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A thesis entitled:

"Mass Spectroscopy of Organic Compounds,"

which is submitted in fulfillment of the regulations for the degree of Doctor of Philosophy in the University of Glasgow.

by

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CONTENTS

SUMMARY of Ph.D. THESIS

MASS SPECTROSCOPY of ORGANIC COMPUNDS

by ANDREW McCORMICK

The mass spectra of twelve dihydro-1,3-benzothiazines and six dihydro-1,3-benzoxazines are recorded. A brief discussion of the particular fragmentations of a few of these is given. The majority of the compounds have a substituted phenyl group on the 2-position of the oxazine or thiazine ring. These compounds fragment in a characteristic fashion in the mass spectrometer which can be described as a retro-Diels-Alder reaction. The relative abundances of the ions produced by this decomposition process are related to the electron donating power of the substituents on the 2-phenyl group and the conclusion is reached that the phenyl ring opens on electron impact - which is in contrast to the findings of previous workers who studied the effect of substituents on the electron-impact-induced dissociation of aromatic ketones. A study of the relative rates of formation of ions produced by the retro-Diels-Alder reaction reveals that this is a two-step process and that a different bond is initially broken in the two series of compounds.

A brief review of the different methods of qualitative analysis of mixtures by mass spectrometry is given and the application of these techniques to the identification of the components of inseparable mixtures of natural products is described. The comparatively new technique of combined gas-chromatography - mass-spectrometry is described in some detail and a novel method of quantitative analysis of mixtures using this method is illustrated.

The use of high resolution mass spectrometry in the rapid identification of naturally occurring compounds of known structure is illustrated. A few of the compounds isolated from the seeds of Mammeia americana L. are described. Some of these were already known. High resolution mass spectrometry has proved to be invaluable in the structure elucidation of others.

CHAPTER I.

INTRODUCTION

Although the basic principles of mass spectroscopy were established at the beginning of the present century[1], and the forerunners of modern instruments were built by Dempster in 1918[2] and Aston in 1919[3], it is only in the last twenty-five years or so that mass spectroscopy has become widely used as a tool in chemical research.

This late development of the science is owed mainly to the fact that early instruments, built in the laboratories of the operators themselves, were extremely unreliable in operation. Improvements in electronic techniques and the demand for rapid, reliable analyses of petroleum fractions during the Second World War led to the production on a commercial basis of mass spectrometers which were reliable and simple to operate. These instruments followed the original design of Dempster, i.e., separation of ions of different mass-to-charge ratios was effected by means of a magnetic field and recording of the spectrum was by electrical means.

In the mass spectrum of a compound run under fixed operating conditions, the relative intensities of the ion beams at different

mass-to-charge ratios are characteristic of that compound, and the absolute intensities of these ion beams are proportional to the partial pressure of the vapour of the compound in the ionization region. Further, the spectra obtained from an instrument run under carefully controlled conditions are highly reproducible, and the use of electrical detection of the ion beams enables a wide range of intensities to be recorded in a single scan. The mass spectrometer, therefore, provides a valuable method of quantitative analysis of mixtures of volatile compounds. Its advantages over traditional methods of hydrocarbon analysis were first demonstrated by Washburn, Wiley and Rock in 1943[4], and for the next decade mass spectrometry was confined almost exclusively to this type of investigation. The complexity of mixtures which could be analysed routinely by mass spectrometry was greatly increased by the introduction of electronic computation of the experimental data.[5] The main disadvantage of the method is the cost of the instrumentation, and for this reason vapour phase chromatography has, to a great extent, superceded mass spectrometry, both as a means of analysing mixtures of known constituents, and for the detection of trace impurities in chemical samples.

As mentioned above, the relative intensities of the ion beams

of different mass-to-charge ratios in the mass spectrum of a compound are characteristic of the structure of the molecule. With a sufficient knowledge of the origin of such ion beams it should be possible to deduce the structure of an "unknown" compound from its mass spectrum, as was pointed out by Rock[6]. The presence of ions in the mass spectrum which could only arise by a re-arrangement of the atoms in the molecule at first seemed to offer a serious drawback to the use of mass spectrometry as a structural tool. It was, however, suggested by McLafferty[7] that occurrence of these re-arrangement ions could be specific for certain atomic groupings. An explanation of their formation could be found in such concepts as ease of formation of the transition state and the stabilities of the charged and neutral fragments formed. Far from hampering structure elucidation, re-arrangement ions might be the most characteristic feature of the mass spectrum of a compound of a given structure. A vast literature has since appeared in which attempts have been made to rationalize the mass spectra of a great variety of chemical structures in terms of the usual concepts of organic chemistry. These concepts, as applied to mass spectrometry, have recently been summarized by Biemann[8]. In many cases "proof" of the mechanisms involved in the formation of fragment ions has been obtained by incorporation of heavy isotopes

in the molecules under investigation.[8,9,11.]

The vast majority of structural investigations by mass spectrometry still make use of a mechanistic approach to the interpretation of spectra. This is particularly useful where hetero-atoms are concerned, the underlying theme being that hetero-atoms such as nitrogen and oxygen may give resonance stabilization of the fragment ions produced. For example, this is used to explain the significant β-cleavages of such compounds as ethers,[10]

$$\left[R - O - CH_2 - R'\right]^{+\cdot} \longrightarrow R - \overset{+}{O} = CH_2 \longleftrightarrow R - O - \overset{+}{C}H_2 \quad + \ {}^{\cdot}R'$$

In spite of the enormous amount of available data correlating mass spectra with particular structures and the wide applicability of mechanistic principles in explaining fragmentations, a great deal of guess work was (and is) still involved in the interpretation of the data obtained. It was soon realized that much of the guesswork could be eliminated if the mass-to-charge ratios (i.e. the mass if the ion is singly charged) of the ions in the spectrum could be determined to a much higher degree of precision than was possible with the single-focusing spectrometers then commercially available. These instruments had resolving powers of one part

in a few hundred, i.e. they were very limited in their ability to separate ions of the same nominal mass but differing in their atomic constitution. Such ions are termed "isobaric" and differ in mass only by the "packing fractions" of the different nuclei involved.[11] This mass difference may only be one part in a few thousands and a mass spectrometer having this resolving power is necessary to produce a distinct signal at the collector for each of the species involved in the isobaric multiplet. Such instruments, specially designed for use in organic chemistry, have become commercially available only in the last few years.[12,13] These instruments are of so-called "tandem" design, i.e., the ions pass through successive electrostatic and magnetic fields. This combination can be designed to have velocity focusing properties and thus one of the factors limiting the resolving power, viz., the energy spread of the ion beam, may be eliminated. The commercial double-focusing instruments follow the designs of either Nier and Johnson or of Mattauch and Herzog. Since an instrument of the former design was used by Beynon in his pioneering work on the application of double-focusing instruments in analytical mass spectrometry,[14] it will be discussed first.

In the Nier-Johnson design of mass spectrometer the ion beam is deflected in the same direction in both the electrostatic and

the magnetic analysers. In such a design the focus at the collector end is not planar: thus photographic detection of the ion currents is not technically possible. This type of instrument, using easily adjustable source and collector slits with an electron multiplier detector has certain advantages in the study of metastable transitions[15] and the use of magnetic scanning enables a very wide mass range to be covered in a single scan. One of the most serious limitations to date is in the method of accurate mass measurement. Since scanning of the spectrum is necessary and the record is produced on a moving paper strip, the method of relating mass to distance along the chart paper has very limited application.[16] The usual method of determining the exact mass-to-charge ratio of an ion is to fix the magnetic field at a constant value and then to measure the ratio of the two voltages necessary to focus at the collector the ion of unknown mass and a known reference mass respectively.[16] Even using the peak-matching technique of Nier[17] this is a time-consuming operation and does not lend itself to automation. Users of these instruments consequently tend to make use of the data obtained under low resolving power in the interpretation of spectra, the accurate masses (and hence composition) of the parent molecule-ion and of only a few "key" fragment ions being used as an aid to

such analyses. Recently, however, a communication has been published[18] which describes a method by means of which high resolution data may be obtained automatically from a Nier-Johnson instrument. Possible advantages over the photographic plate method described below are discussed.

Deflexion of the ion beam in opposite directions in the two analyser systems is the basic feature of the Mattauch-Herzog design. This gives a focal plane at the collector end and thus a photographic plate can be used as detector. Use can also be made of the very high sensitivity of the electron multiplier by positioning a conventional slit assembly at a point in the focal plane. When the photographic plate method is used no scanning of the spectrum is necessary. This simultaneous collection of all ions in the mass spectrum is essential if sources which give an ion beam of fluctuating intensity are used; examples of these are the field emission[19] and spark sources.[20] With such sources integration of the collected ion currents, as is done by the photographic plate, is necessary.

If the normal electron bombardment source is used, the distance of the lines along the photographic plate can be related with a high degree of precision to the mass of the ions which produced them if simultaneous calibration of the plate by means of a reference

compound is employed.[21] Thus it is practicable to obtain on a single exposure all of the high resolution data obtainable from the instrument. This method lends itself to automatic computation of the data. Using computer methods the chemical formulae of all of the ions in the mass spectrum can readily be obtained. A novel approach to the interpretation of spectra using such techniques has been introduced by Biemann. This is the "element-mapping" system[21] and although it has been little used to date it should prove to be one of the most important advances made in analytical mass spectrometry and may be the key to interpretation of spectra by electronic computers.

For problems requiring only low resolving power, e.g., the routine detection of known compounds, expensive double-focusing mass spectrometers are superfluous. A great variety of single-focusing instruments are now readily available for this and other special purposes. These may use magnetic dispersion, time-of-flight, quadrupole resonance or radio-frequency methods for separation of ions of different mass-to-charge ratios and may have certain advantages, such as portability or faster scan speeds, not common to the large double-focusing instruments. A description of some of these instruments is given in reference 22.

One of the main factors limiting the use of mass spectrometry

in organic chemistry was, and to a lesser extent still is, the requirement that the sample must be vaporized prior to ionization. The conventional type of sample-handling system consists of a glass flask about three litres in volume. The sample is allowed to expand into this vessel and the vapour flows through a molecular leak and then along a glass tube into the ionization chamber. The pressure of sample in the ionization chamber required in order to produce a useful spectrum is of the order of 10^{-5} torr. The large size of the sample reservoir, the comparatively high sample pressure in it (about 10^{-2} torr) and the molecular leak are necessary to ensure a steady flow of sample vapour at the required pressure into the ionization chamber. The vapour pressure requirement of 10^{-2} torr or more means that such a system can only be used for gases or very volatile liquids, such as the hydrocarbon mixtures mentioned above.

The range of samples which can be examined in the mass spectrometer was greatly extended by the introduction of heated reservoir systems by O'Neal and Wier[23] and by Caldecourt.[24] Such systems can be operated at temperatures up to 400°C: thus involatile liquids and solids may be vaporized. One limitation of the method is that many samples decompose thermally at temperatures below that required to produce a sufficient vapour pressure. Further, modern

techniques of organic chemistry permit the study of sub-milligram quantities of material. In such circumstances the amount of sample available for mass-spectrometric examination may be much less than the half-milligram or so required for the normal type of heated reservoir system. If only a qualitative analysis is required this second difficulty may be overcome by reducing the volume of the heated reservoir or by increasing the leak rate. The resultant greater depletion in sample pressure during recording of the spectrum is not so important in qualitative as in quantitative work.

The first difficulty mentioned in connexion with reservoir systems, i.e., insufficient vapour pressure below the thermal decomposition point, is not so readily countered. One method commonly used is to make a more volatile derivative of the sample, e.g., by formation of ethers from alcohols. This, however, has drawbacks; for example, not all of the hydroxyl functions of a molecule may etherify. The reaction would then have to be repeated using a different alkyl group in order to ascertain the number of such groups introduced and hence the true molecular weight of the original compound. Further, preparation of derivatives will generally result in mixtures; thus purification techniques will have to be used prior to mass spectrometric examination. A more generally useful means of examining involatile or thermally unstable compounds is

to use a direct evaporation technique. In this method the sample is sublimed directly into the ionization chamber without having to pass through a leak. This means that the vapour pressure need only be of the order of 10^{-5} torr instead of the 10^{-2} torr required for a reservoir system. By direct evaporation the spectra of thermally sensitive compounds can thus be obtained at much lower temperatures. One method of direct evaporation is the probe technique introduced by Reed.[25] Using this method the spectra of a great variety of compounds such as sugars and polyhydroxy alcohols,[26,27] which had hitherto been thought unsuitable for mass spectrometry, were obtained. A later version of this probe made use of a vacuum lock for rapid introduction of samples. Such has been the success of this technique that most commercial instruments designed for use in the mass spectrometry of organic compounds have a modified version of Reed's probe as a standard attachment. The chief advantage of this method is that the sample is placed close to the mass spectrometer filament. Thus collisions prior to ionization between sample molecules and surfaces are reduced to a minimum.

CHAPTER II

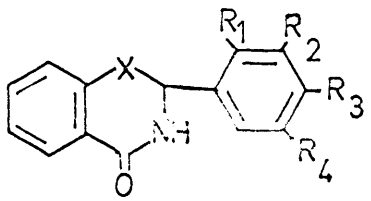
THE MASS SPECTRA OF SOME DIHYDROBENZOTHIAZINES AND DIHYDROBENZOXAZINES

Investigation of these compounds was undertaken for two reasons. Firstly because no spectra of compounds of these classes had previously been reported, and secondly because it was thought that it would be of interest to compare the spectra of substances differing only in the nature of a single hetero-atom at a given position in the carbon skeleton.

The structures of the species examined are given in Fig. IIa. The author is indebted to Mr. M. Kaisin of the Université Libre de Bruxelles for purifying and supplying these compounds.

Owing to the difficulty of obtaining reproducible spectra by the probe method it was decided to try to obtain spectra using the heated reservoir. It was found, however, that in most cases pyrolysis of the samples had taken place. The spectra reproduced in Table IIa were obtained by means of a gallium reservoir system*. This is made entirely of glass except for the gallium cut-offs and seems to be less destructive to samples than the normal reservoir which has a metal valve manifold. Pyrolysis of some of the samples

*The author is greatly indebted to Dr. H.C. Hill and Mr. R. Coombes of I.C.I., Billingham, for these spectra.

