 184–189, 193, 194
  constant, 184–193
  in esr spectra, 205–207
  in nmr spectra, 183–193
  structural and stereochemical significance, 188–203
  theory of, 189, 190
Squalane, ir spectrum, 89
Staudinger equation, 272
Stearic acid, ir spectrum, 98
Steroids, 17
  circular dichroism, 233
  dipole moments, 333
  ir spectra, 45, 57, 70, 84–89, 92, 106, 107
  mass spectra, 318
  optical rotation and ord, 211, 221, 223–234, 238, 240
  stereochemical nomenclature, 225
  uv spectra, 135–137, 139–142, 156, 160, 164
Stokes lines, 118
Stray light, 132
Stretching vibrations, 37–39, see also under individual bonds
Structure amplitude, 292–300
Sulphur compounds:
  dipole moments, 327
  ir spectra, 54, 56, 59
  ord of, 231, 232
  Raman spectra, 121

Sulphur compounds (*cont.*):
uv spectra, 127, 143–146, 164
Sum and difference spectra, 157
Surface pressure, 262
Svedberg's equation, 268
Sydnones, dipole moments, 330
Symmetry, 279–284
centre of, 281
mirror plane of, 279
operation, 279
and uv spectra, 127, 129
Systematic absences, 290

τ (tau)-values, 173–178
Tautomerism, 18
and ir spectra, 104–106
and uv spectra, 142, 153, 161
Terpenes, 17
ir spectra, 49, 59, 72, 88–92, 95–98
optical rotation and ord, 211, 221, 223, 225–231, 241
uv spectra, 135, 139–142
X-ray structure analysis, 279, 289, 294
Testosterone, ord curve, 230
Tetracene, ir and uv spectra, 95, 148
Tetragonal crystal, 282
Tetramethylsilane, 172
Tetrazine (sym-), uv spectrum, 128
Theta-solvent, -temperature, 262
TMS, see tetramethylsilane
Tracer studies, 311
Transition:
allowed, 129, 159
forbidden, 38, 41, 129, 146, 160
moment, 40, 129
Transmittance, 25
Transoid:
dienes, 135
unsaturated ketones, 65, 142
Triclinic crystal, 282, 288
Trienes, uv spectra, 134–137
Triplet state:
and esr spectra, 205
and uv spectra, 127
Tropane derivatives, 68
Tryptophane, ir spectrum, 111
Turbidity, 267

Ultracentrifugation:
pseudo-equilibrium (Archibald) method, 256–259, 261, 272
sedimentation equilibrium, 256, 271
sedimentation velocity, 256, 268–271
Ultraviolet and visible spectra, 10, 22–34, 126–167
absorptions of common structural types, 132–155
alkyl substitution and, 133–135, 139–141, 144, 146, 151
analytical applications of, 160–164
conjugation and, 19, 127, 128, 132–156, 159
experimental technique, 129–133
literature of, 164
solvent effects on, 129, 130, 133, 140, 153
solvents for, 130, 131, 140, 145
steric effects on, 135, 138, 145, 153, 159, 160
structural applications of, 155–160
tautomerism and, 142, 153, 161
theoretical aspects of, 126–129, 132, 134, 151, 164
Unit cell, 278
centred, 281
dimensions, 286, 291
primitive, 281
Unsaturated compounds, see olefins, acetylenes or under main functional group (e.g. ester)

Van der Waals radii, 301
Vapour density, 248
Vapour pressure, depression of, 248–250, 259
Verbenone, ir spectrum, 77, 95
Vibration, molecular, 23, 36, see also stretching and bending
Vicinal action, 220, 240
Vinyl chloride, nmr spectrum, 188
Vinyl ethers:
ir spectra, 69
uv spectra, 133
Virial coefficients, 263, 267
Viscosity, 247, 270, 272–274
intrinsic, 272
limiting viscosity number, 272
specific, 272
variation with shear-force, 273
Viscosity-average mol. wt., 248
Vitamins:
uv spectra, 164
uv spectra of A group, 134, 164
uv spectrum of pyridoxine, 152, 153
X-ray structure analysis of B_{12}, 302

Water, in ir spectra, 43, 79, 106

Wavenumber, 6
Weight-average mol. wt., 247, 258, 267, 271
Weissenberg photograph, 288
Woodward and Fieser rules:
  for polyenes, 135–137
  for unsaturated ketones, 140–142

X-ray diffraction, 6, 12, 276–304
X-ray diffraction (*cont.*):
  applications, 300–304
  by crystals, 284–300
  experimental procedure, 285–292
  mol. wt. determination by, 254, 291
  structure analysis by, 292–300
Xylenes, 46, 147

Z-average mol. wt., 248
Zimm extrapolation method, 268