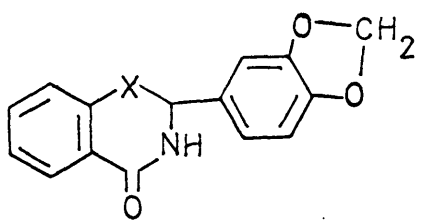


X = S

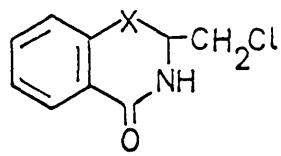
(I) $R_1 = R_2 = R_3 = R_4 = H$
(II) $R_1 = Cl$: $R_2 = R_3 = R_4 = H$
(III) $R_3 = OCH_3$: $R_1 = R_2 = R_4 = H$
(IV) $R_2 = R_3 = OCH_3$: $R_1 = R_4 = H$
(V) $R_2 = R_3 = R_4 = OCH_3$: $R_1 = H$
(VI) $R_2 = OCH_3$: $R_3 = OH$: $R_1 = R_4 = H$
(VIII) $R_3 = N(CH_3)_2$: $R_1 = R_2 = R_4 = H$
(IX) $R_2 = NO_2$: $R_3 = Cl$: $R_1 = R_4 = H$
(X) $R_2 = OCH_3$: $R_3 = OCH_2Ph$: $R_1 = R_4 = H$

X = O

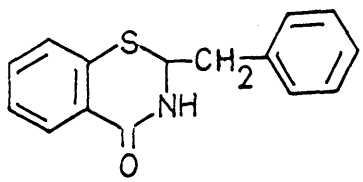
(XIII) $R_1 = R_2 = R_3 = R_4 = H$
(XIV) $R_3 = OCH_3$: $R_1 = R_2 = R_4 = H$
(XV) $R_2 = R_3 = R_4 = OCH_3$: $R_1 = H$
(XVII) $R_2 = NO_2$: $R_3 = Cl$: $R_1 = R_4 = H$



(VII) X = S : (XVI) X = O



(XI) X = S : (XVIII) X = O



(XII)

Fig. IIa.

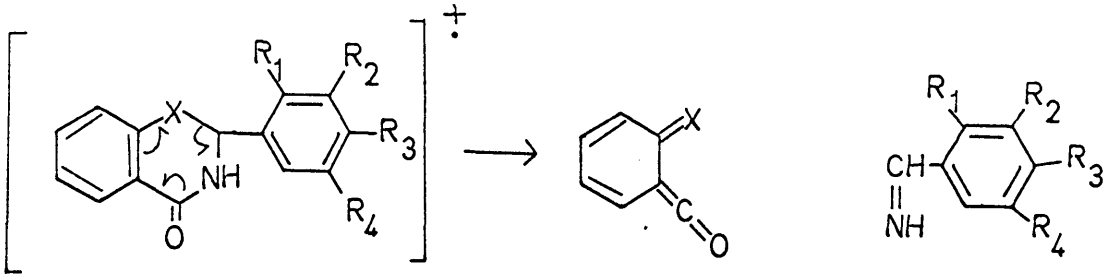
still occurred, however, and it was found necessary to re-run these by a direct insertion technique.

To facilitate visual comparison of spectra the data of Table IIa have been reproduced in line-diagram form in Fig. II(b). Spectra obtained by the probe method are also included here and are distinguished by the letter P.

The spectra show the expected fragmentations of the functional groups[8,11,28] and in addition there are certain features characteristic of these particular structures, especially in the case of the compounds with 2-phenyl substituents.

One of the principle degradation paths in both the 2-phenyldihydrobenzothiazine and 2-phenyldihydrobenzoxazine series can be described as a retro-Diels-Alder reaction. This has been found to explain fragmentation in many other classes of compounds[8]. A recent communication discusses the factors influencing such a method of decomposition[29]. Either of the two fragments resulting from the retro-Diels-Alder reaction may retain the positive charge. These will be referred to as A and B (Fig. II(c)). The extent to which the positive charge remains with A or B depends on the nature of the substituents attached to the "B" part of the molecule.

In addition to A^+ and B^+, ions corresponding to $(A + H)^+$



A

B

mass 136, X=S

mass 120, X=O

FIG. IIc

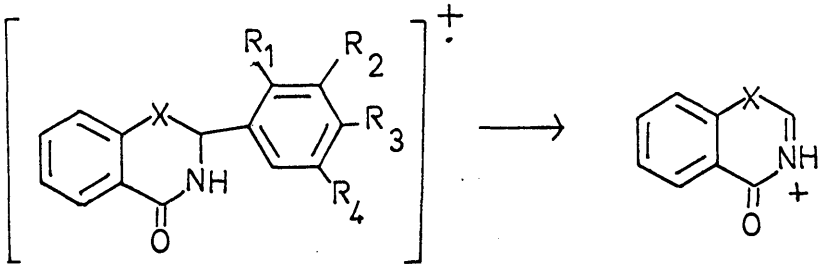


FIG. IId

are important, especially in the dihydrobenzoxazine series where $(A + H)^+$ is often the base peak in the spectrum. Deuterium labelling shows that the "extra" hydrogen in this ion is derived from the nitrogen atom of the azine ring.

A rather unexpected feature of the spectra of the 2-phenyl-dihydrobenzothiazines is the presence of abundant ions at $^m/_e$ (B - 14). Accurate mass measurement shows these to have the formulae of the corresponding B^+ ion less one nitrogen atom. The hydrogen attached to nitrogen in the unionized molecule is retained in this fragment ion. Like that of the B^+ ions, the abundance of the $(B - 14)^+$ particles seems to depend on the electron-donating power of the substituents on the phenyl group.

In the mass spectra of compounds containing nitrogen or sulphur, e.g., amino acid esters[30], amides[31], amines[32], thiols[33] and thioethers[34], abundant ions formed by fission of bonds beta to the hetero-atom are characteristic features. It might be expected that this fission would also be common to the spectra of the dihydrobenzoxazines and dihydrobenzothiazines, giving rise to peaks at $^m/_e$ 148 or $^m/_e$ 164 respectively (Fig. IId). However, it is negligible in the fragmentation of the 2-phenyl compounds. The small abundance of $^m/_e$ 164 and $^m/_e$ 148 may be explained on the

basis of unfavourable cleavage alpha to the phenyl group and to the attachment of electron-withdrawing groups to the nitrogen and sulphur atoms. Alkylacetamides[31], for example, show a reduced tendency to cleave beta to the nitrogen atom as compared with the corresponding amines. $^{m}/e$ 164 in fact is the base peak in the spectrum of the 2-benzyldihydrobenzothiazine (XII) and is the second most abundant ion in the spectrum of the 2-chloromethyl compound (XI). This same fission produces the most abundant species in the 2-chloromethyldihydrobenzoxazine spectrum (XVIII) at $^{m}/e$ 148.

4-oxo-2 (-3 , 4-dimethoxy) phenyl-2, 3-dihydro-1, 3-benzothiazine (IV).

This compound is discussed in some detail as its mass spectrum has all of the characteristic features mentioned above. General decomposition pathways given for this molecule apply also to the other members of the 2-phenyldihydrobenzothiazine series. Only in this particular case has deuterium labelling been used in the investigation of fragmentations involving migration of hydrogen atoms in the 2-phenyldihydrobenzothiazines.

Fragment $B^{+\cdot}$ gives rise to the base peak at $^{m}/e$ 165. Loss of one hydrogen atom from $B^{+\cdot}$ gives $^{m}/e$ 164 (34%, metastable observed 163.0, calculated 163.0). Elimination of hydrogen from $B^{+\cdot}$ is a common feature of the spectra of the 2-phenyl compounds. That the hydrogen attached to nitrogen is retained in this decomposition process is shown by examination of the spectrum of the N-deutero compound in which $^{m}/e$ 164 of the non-deuteriated species has become $^{m}/e$ 165. Loss of 27 mass units occurs from $(B - 1)^{+}$ as shown by the presence of a "metastable peak" at $^{m}/e$ 114.5 (calculated for 164 $\longrightarrow$ 137 is 114.5). This is in agreement with the behaviour of certain other types of aromatic nitrogen-containing compounds, expulsion of HCN being a common feature of their mass spectra[34,35].

A second degradation of $B^{+\cdot}$ involves the loss of a methyl radical to give $^{m}/e$ 150 (metastable observed 136.5; calculated for 165 $\longrightarrow$ 150 is 136.4), an entirely normal reaction of aromatic methyl ethers[36,37]. Aromatic methyl ethers show a characteristic loss of 30 mass units[36] (CH_2O). Although such a fragmentation from the parent molecule-ion is not evident in the present case a peak does occur at $^{m}/e$ 135 (4.4%) which could be due to

$$^{m}/e\ 165\ (B^{+\cdot}) \xrightarrow{-CH_2O} {}^{m}/e\ 135,$$

although no metastable (calculated 110.5) is observed. Aromatic methyl ethers also exhibit cleavage of the aryl-oxy bond giving rise to (p - 31) peaks. Peaks at $^{m}/e$ 270 (1.2%) and $^{m}/e$ 134 (7.6%) probably arise by such a fission from the parent molecule-ion and from the B^{+} fragment respectively. This type of fission has been shown to be a two-step process, involving loss of formaldehyde followed by elimination of hydrogen[36].

All of the 2-phenyldihydrobenzothiazines which give rise to abundant B^{+} ions also have in their mass spectra a peak at $^{m}/e$ (B - 14). Accurate mass measurement shows that the nitrogen atom of the azine ring is not retained in the $(B - 14)^{+}$ fragment. Deuterium labelling shows that this ion is formed by transfer of the hydrogen from the nitrogen. $(B - 14)^{+}$ has the constitution of a substituted tropylium ion. The enhanced stability associated with the tropylium structure[38,39,40,41] is probably a major factor in the formation of the $(B - 14)^{+}$ species. Evidence of the stability of the $(B - 14)^{+}$ fragment is the lack of ions of even moderate abundance arising from its decomposition. In the spectra of the compounds with methoxyl groups on the phenyl ring, e.g. (III), (IV) and (V) a peak does occur one mass unit below the mass of $(B - 14)^{+}$. However in each case a metastable peak indicates that this arises from the

loss of a methyl radical from the $B^{+\cdot}$ ion. In the high resolution spectrum this peak is a singlet and therefore is due to $(B - CH_3)^+$ only. In the spectra of the compounds which do not have an easily eliminated methyl group the peak at $^m/e$ (B - 15) is absent.

In the mass spectrum of (IV) $^m/e$ 136 due to fragment $A^{+\cdot}$ is 48% of the base peak. The abundance of the $A^{+\cdot}$ ions, like those of $B^{+\cdot}$ and $(B - 14)^+$ is affected by the substitution of the B part of the molecule. A discussion of the abundances of these ions in relation to the possible structures of the transition state of the parent molecule-ion is given below.

Accurate mass measurement and the use of metastable peaks shows the decomposition pathway of $A^{+\cdot}$ to be

$$\underset{A^{+\cdot}\ ^m/e\ 136}{C_7H_4SO^{+\cdot}} \xrightarrow[(\text{calc } 85.8)]{m^x 85.8} \underset{^m/e\ 108}{C_6H_4S^{+\cdot}} + CO \xrightarrow[(62.3)]{m^x 62.3} \underset{^m/e\ 82}{C_4H_2S^{+\cdot}} + C_2H_2$$

Expulsion of small, thermodynamically stable molecules such as carbon monoxide and acetylene has been postulated as the driving force of many decompositions observed in the mass spectrometer[8,11]

4-oxo-2 (-3 -methoxy, 4 -hydroxy) phenyl-2, 3-dihydro-1, 3-benzothiazine (VI)

This compound exhibits the same general fragmentation pattern as (IV). One interesting point is that the $(A + 1)^+$ fragment is isobaric with the $(B - 14)^+$ ion. Under high resolving power $^m/e$ 136 was found to be a singlet of $^m/e$ 135.9990, i.e., is due entirely to A^+ (C_7H_4SO = 135.9983). $^m/e$ 137 was a doublet comprised of $^m/e$ 137.0059 and 137.0603 in the abundance ratios of 1:2 respectively. The higher mass constituent is therefore $(B - 14)^+$ ($C_8H_9O_2$ = 137.0602). The lower mass part was too abundant (25% of $^m/e$ 136 to be due entirely to the heavy isotope contribution of A^+ and must also have included $(A + H)^+$, (C_7H_5SO = 137.0061; $C^{13}C_6H_4SO$ = 137.0106). These would not be separated at the resolving power used (about 1 part in 8,000) since $M/\Delta M \approx 30,000$. Such considerations of isotope abundance and high resolution data show that $(A + H)^+$ ions are common to the spectra of all of the compounds examined.

It is interesting to note that loss of $CH_3^{\cdot}$ does not occur from the B^+ ion of (VI). A metastable at $^m/e$ 121.5 shows that in this instance the methyl group is eliminated from $(B - 1)^+$ (calculated for 150 $\longrightarrow$ 135 is 121.5). Loss of CH_2O from B^+ gives rise to $^m/e$ 121.0528 (calculated for C_7H_7NO is 121.0528). $^m/e$ 122 is more abundant than can be accounted for by the heavy isotope contribution of $^m/e$ 121. Accurate mass measurement showed it to

have the formula C_7H_8NO and a metastable at $^m/e$ 98.6 (calculated 98.6) accounts for its formation by expulsion of CHO from $^m/e$ 151 (B^+). Loss of CHO from phenols is of general occurrence[42].

4-oxo-2 (-3 -methoxy - 4 -benzyloxy) phenyl -2, 3-dihydro -1, 3-benzothiazine (X).

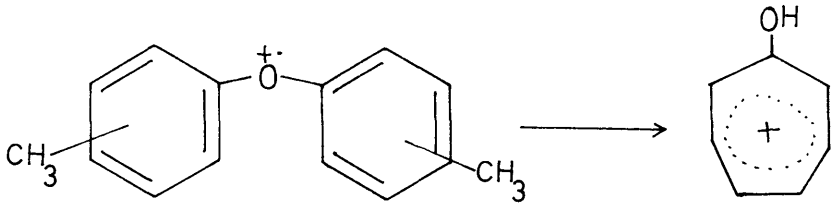
Cleavage of the carbon-oxygen bond of ethers results in charge retention either by the oxygenated fragment or by the hydrocarbon entity. This fragmentation process may be visualised as initial removal of one of the lone pair electrons of the ether oxygen followed by either a one or two electron shift[43].

$$R' - O - R + e \longrightarrow R' - \dot{O}^+ - R + 2e$$

(a) $R'-\dot{O}^+-R \qquad R' - O^{\cdot} + R^+$

(b) $R-\dot{O}^+-R \qquad R'-O^+ + {\cdot}R$

The fragment $R'O^+$ occurs to a negligible extent[44] except in the case of aryl methylethers[36,37] and in diaryl ethers[45] where the structure of R′ may confer special stability on this ion, e.g., in the case of tolyl ethers a hydroxytropylium cation may be formed[46].



Fragmentation of the carbon-oxygen bond in (X) could give a R^+ ion (reaction (a) above) of tropylium structure. It is not surprising therefore that $^m/_e$ 91 accounts for 41% of the total ion current whereas reaction (b) gives a $R' - O^+$ fragment at $^m/_e$ 286 which is only 0.06% of Σ_{39}.

The B^+ fragment in this case ($^m/_e$ 241) is only 1.6% of the abundance of $^m/_e$ 91. $(B - 14)^+$ is more predominant ($^m/_e$ 227, 16%). The peaks at $^m/_e$ 136 ($A^{+\cdot}$) and $^m/_e$ 137 ("isotope peak" of $A^{+\cdot} + (A + H)^+$) are 6.5% and 4.9% respectively. A peak at $^m/_e$ 150 (5.3%) corresponds to the $B^{+\cdot}$ ion less the benzyl group. A metastable peak at $^m/_e$ 93.5 confirms the formation of this from $^m/_e$ 241. The fragmentation pathways of (X) are summarised in Fig. IIe.

4-oxo-2-benzyl-2, 3-dihydro-1, 3-benzothiazine (XII)

This compound differs from the 2-phenyldihydrobenzothiazines discussed above in that loss of the 2-substituent involves "favourable"

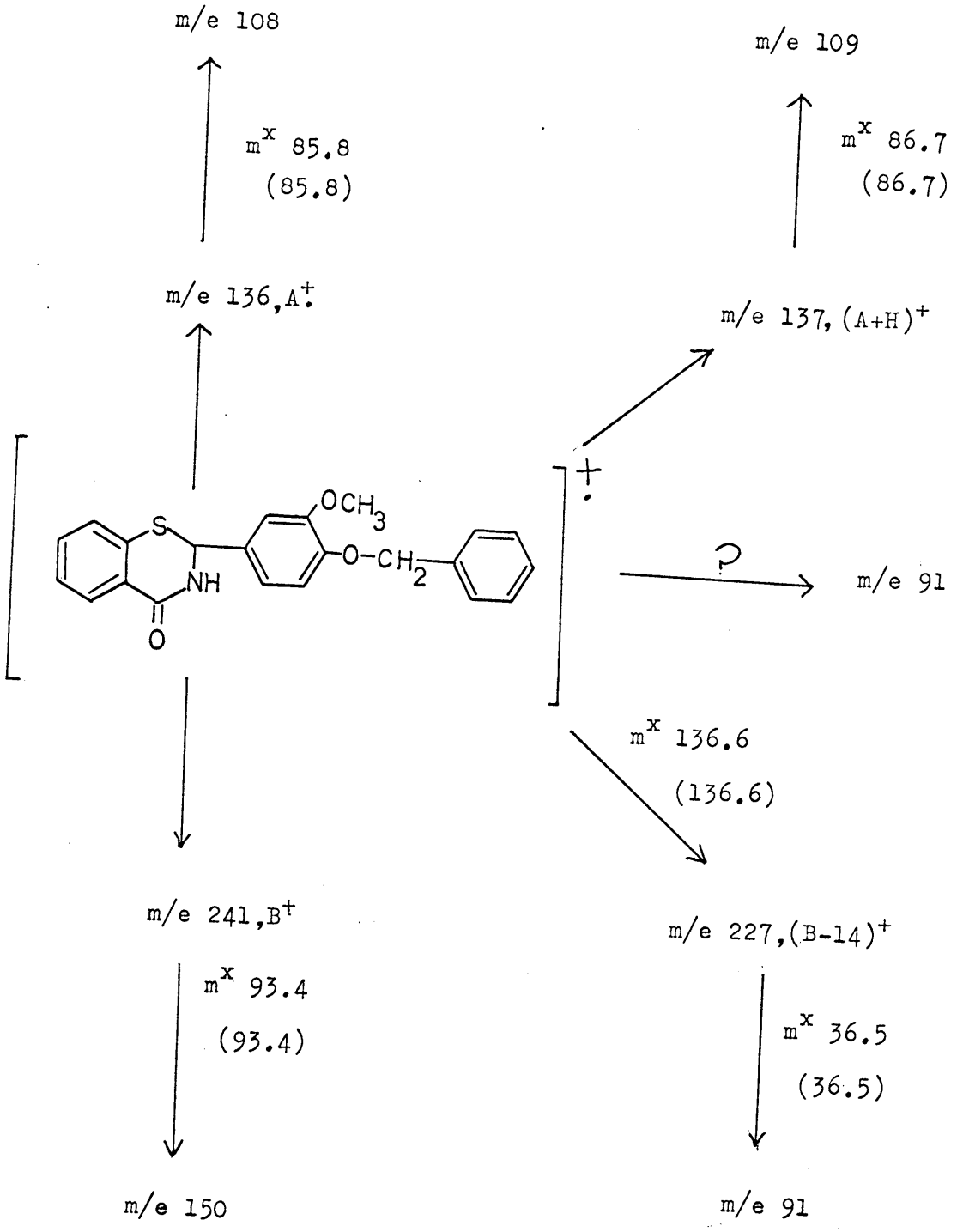

m/e 108
m/e 109
m^x 85.8
(85.8)
m^x 86.7
(86.7)
m/e 136, A+·
m/e 137, (A+H)+
OCH3
S
NH
O-CH2
O
+·
?
m/e 91
m^x 136.6
(136.6)
m/e 241, B+
m/e 227, (B-14)+
m^x 93.4
(93.4)
m^x 36.5
(36.5)
m/e 150
m/e 91

FIG. IIe

cleavage of a bond beta to both the benzene ring and to the sulphur and nitrogen atoms of the thiazine ring. Retention of the positive charge by the fragment containing the hetero-atoms gives the base peak of the spectrum at $^{m}/e$ 164. The charged hydrocarbon fragment ($^{m}/e$ 91) is present in the spectrum to the extent of only seven per cent of the base peak. The predominance of $^{m}/e$ 164 can be attributed to the stabilization of the positive charge by both the nitrogen and sulphur atoms (Fig. IIf). The virtual absence of $^{m}/e$ 164 in the spectra of the 2-phenyl compounds indicates that the driving force for its formation in this case is due to the increased stability of the benzyl radical compared with that of the phenyl radical.

The parent molecule-ion of (XII) has a relative abundance of only 0.6% whereas in the majority of the spectra of the 2-phenyl compounds it is of the order of 40%. Peaks with $^{m}/e$ values corresponding to the $B^{+\cdot}$ and $(B - 14)^{+}$ ions of the 2-phenyl compounds are present to a negligible extent in the spectrum of (XII) ($^{m}/e$ 119 and $^{m}/e$ 105). $A^{+\cdot}$ and $(A + H)^{+}$ ions are still of considerable importance (17.5% and 20% respectively). No possibility exists in this instance for the formation of a doublet at $^{m}/e$ 137. Subtraction of the isotope contribution of $^{m}/e$ 136 leaves 18.7%

of the intensity of the base peak attributable to $(A + H)^+$. This is much greater in comparison with $A^{+\cdot}$ than in the spectra of the 2-phenyl compounds. There are two possibilities for transfer of a hydrogen to the sulphur atom through a four-membered transition state (Fig. IIg). Deuterium labelling of (XII) by the method described in the experimental section gave a sample of 84% isotopic purity. From the spectrum it was calculated that one third of the $(A + H)^+$ ions are formed in a process involving transfer of hydrogen from the nitrogen atom (reaction (a), Fig. IIg). It is assumed that the remaining two thirds are formed by transfer of hydrogen from the $-CH_2-$ of the benzyl substituent (reaction (b), Fig. IIg). The $-CH_2-$ hydrogens are not exchanged in the labelling procedure as shown by the fact that $^m/_e$ 91 remains unshifted in the spectrum of the deuteriated material. Participation of the -CH- group of the thiazine ring in the hydrogen transfer reaction is unlikely since, as has been described above, this hydrogen atom does not contribute to $(A + H)^+$ in the spectra of the 2-phenyl compounds.

4-oxo-2-chloromethyl-2, 3-dihydro-1, 3-benzothiazine (XI)

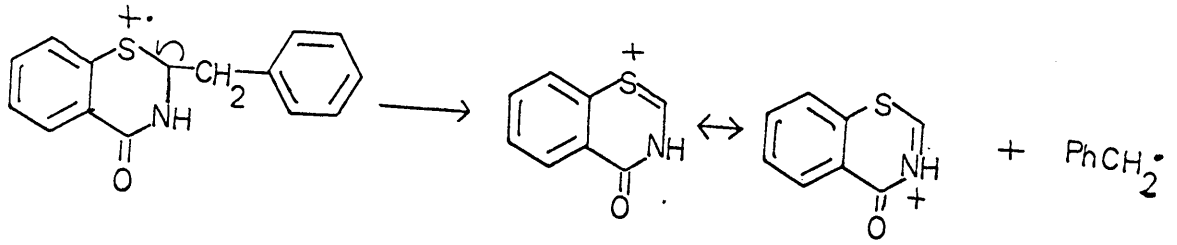
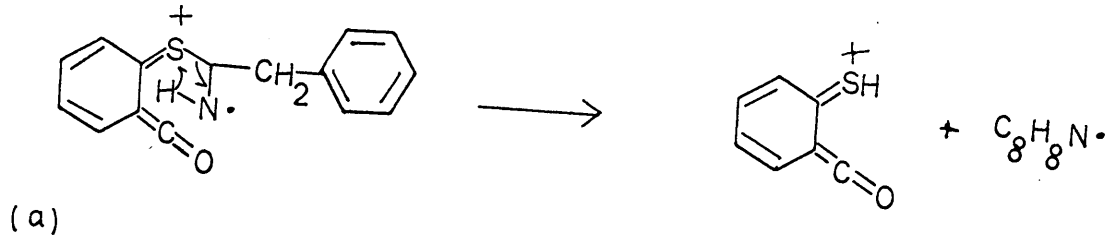
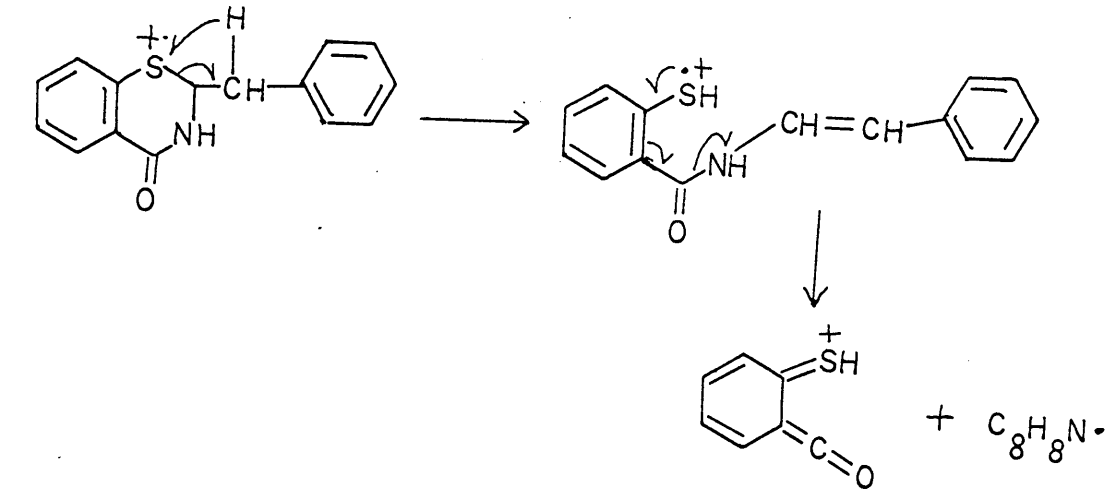


FIG. IIf



(a)



(b)

FIG. IIg

Attempts to obtain a spectrum of this compound by means of a heated reservoir system resulted in complete pyrolysis of the sample. The spectrum obtained by the direct probe method has $^{m}/e$ 136 ($A^{+\cdot}$) as the base peak whilst $^{m}/e$ 137 has a considerable contribution from $(A + H)^{+}$ ions. Fission of the bond to the 2-chloromethyl substituent is an important process, giving $^{m}/e$ 164 (70%), while loss of hydrogen chloride also occurs, as is shown by the peak at $^{m}/e$ 177 (M - 36, 16%).

THE DIHYDROBENZOXAZINES

The spectra of these compounds are similar to those of the corresponding dihydrobenzothiazines with, however, some important differences. Some data relative to the characteristic fragmentations of the 2-phenyldihydrobenzoxazines and 2-phenyldihydrobenzothiazines is presented in Table II(b). From this it is seen that the dihydrobenzoxazines all have the $A^{+\cdot}$ ion ($^{m}/e$ 120) in their spectra but that this is invariably less important in its contribution to the total ion current than is $^{m}/e$ 136 in the spectra of the corresponding 2-phenyldihydrobenzothiazines. $B^{+\cdot}$ has exactly the same constitution for corresponding compounds from both series

TABLE IIb.

Compound	A%Σ	B%Σ	(B - 14)%Σ	$\frac{A + H}{A}$%	$\frac{B - 1}{B}$%	Σσ
I	36.4	0.7	-	9.2	-	0
II	39.7	1.1	-	8.4	-	-
III	20.4	13.6	9.2	17.2	51.5	-0.27
IV	8.0	16.9	14.4	37.9	34.4	-0.19
V	5.0	15.1	16.0	71.3	15.7	-0.11
VI	12.4	11.4	8.4	17.3	40.0	-0.29
VII	13.8	22.6	7.8	21.9	29.7	-
VIII	1.96	21.0	29.0	69.8	52.2	-0.83
IX	50.6	-	-	5.1	-	+0.94
X	2.6	0.7	6.7	67.7	31.3	-.34
XIII	11.3	4.5	-	214.9	129.8	0
XIV	8.3	9.8	-	274.1	105.1	-0.27
XV	1.2	18.3	-	1554.7	12.7	-0.11
XVI	5.4	15.8	-	312.8	65.5	-
XVII	22.5	-	-	10.5	-	+0.94

and its relative abundance shows the same trend within each set of compounds. $B^{+\cdot}$ is absent in the spectra of both of the compounds with m-nitro, p-chloro substituents on the 2-phenyl group. $(A + H)^+$ is vastly greater in abundance in the spectra of the 2-phenyldihydrobenzoxazines than in those of the 2-phenyldihydrobenzothiazines. It is the base peak in four of the five spectra recorded and its contribution to the total ion current shows the same trend as is observed for $B^{+\cdot}$. $(B - 1)^+$ is again important; relative to the corresponding $B^{+\cdot}$ its abundance varies in the same way in each series but it is generally more important in the dihydrobenzoxazine spectra. It should be noted that the corresponding compounds (V) and (XV) are not strictly comparable since one spectrum was obtained by the reservoir system whilst the other had to be determined by means of the direct insertion technique (see below).

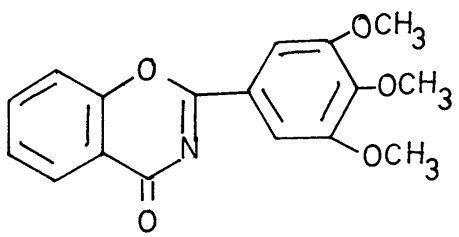
4-oxo-2-phenyl-2, 3-dihydro-1, 3-benzoxazine (XIII)

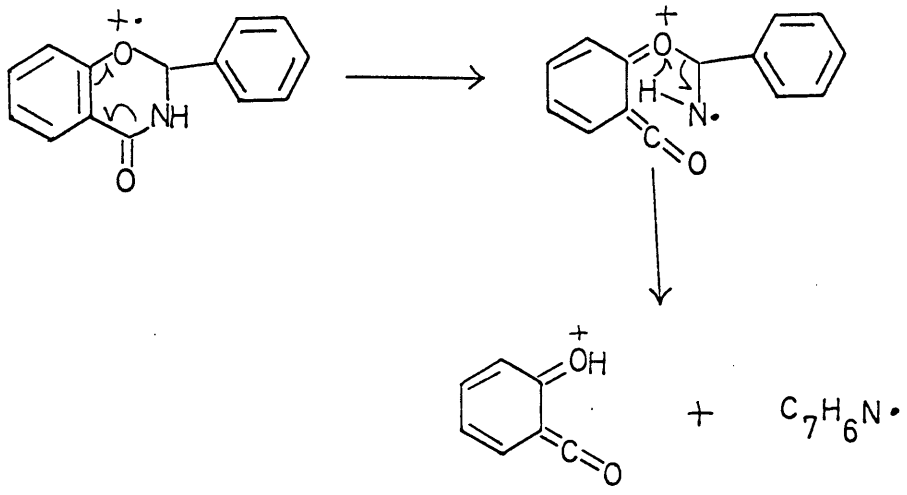
The base peak of this spectrum is $^m/_e$ 121. This is the $(A + H)^+$ ion of the dihydrobenzoxazine series, formed by a retro-Diels-Alder type of fragmentation with transfer of one hydrogen.

The spectrum of the N-deutero species shows that the hydrogen is transferred from the nitrogen atom of the azine ring, as was expected by comparison with the dihydrobenzothiazines. A plausible mechanism for the formation of $(A + H)^+$ is given in Fig. IIh.

4-oxo-2 (3 , 4 , 5 -trimethoxy) phenyl-2, 3-dihydro-1, 3-benzoxazine (XV).

The formula of this compound is $C_{17}H_{17}NO_5$, giving a molecular weight of 315. By introducing the sample into the mass spectrometer via the gallium reservoir a spectrum was obtained (Table II(a)) which indicated a molecular weight of 313. The peaks at $^m/e$ 314 and 315 are approximately the calculated intensity for the (M + 1) and (M + 2) peaks of $C_{17}H_{15}NO_5$. The base peak of the spectrum, $^m/e$ 193, does not resemble the normal B^+ fragment as there is no $(B - 1)^+$ at $^m/e$ 192. A^+ occurs at $^m/e$ 120 but there is no $(A + H)^+$, in marked contrast to the spectra of the other compounds of the series. It was therefore suspected that the compound had been pyrolysed to the dehydro derivative having a 2, 3 double bond in the oxazine ring,





m/e 121, $(A+H)^+$

FIG. IIh

In a study of the mass spectra of some monoterpene hydrocarbons, von Sydow and Ryhage[47] noted that at higher temperatures of the introduction system certain compounds having a cyclohexadiene ring, e.g., ∝-phellandrene, gave spectra with prominent (M - 2) peaks. Djerassi, Budzikiewicz and Williams[48] state that "(M - 2) peaks are sufficiently rare that their occurrence can usually be considered prima facie evidence for the presence of an unsaturated contaminant". Since the spectra of von Sydow and Ryhage were obtained by means of direct coupling of a gas chromatograph to a mass spectrometer the mixtures whose spectra were obtained could not have been introduced as such, since the components had different retention times under the conditions used. Thermal decomposition in the gas transfer system was immediately suspected. On lowering the introduction temperature the abundance of the (M - 2) fragments was greatly reduced.

The spectrum of (XV) was re-determined using the direct insertion technique (Fig. II(a), XV P). From this it can be seen that the true molecular weight of 315 has been obtained; $B^{+\cdot}$ (98%) and $(B - 1)^+$ (13%) are present and $(A + H)^+$ is the base peak of the spectrum.

These results indicate that caution must be used in the

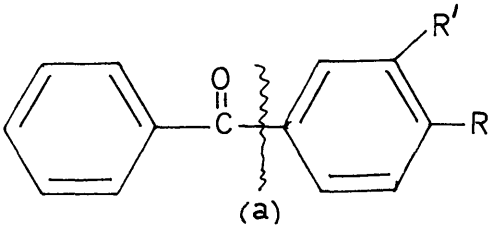
interpretation of spectra obtained by means of a heated reservoir when the compounds in question may easily dehydrogenate on heating. Although, as stated by Djerassi and his co-authors, (M - 2) peaks may be indicative of a mixture, it does not follow that the compound introduced is impure. Re-determination of the spectrum at a lower reservoir temperature or by means of a direct insertion technique should first be attempted. If an abundant (M - 2) peak persists under these conditions even when the lowest possible temperature of the ionization chamber is used, a final check on the purity of the sample should be made by some other means, e.g., by thin layer chromatography. The use of low electron volts would not be helpful as this would not distinguish between a mixture introduced as such and one produced in the mass spectrometer by a thermal process.

FORMATION OF THE CHARACTERISTIC IONS OF THE 2-PHENYL COMPOUNDS

Examination of Table IIb shows that, if the abundance of A^+ in the 2-phenyldihydrobenzothiazine spectra is expressed as a percentage of the total ion current (Σ_{39}) then this abundance varies from compound to compound although the only difference in structure between the various members of the series is in the

substitution of the 2-phenyl group. A parallel varation of A^+ occurs in the spectra of the 2-phenyldihydrobenzoxazines. The abundance of A^+ must then depend on the substituents on the 2-phenyl group, a similar effect of these substituents being present in the fragmentation of corresponding compounds from the two series.

McLafferty[49] showed that, in the mass spectra of substituted benzoyl compounds,



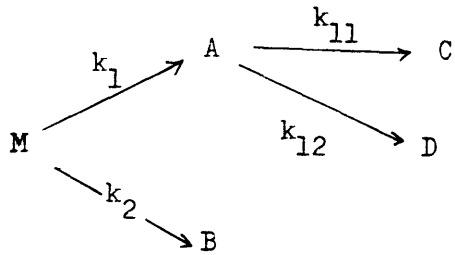
(a)

the abundance of the $PhCO^+$ ion formed by cleavage (a) could be quantitatively related to the electron donating power of the substituents R and R^1 expressed by their Hammett[50] sigma values. Using ionization and appearance potential data, similar correlations have been observed for substituted benzyl radicals[51], acetophenones[52], benzenes[53] and phenoxy ions[54].

The earlier work of McLafferty has been expanded and refined in a more recent communication[55]. In this the basic argument of the "quasi-equilibrium" theory[56] is used to develop a free energy relationship which can be applied to the formation of ions common

to a series of mass spectra.

The quasi-equlibrium theory considers mass spectral processes as a set of competing, consecutive, unimolecular, decompositions,



and for the decomposition of a parent molecule ion M yielding among other products an ion A formed only by a single process from M, the rate of change in the concentration of A in the ion source is given by

$$\frac{d}{dt}[A] = k_1[M] - k_h[A] - k'_{inst}[A] \qquad (1)$$

In equation (1), the second term on the right hand side includes the terms for all decomposition pathways of A; the third includes terms for real or apparent loss of A because of instrumental parameters.

If the steady state approximation is applied to the concentrations of all the ions in the source, the concentration of A relative

to the molecular ion is described by equation (2)

$$\frac{[A]}{[M]} = \frac{k_1}{k_{1n} + k'_{inst}} = Z \qquad (2)$$

Provided that the recorded intensities are proportional to the concentrations of ions in the source, Z is the ratio of the rate of formation of A to the sum of its rates of removal. Mass spectra in which the common fragment ion A may be formed from different parent molecule-ions can be related; for if A is formed with the same energy distribution from each molecular ion, the denominator of the term on the right is not dependent on the method of formation of A. The magnitudes of Z in a series of spectra will thus parallel the different rates of formation of A from each parent molecule-ion, and may therefore be related to a basic rate constant for a standard compound, as in equation (3).

$$\frac{Z}{Z_o} = \frac{\dfrac{[A]}{[M]}}{\dfrac{[A]^o}{[Mo]}} = \frac{k_1}{k_o} \qquad (3)$$

Suppose that two parent molecule-ions M and M' from two

different series of compounds bear the same substituent y and give rise to fragment ions A and A', A being common to one series of spectra and A' to the other. If the effect of y on the rate of formation of A from M is related to its effect on the rate of formation of A' from M' by

$$\log \frac{k_1^y}{k_1^o} = c \log \frac{k_1'^y}{k_1'^o} \qquad (4)$$

then this free energy relationship must be reflected in the magnitudes of Z and Z', as in equation (5)

$$\log \frac{Z}{Zo} = c \log \frac{Z'}{Z'o} \qquad (5)$$

The value of this relationship in interpreting data occurs when c, the proportionality factor, is constant for a wide variation of y. In this case the substituent y exerts a similar effect in the two series of reactions and the free energy relationship is linear.

In the case of the 2-phenyldihydrobenzothiazines, A of

equations(1) — (3) is $^{m}/e$ 136 and M depends on the particular compound. The "standard" substance is taken as the unsubstituted compound (I), so that:-

$$Zo = \frac{\text{Abundance of } ^{m}/e\ 136}{\text{" " } ^{m}/e\ 241} \quad \text{in the spectrum of I}$$

$$Z = \frac{\text{Abundance of } ^{m}/e\ 136}{\text{" " } ^{m}/e\ M} \quad \text{in the spectrum of each species of mol. wt. M.}$$

Similarly for the 2-phenyldihydrobenzoxazines,

$$Z'o = \frac{\text{Abundance of } ^{m}/e\ 120}{\text{" " } ^{m}/e\ 225} \quad \text{in the spectrum of XIII}$$

$$Z' = \frac{\text{Abundance of } ^{m}/e\ 120}{\text{" " } ^{m}/e\ M'} \quad \text{in the spectrum of each species of mol. wt. M'}$$

If the substituents on the 2-phenyl group exert a similar effect on the formation of the A^{+} ions in the two series of spectra, then the relationship (5) should be linear. It was necessary to

obtain accurate relative abundances of the ions involved to test the applicability of (5) to the series of compounds discussed here. As mentioned above, this was impossible from the spectra obtained by means of the gallium reservoir system owing to the pyrolysis of some of the compounds. Spectra on which accurate measurements had to be carried out were, therefore, re-determined by introducing the samples by means of the direct insertion probe. Highly reproducible results were obtained using the method described below (see experimental section). Measured peak heights for $^{m}/e$ 136 and $^{m}/e$ 120 were corrected as required for contributions from the "isotope peaks" of $^{m}/e$ 135 etc. The average results from six spectra of each compound were plotted as in Fig. IIi.

The investigation was somewhat hampered in that only a few pairs of identically substituted compounds were available. However, the approximate linearity of the plot obtained indicates that the substituents exert a similar influence on the fragmentation of corresponding compounds from each series. The much greater contribution of A^{+} to the total ion current in the spectra of the sulphur compounds (Table IIb) must then depend on other factors. Formation of these ions involves rupture of the 1, 3 carbon-sulphur or carbon-oxygen bond and of the 3, 4 carbon-nitrogen bond. The order of

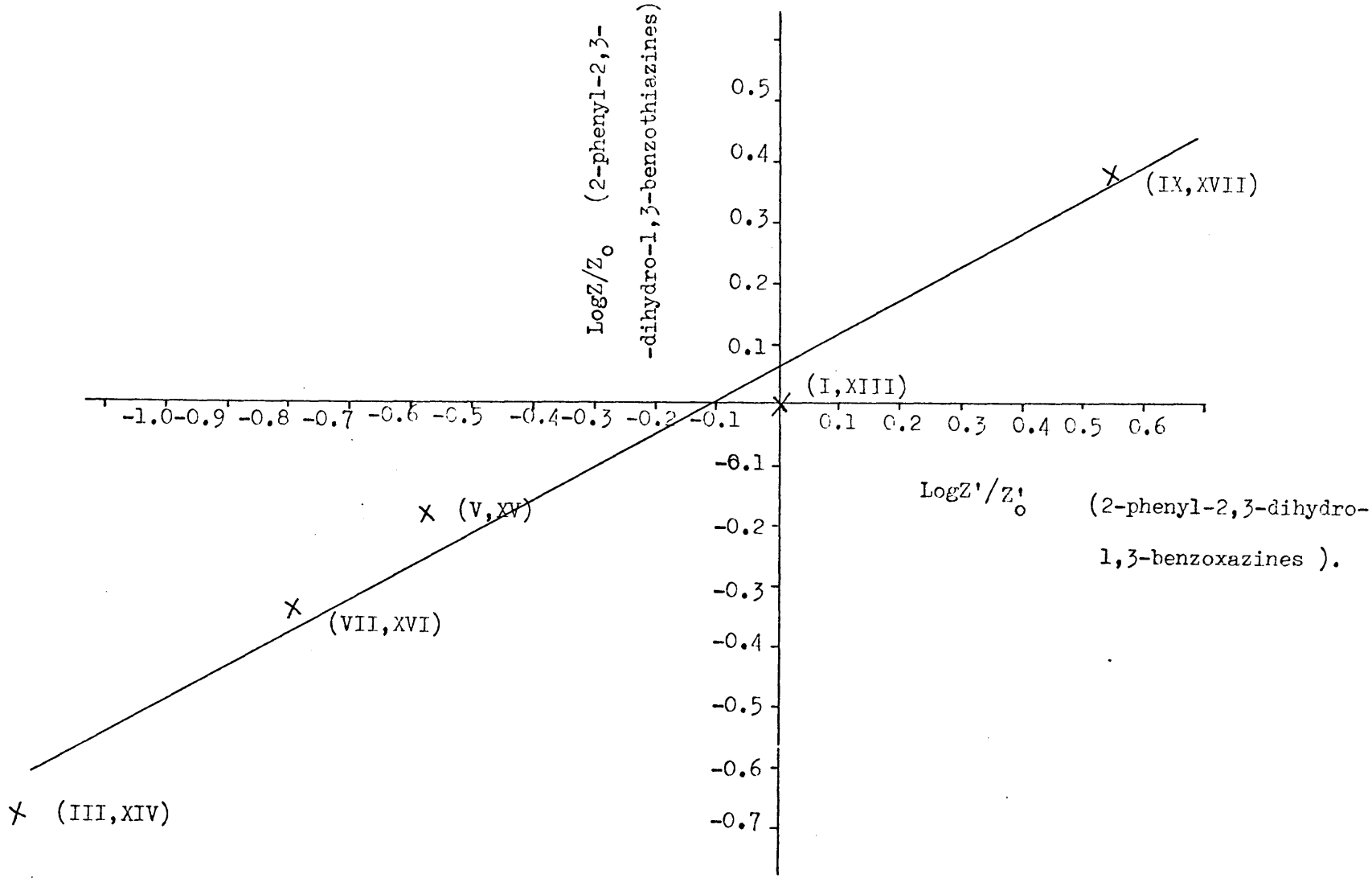

LogZ/Z$_o$ (2-phenyl-2,3-dihydro-1,3-benzothiazines)
0.5
0.4
0.3
0.2
0.1
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-1.0 -0.9 -0.8 -0.7 -0.6 -0.5 -0.4 -0.3 -0.2 -0.1
0.1 0.2 0.3 0.4 0.5 0.6
(I, XIII)
(IX, XVII)
(V, XV)
(VII, XVI)
(III, XIV)
LogZ'/Z'$_o$ (2-phenyl-2,3-dihydro-1,3-benzoxazines).

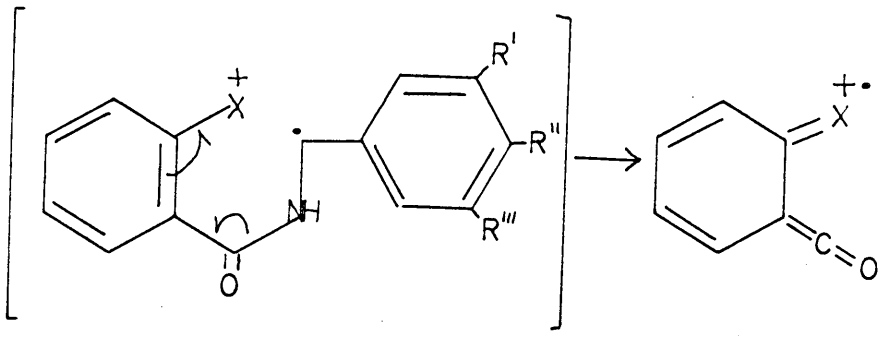
Fig. IIi.

dissociation energies of these bonds is[57]

$$C - O > C - S > C - N$$

The greater abundance of the $A^{+\cdot}$ ions in the spectra of the dihydrobenzothiazines can be readily explained, therefore, by the more facile cleavage of a carbon-sulphur than of a carbon-oxygen bond. Further, ions containing sulphur are generally more stable than their oxygen analogues[58]. A plausible mechanism for the formation of the $A^{+\cdot}$ ions, involving initial cleavage of the 1, 2 bond, is given in Fig. IIj. In all of the spectra examined the loss of 28 mass units from $A^{+\cdot}$ is shown by the presence of "metastable peaks" at $^{m}/e$ 70.5 (120 $\longrightarrow$ 92; calc. 70.5) and $^{m}/e$ 85.8 (136 $\longrightarrow$ 108; calc. 85.8). The fragment lost was proved in a few cases to be carbon monoxide by accurate mass measurement.

On the other hand, the abundance of $(A + H)^{+}$ ions is very much greater in the spectra of the 2-phenyldihydrobenzoxazines than in the other series. It is proposed that, in this case, initial fission of the 3, 4 carbon-nitrogen bond occurs, as in Fig. IIh. Cleavage of the 1, 2 carbon-oxygen bond can then be accompanied by simultaneous formation of an oxygen-hydrogen bond;



Fig, IIj.

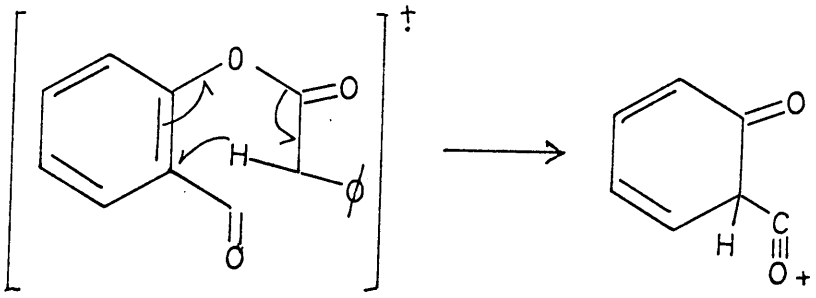


Fig. IIk.

an energetically favourable process. A similar explanation for one of the decompositions of 4-hydroxy-3-phenyl coumarins has been given by Barber and co-workers[59]. Transfer of the hydrogen to the aromatic ring was proposed, as in Fig. IIk. In the present case transfer of the hydrogen to the oxygen through a four-membered transition state is preferred as formation of an oxygen-hydrogen bond would be energetically more favourable than formation of a carbon-hydrogen one.

$(A + H)^+$ ions also occur to a small extent in the spectra of the 2-phenyldihydrobenzothiazines. The fragmentation scheme of Fig. IIh may play a minor role in the fission of these compounds.

The reaction schemes of figures IIj and IIh can also give rise to the $B^{+\cdot}$ ions by retention of the positive charge on the appropriate part of the molecule; provided, of course, that the hydrogen rearrangement of Fig. IIh does not take place in this instance. There was no evidence for $(B - 1)^+$ being formed in a one-step process from the parent molecule-ion in any of the spectra; in each case a metastable showed its formation from $B^{+\cdot}$.

It is of interest to compare the abundance of the characteristic ions from the A and B parts of the 2-phenyldihydro-1, 3-benzothiazine molecules with the electron donating power of the

substituents on the 2-phenyl group. The overall electron donating power of a number of substituents[60] may be expressed by the sum, $\Sigma\sigma$, of the Hammett σ values of the individual substituents. The last column of Table IIb gives $\Sigma\sigma$ for the 2-phenyl compounds with meta- and para- substituents.

Compound VIII which has the largest negative value of $\Sigma\sigma$ (greatest electron donating power) has the smallest combined abundance of $A^{+\cdot}$ and $(A + H)^+$ ions in its mass spectrum and the largest abundance of $B^{+\cdot}$ and $(B - 14)^+$. On the other hand IX, which has the greatest positive value of $\Sigma\sigma$ has also by far the greatest abundance of ions from the "A" part of the molecule; ions from the B part being absent in its mass spectrum. The ratio of $(A + H)^+$ to $A^{+\cdot}$ is also greatest in the spectrum of VIII and least in that of IX.

It would seem, therefore, that electron withdrawing substituents on the 2-phenyl group favour initial fission of the 1, 2 carbon-sulphur bond with formation of $A^{+\cdot}$ ions ($^m/e$ 136), Fig. IIj. Electron donating substituents give an increased abundance of ions from the B part of the molecule at the expense of the A-type ions. Further, substituents with a negative value of $\Sigma\sigma$ stabilize the 1, 2 bond so that the alternative primary process, fission of the

3, 4 bond, becomes more important. This leads to formation of $(A + H)^+$ ions, according to the scheme of Fig. IIh.

From the data of Table IIb it is seen that compounds III, IV, V and VI give results which are in the reverse order expected from the preceding arguments. These compounds have substituents which are electron donating by a resonance effect when in the para- position, whereas they are electron withdrawing by an inductive effect when in the meta- position[60]. It is suggested that, on electron impact of these molecules, the substituents all become electron donating, i.e., expansion of the phenyl ring may take place to some extent. Stabilization of the delocalised charge on the seven-membered ring by the non-bonded electrons of all the substituents is then possible (Fig. III). Of these four compounds, V, with three methoxyls on the phenyl group would then be expected to have the greatest abundance of B^+ and $(B - 14)^+$ and the least abundance of "A" ions, of which $(A + H)^+$ should be more important in comparison with A^+ than in the other three compounds. This is exactly what is observed even though the greater number of substituents would make available more possible decomposition pathways to the ions from the B part of the molecule. It is interesting that the ratio of $(B - 14)^+$ to B^+ also increases

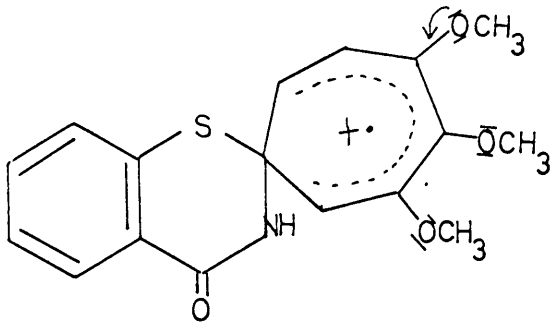


Fig.III.

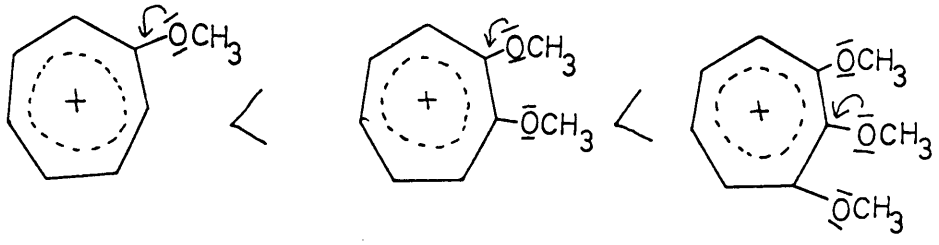


Fig.IIm. Stabilization of tropylium ions by methoxyl groups.

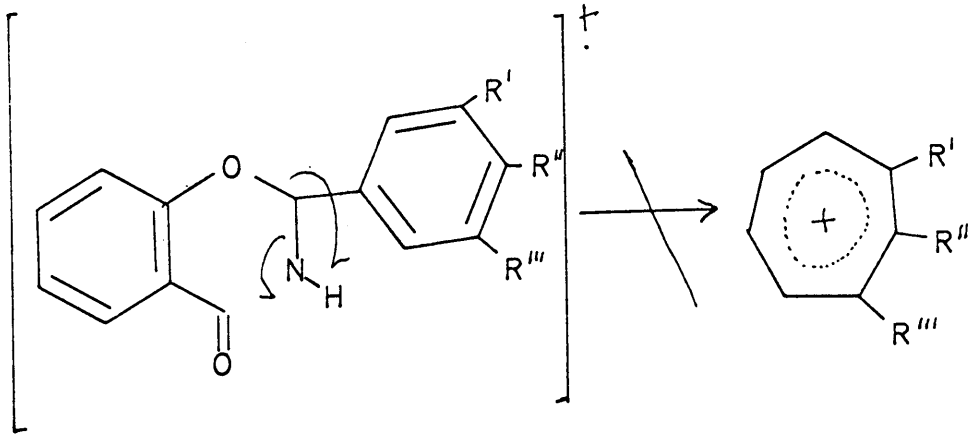


Fig. IIn.

along the series of compounds with one, two and three methoxyl groups. This is further evidence that $(B - 14)^+$ has the tropylium ion structure which can be increasingly stabilized by addition of methoxyl groups (Fig. IIm).

The same trend is observed in the spectra of the dihydrobenzoxazines. For reasons discussed above $(A + H)^+$ is in most cases much more important in the spectra of these compounds than in those of the other series. Again, however, this ion is of a relatively low abundance in the spectrum of XVII which has the powerful electron withdrawing nitro- and chloro- substituents on the phenyl group. $(B - 14)^+$ is absent in all of these spectra. Initial cleavage of the 3, 4 bond, as has been postulated for these compounds, could lead to $(B - 14)^+$ ions only by expulsion of a nitrogen atom (Fig. IIn); an unlikely process. In the compound XVII for which fission of the 1, 2 bond is proposed, $B^{+\cdot}$ and $(B - 14)^+$ are absent. In this case the electron withdrawing substituents attached to the phenyl group would destabilize any ion formed from the B part of the molecule; the spectrum is thus more like that of the corresponding dihydrobenzothiazine, $A^{+\cdot}$ being the major decomposition product.

CONCLUSIONS.

The spectra of the 2, 3-dihydro-1, 3-benzothiazines and 2, 3-dihydro-1, 3-benzoxazines show the usual fragmentations of the functional groups. Fissions of such groups as are present in the compounds described in this chapter have been amply commented on in other publications[61] and will not be further discussed.

In addition to minor decompositions which are due to the presence of functional groups such as methoxyl and hydroxyl, the compounds with substituted phenyl groups on the 2-position of the thiazine and oxazine rings have in their spectra major ions a study of whose formation leads to some interesting conclusions.

The A^+ ions are formed by initial cleavage of the 1, 2 bond of the azine ring. They are much more important in the sulphur compounds than in their oxygen analogues owing to the lower energy requirements for the fission of a carbon-sulphur compared with a carbon-oxygen bond; and to the better stabilization of a positive charge by sulphur than by oxygen. Electron withdrawing substituents on the 2-phenyl group favour this cleavage. Conversely, electron donating groups favour charge retention on the B part of the molecule. Groups, such as methoxyl, which have lone pair electrons tend to donate electrons to the phenyl ring even though they are

in the meta- position of the neutral molecule. This is thought to be the result of the equivalence of the substituent positions due to the expansion of the ring in the transition state of the parent molecule-ion.

Only the sulphur compounds have the substituted tropylium ions at $^{m}/e$ $(B - 14)^{+}$ in their mass spectra. Predominant fission of the 1, 2 bond as the initial step in the decomposition of these compounds is given as the reason for the formation of $(B - 14)^{+}$. The abundance of $(B - 14)^{+}$ is enhanced by the addition of groups such as methoxyl to either the meta- or para- positions of the 2-phenyl ring. This is considered evidence for the tropylium structure assigned to these ions.

Fission of the 3, 4 bond is predominantly the initial process in the decomposition of the 2-phenyldihydrobenzoxazines. Subsequent cleavage of the 1, 2 carbon-oxygen bond can then take place simultaneously with the formation of a hydrogen-oxygen bond; thus the energy required is smaller than for simple fission of the carbon-oxygen bond. The conclusion that the 3, 4 bond breaks first in the dihydrobenzoxazines is supported by the fact that $(B - 14)^{+}$ ions are absent in all of the spectra, in contrast to the majority of the spectra of the dihydrobenzothiazines.

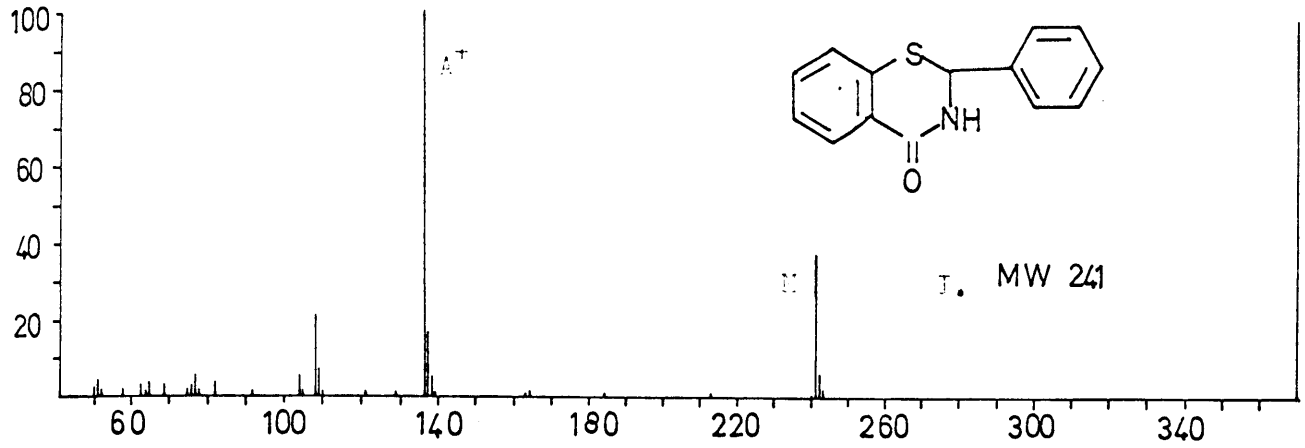
From the precise correlation between log $^{Z}/_{Zo}$ and Hammett sigma for the acyl ion formation which he obtained in the study of substituted benzophenones and acetophenones, McLafferty concludes[55] that both parent and fragment ions are formed in the ground state; opening of the aromatic ring does not occur and the structures of the ions are exactly as would be found in solution chemistry. If, however, ions are formed in an excited state this would be reflected in an increased contribution of the resonance effect in meta- substituents. The observed Hammett sigma is the sum of the resonance and inductive effects of the substituent[62]. An increase in the resonance contribution of a methoxyl group would result in greater electron donating power than expected from the normal sigma value. This is precisely the conclusion reached from a study of the ion abundances in the 2-phenyldihydro-1, 3-benzothiazines and 2-phenyldihydro-1, 3-benzoxazines. Where ions are formed with excess energy, a broad metastable for the transition is often observed[63]. No metastables were observed in any of the spectra for the formation of the $A^{+\cdot}$ ions. However the metastable for the loss of carbon monoxide from $A^{+\cdot}$ in the dihydrobenzoxazine spectra was invariably broad and flat-topped.

EXPERIMENTAL

N-deuterio specimens were prepared by heating the samples in D_2O to which a little sodium had been added. The minimum quantity of dry dioxan necessary to effect solution was added and some of the precipitate obtained on cooling was placed on the direct insertion probe. By this means samples containing greater than 80% of the N - d_1 species (mass spectral measurement) were obtained.

It was often quite difficult to obtain reproducible spectra by direct insertion of the sample into the ion source of some older types of mass spectrometer. Variation of the vapour pressure of the sample during the mass scan was the most serious obstacle, and as the instruments were not provided with a method of measuring total ion currents this could only be checked by recording several spectra at short intervals and noting the differences, if any, in relative peak heights on the records (R.I. Reed, personal communication). The aforementioned problem was aggravated by the relatively long scan times required owing to the slow response of the conventional recording system. The modern mass spectrometer (M.S. 9) used for the experiments in this chapter is equipped with a total ion current monitor, and

an adjustable probe, and has an electron multiplier detector. By careful adjustment of the probe position until a steady total ion current reading was observed followed by very quickly scanning through the unwanted regions of the mass spectra, results were obtained for the A^{+} to parent molecule-ion ratios which differed by less than 1% in successive spectra. The average of six such results was taken as the "Z" value for each of the 2-phenyl compounds used in obtaining the graph of Fig. IE.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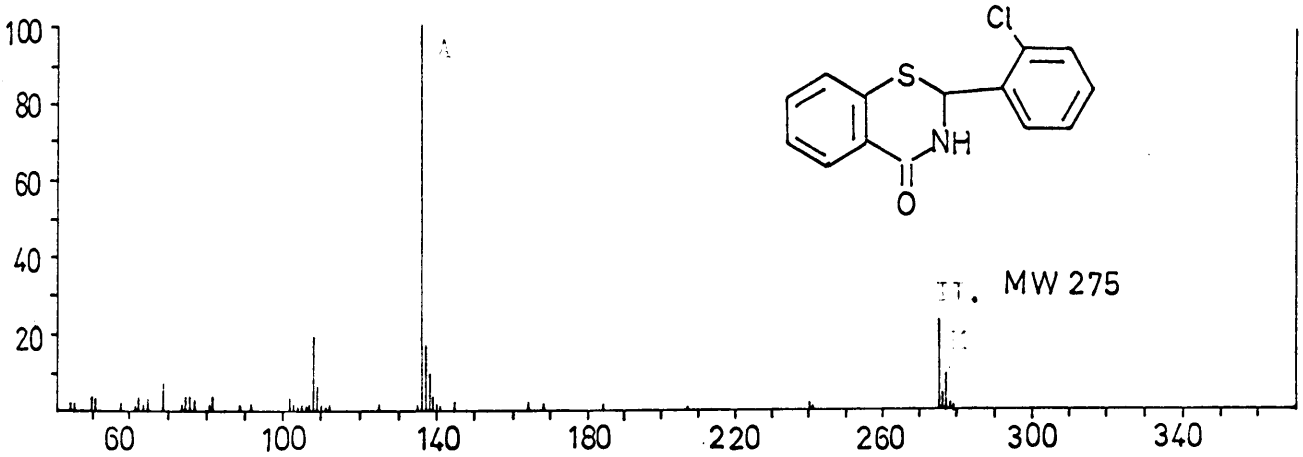
80
60
40
20
60
100
140
180
220
260
300
340
S
NH
O
MW 241


100
80
60
40
20
60
100
140
180
220
260
300
340
Cl
S
NH
O
MW 275

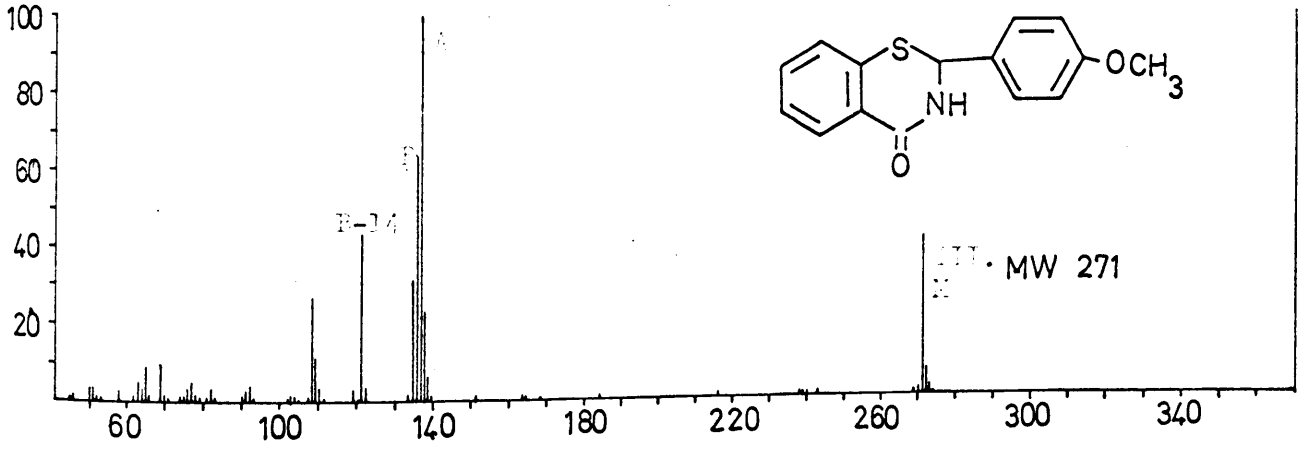
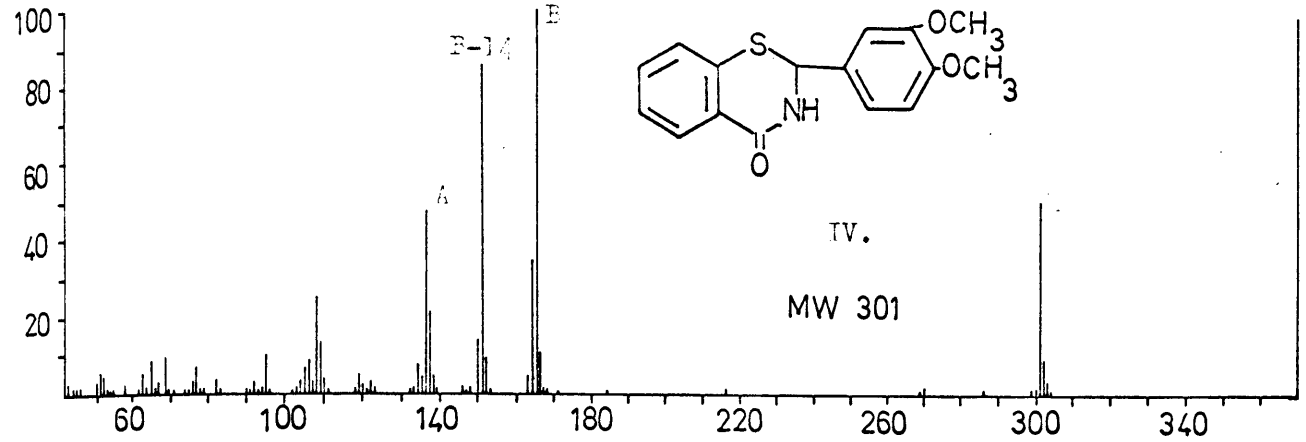
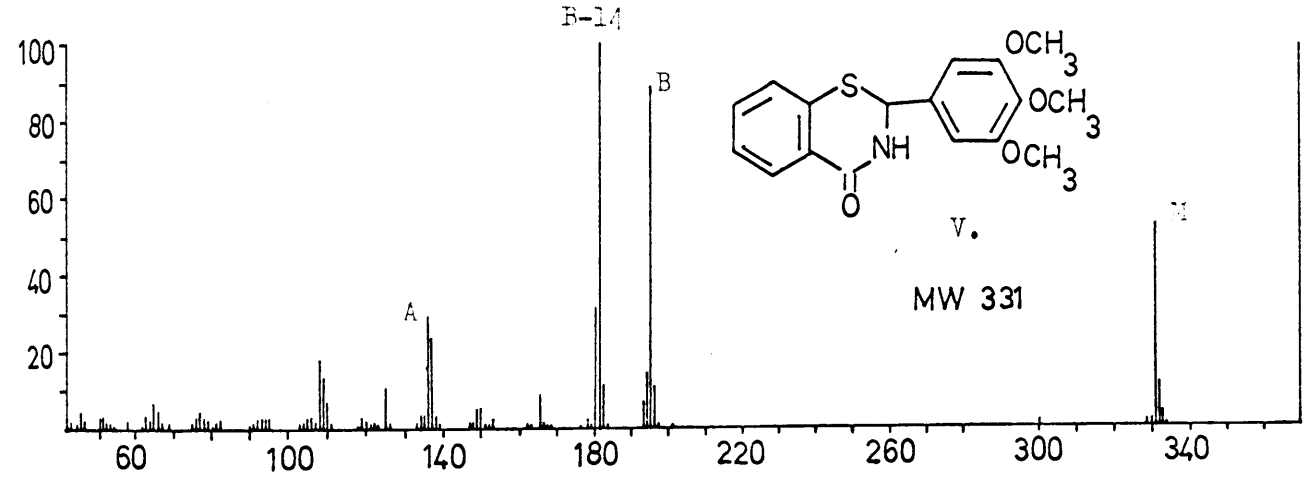

100
80
60
40
20
60
100
140
180
220
260
300
340
S
NH
O
OCH3
MW 271

Fig. IIb.



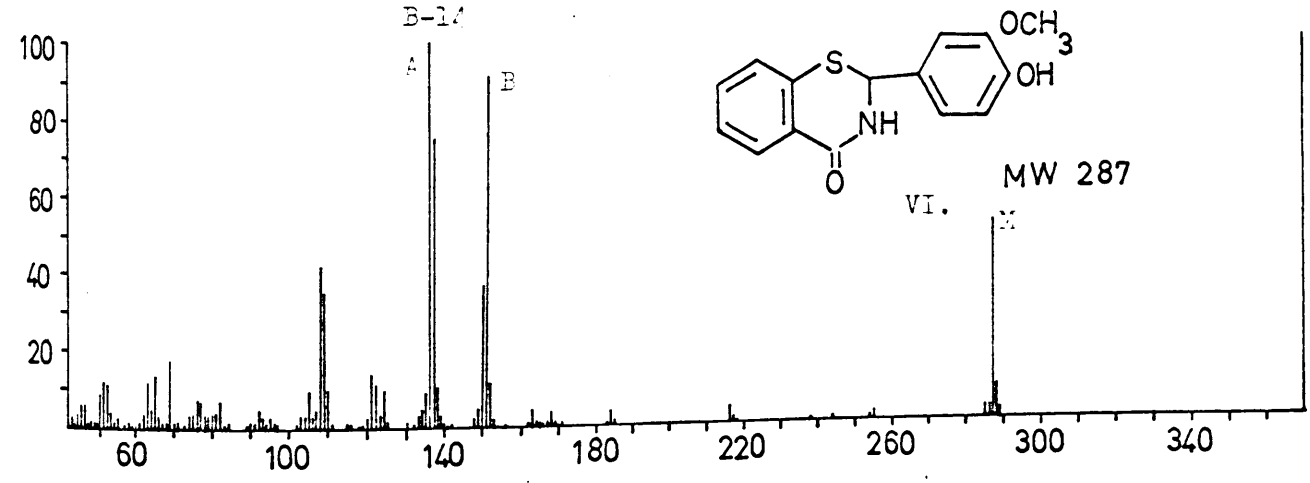
IV.

MW 301



V.

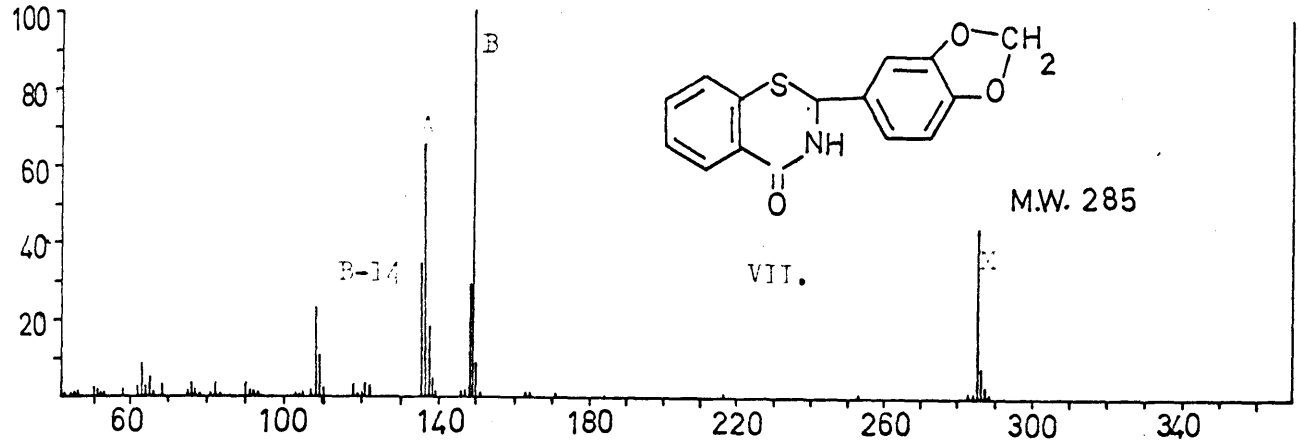
MW 331

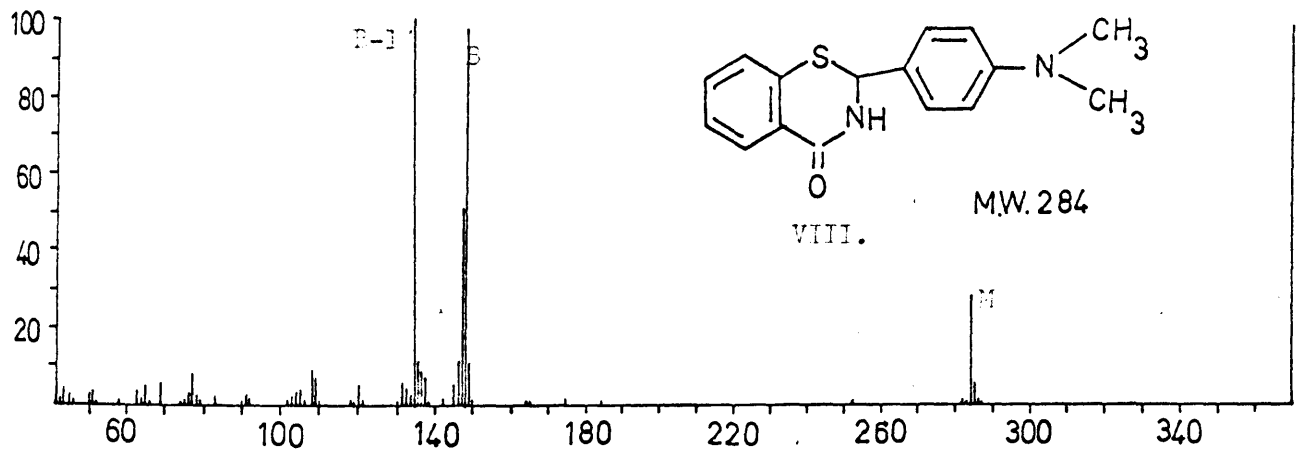


VI.

MW 287

Fig. IIb. cont'd.





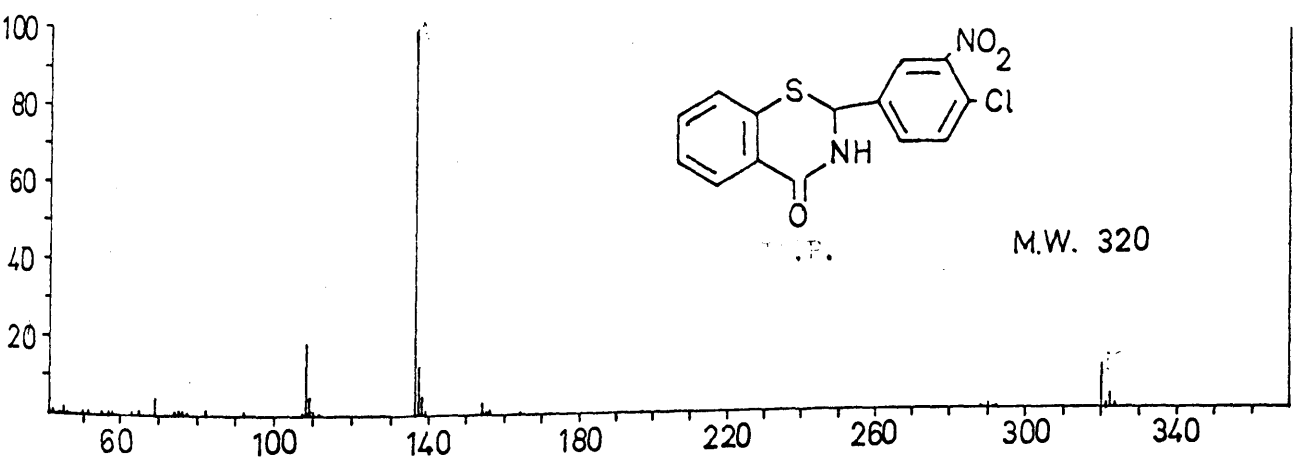
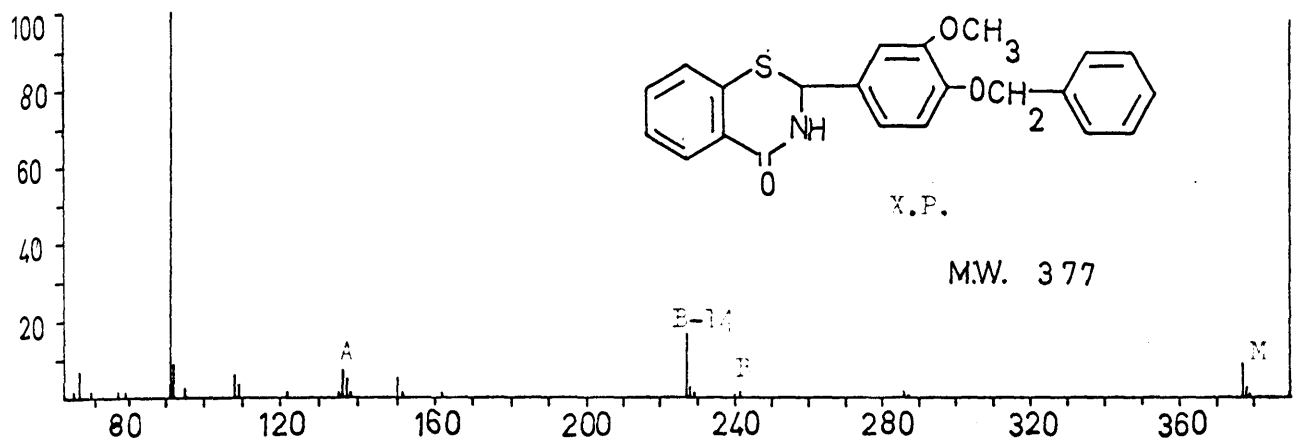
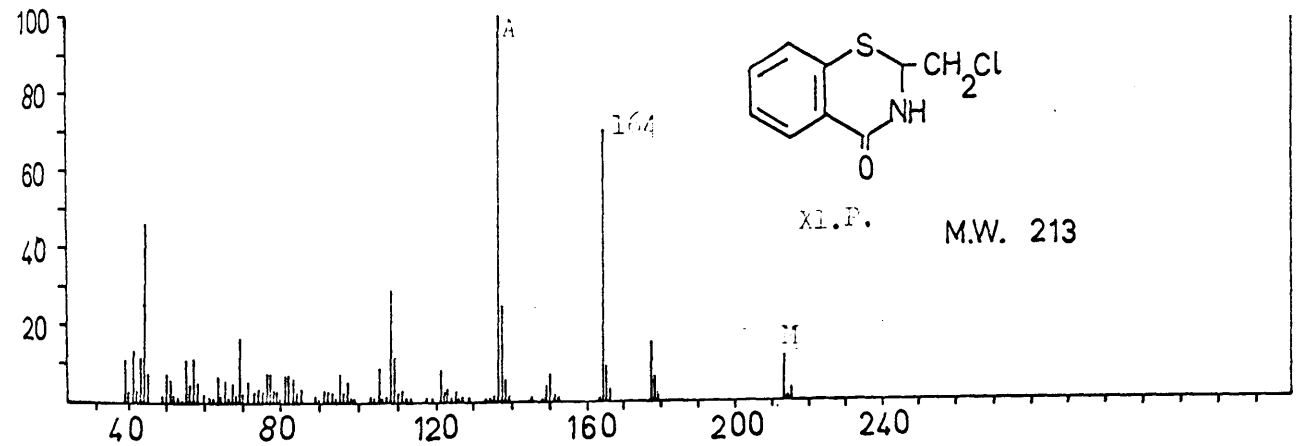


Fig. III. contd.





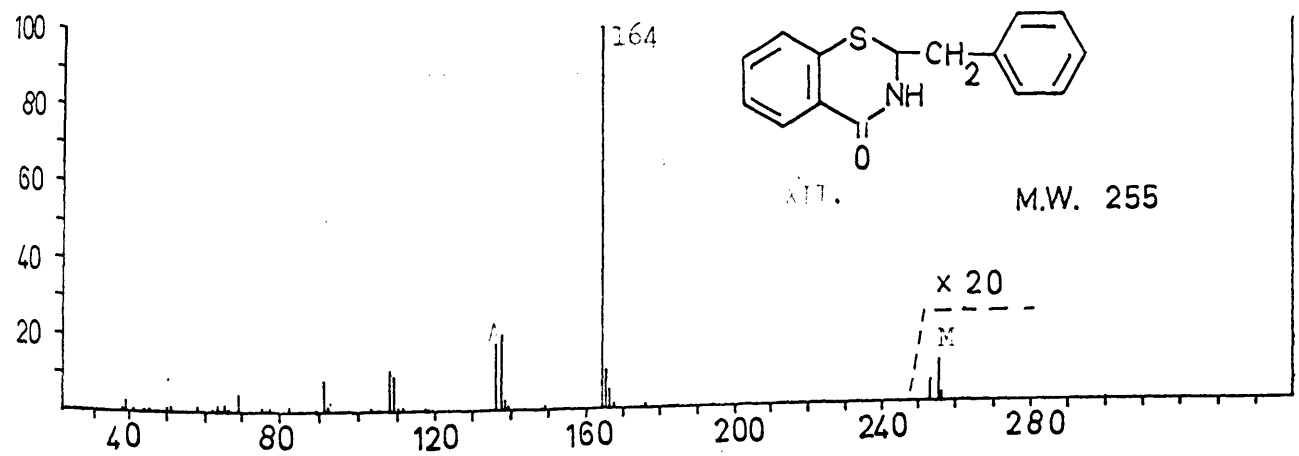
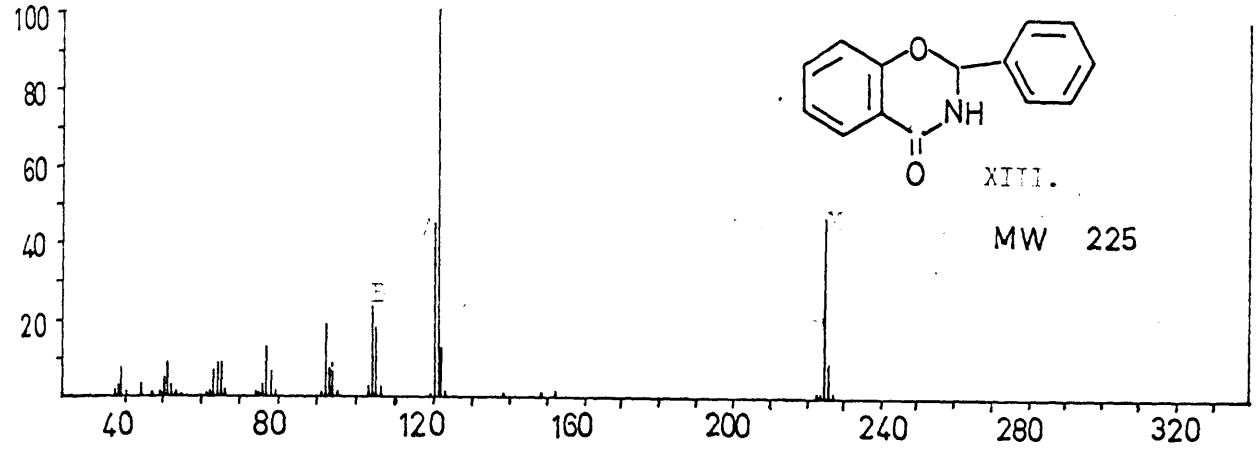
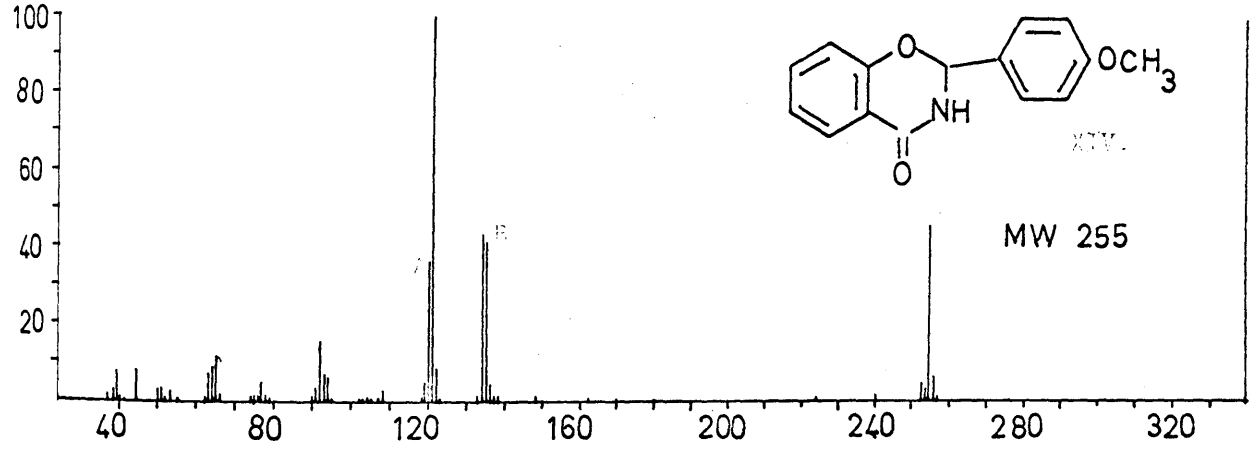


Fig. IIb. cont'd.





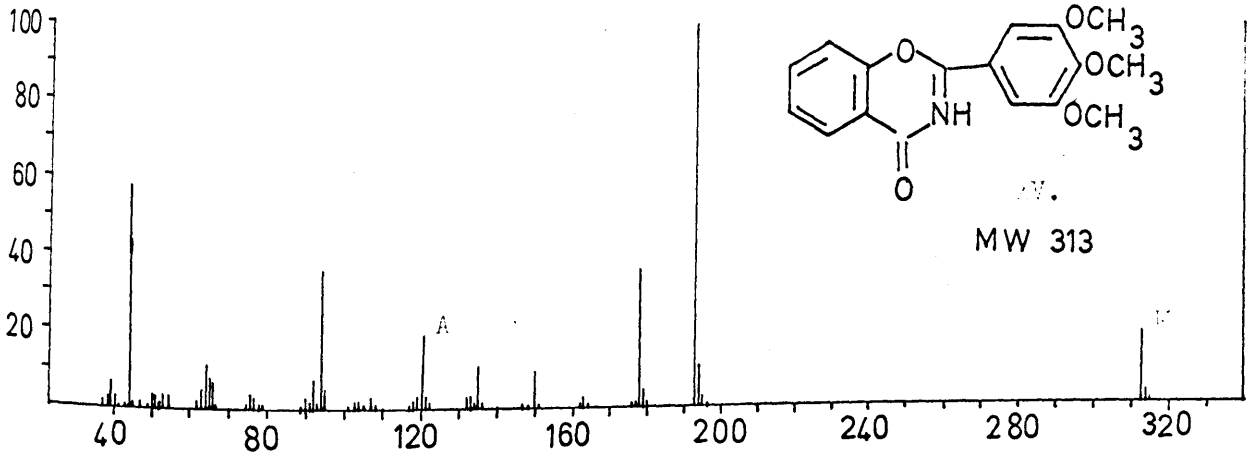
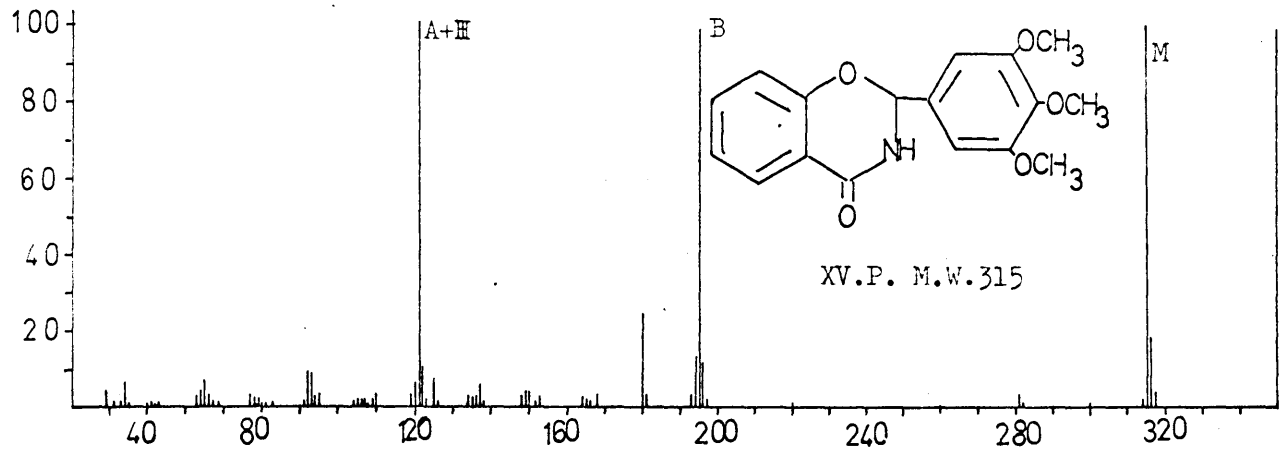
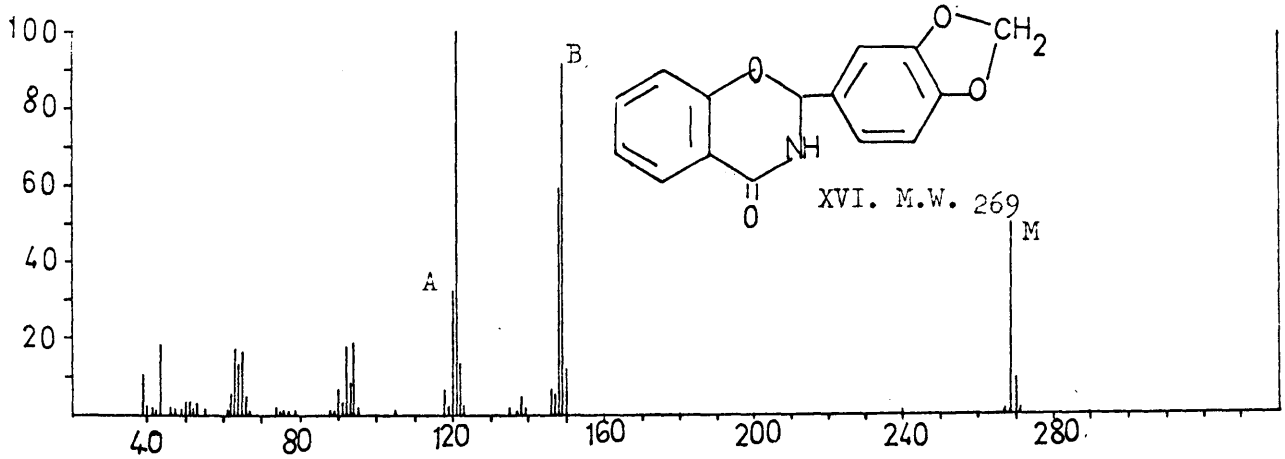


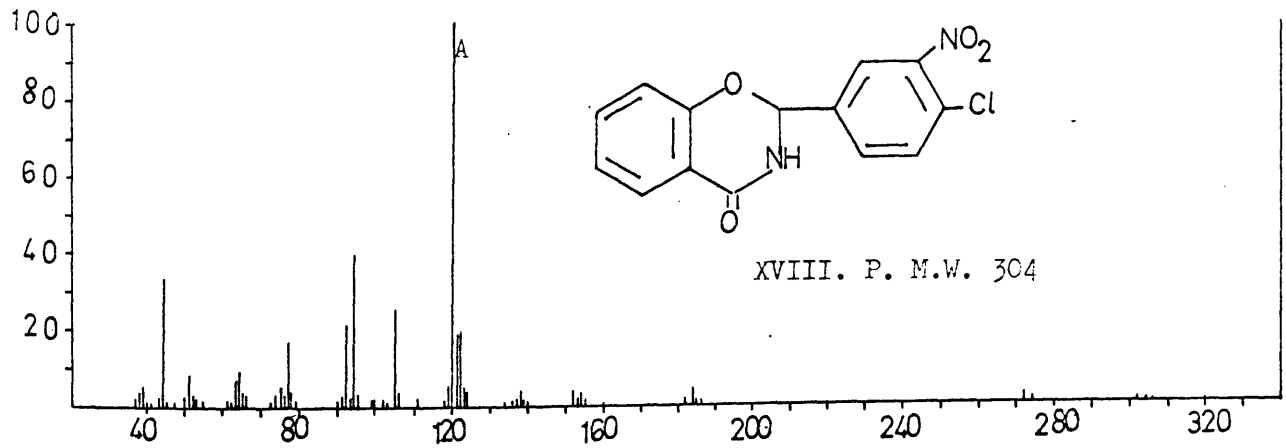
Fig. IIb. cont'd.



XV.P. M.W.315



XVI. M.W. 269



XVIII. P. M.W. 304

Fig. IIb. cont'd

TABLE II(a).

COMPOUND (I).

M/e	%Ab	M/e	%Ab
38	0.7	109	7.1
39	3.0	110	1.6
45	1.3	121	1.1
50	2.3	122	0.6
51	4.3	129	0.8
52	1.1	136	100.0
58	1.9	137	16.9
63	2.7	138	5.8
64	0.9	139	0.8
65	3.5	163	0.6
69	2.6	164	1.4
75	1.0	184	0.4
76	2.1	213	0.4
77	5.4	240	1.0
78	1.6	241	37.7p
82	3.0	242	6.4
92	1.6	243	2.2
104	5.0	244	0.3
105	1.8		
108	21.0		

TABLE II(a). cont'd.

COMPOUND (II).

M/e	%Ab	M/e	%Ab	M/e	%Ab
39	2.5	92	1.7	141	0.6
44	0.6	93	0.4	145	1.1
45	1.3	102	2.9	164	1.5
50	2.8	103	1.5	168	0.8
51	2.7	104	0.6	184	0.4
52	0.5	105	0.6	240	1.2
58	1.9	106	0.5	241	0.4
62	0.7	107	0.6	275	23.5p
63	2.7	108	19.4	276	4.1
64	0.9	109	6.0	277	9.1
65	2.9	110	1.5	278	1.5
69	7.4	111	0.8	279	0.5
74	1.0	112	0.9		
75	2.7	125	0.5		
76	3.1	135	0.6		
77	2.5	136	100.0		
81	0.6	137	16.1		
82	2.8	138	9.0		
89	0.5	139	2.7		
90	0.4	140	1.4		

TABLE II(a) cont'd.

COMPOUND (III)

M/e	%Ab	M/e	%Ab	M/e	%Ab	M/e	%Ab
39	5.7	78	1.7	121	42.4	270	1.9
44	0.5	79	0.7	122	3.9	271	42.0p
45	1.8	81	0.7	133	1.4	272	7.6
50	2.7	82	3.9	134	32.3	273	2.7
51	3.5	83	0.5	135	62.7	274	0.4
52	0.8	90	1.0	136	100.0		
53	0.4	91	2.7	137	23.5		
58	2.5	92	4.2	138	6.4		
62	1.0	93	1.0	139	1.2		
63	5.2	102	0.5	151	0.5		
64	3.5	103	1.0	163	1.1		
65	9.2	104	1.0	164	0.7		
66	1.0	105	0.5	168	0.4		
69	9.8	107	1.0	184	0.9		
70	0.5	108	27.7	216	0.9		
71	0.5	109	11.3	238	0.7		
74	0.9	110	2.5	239	0.8		
75	1.4	111	0.6	240	1.0		
76	3.2	119	2.6	243	0.9		
77	4.7	120	0.6	269	0.9		

TABLE II(a) cont'd.

COMPOUND (IV)

M/e	%Ab	M/e	%Ab	M/e	%Ab	M/e	%Ab
39	4.3	71	0.5	104	2.1	138	4.6
41	2.3	74	0.8	105	6.7	139	1.2
43	0.8	75	1.4	106	8.4	146	0.4
44	1.4	76	3.3	107	3.7	147	0.3
45	1.7	77	6.3	108	24.6	148	2.0
50	2.8	78	1.7	109	13.6	149	0.7
51	5.1	79	1.9	110	2.7	150	13.9
52	4.1	80	1.3	111	0.8	151	85.2
53	1.1	81	0.6	118	1.2	152	8.7
54	0.4	82	3.4	119	4.6	153	0.9
55	1.0	83	0.5	120	2.3	163	4.3
58	2.2	90	0.7	121	0.9	164	34.4
62	1.0	91	1.0	122	2.3	165	100.0
63	4.8	92	3.5	123	1.3	166	10.3
64	2.1	93	1.0	132	0.5	167	1.1
65	8.3	94	1.1	133	0.5	168	0.4
66	1.2	95	8.7	134	7.6	171	0.4
67	2.3	96	0.7	135	4.4	184	0.6
69	9.1	102	0.5	136	47.5	216	0.8
70	0.5	103	1.1	137	21.7	269	0.5

TABLE II(a) cont'd.

COMPOUND (IV) cont'd.

M/e	%Ab
270	1.2
299	1.0
300	1.7
301	51.2p
302	9.3
303	3.4
304	0.5

TABLE II(a) cont'd.

COMPOUND (V)

M/e	%Ab	M/e	%Ab	M/e	%Ab	M/e	%Ab
38	1.6	76	2.8	110	6.5	150	4.4
39	6.5	77	4.0	111	1.0	151	1.4
41	1.0	78	2.5	118	0.7	152	1.6
43	1.0	79	2.0	119	2.3	153	2.5
44	4.8	80	1.1	120	2.2	154	0.5
45	2.4	81	1.7	121	1.1	162	1.0
50	2.6	82	2.7	122	1.5	163	0.8
51	3.3	90	0.6	123	0.7	165	9.5
52	1.8	91	0.8	125	10.2	166	2.0
53	1.5	92	2.6	126	0.8	167	1.1
54	0.8	93	2.4	133	1.0	168	1.7
58	1.7	94	2.7	134	3.3	176	0.6
62	0.7	95	2.7	135	4.0	178	2.1
63	3.5	103	1.4	136	29.3	179	0.8
64	2.0	104	1.5	137	23.2	180	30.5
65	7.0	105	2.8	138	3.7	181	100.0
66	4.4	106	1.5	139	1.2	182	11.3
67	1.7	107	2.1	147	0.7	183	1.2
69	2.0	108	18.0	148	1.5	193	7.3
75	1.4	109	13.4	149	4.5	194	14.0

TABLE II(a) cont'd.

COMPOUND (V) cont'd.

M/e	%Ab
195	89.2
196	10.5
197	1.3
201	0.4
300	1.4
301	0.4
316	0.4
317	0.3
329	1.6
330	1.9
331	52.9p
332	10.8
333	3.8
334	0.6

TABLE II(a) cont'd.

COMPOUND (VI)

M/e	%Ab	M/e	%Ab	M/e	%Ab	M/e	%Ab
39	11.7	60	0.4	81	4.0	109	34.8
40	0.9	61	1.2	82	6.7	110	9.2
41	2.0	62	3.1	83	1.0	111	1.4
42	0.6	63	10.6	84	1.1	115	0.5
43	3.2	64	4.5	89	0.6	116	0.6
44	5.4	65	12.9	90	1.4	118	0.6
45	5.6	66	3.0	91	1.7	119	0.6
46	0.4	67	1.1	92	4.5	120	2.2
47	1.3	68	0.4	93	2.3	121	13.7
48	0.9	69	16.8	94	1.1	122	11.0
49	1.0	70	1.3	95	2.6	123	3.0
50	8.2	71	1.1	96	1.5	124	9.1
51	11.0	73	0.5	97	0.6	125	0.8
52	10.2	74	2.3	102	0.7	130	0.7
53	3.9	75	3.1	103	3.1	132	1.0
54	1.5	76	6.1	104	2.6	133	3.0
55	2.3	77	7.5	105	8.9	134	4.4
57	0.7	78	2.7	106	3.6	135	8.6
58	0.8	79	3.4	107	4.3	136	100.0
59	0.6	80	3.1	108	40.6	137	74.9

TABLE II(a) cont'd.

COMPOUND (VI) cont'd.

M/e	%Ab	M/e	%Ab	M/e	%Ab
138	10.1	169	0.5	244	0.6
139	2.7	170	0.5	253	0.5
140	0.8	171	1.1	254	1.2
141	0.4	183	0.5	255	2.0
142	0.4	184	3.0	256	0.8
148	0.8	185	0.6	259	0.5
149	5.1	186	0.4	270	0.5
150	36.5	196	0.4	285	3.1
151	91.2	209	0.4	286	2.2
152	10.5	211	0.4	287	50.7p
153	1.9	212	0.4	288	9.3
154	0.4	215	0.4	289	3.3
156	0.5	216	4.1	290	0.5
162	0.6	217	0.6		
163	4.6	218	0.5		
164	1.7	224	0.4		
165	1.5	226	0.4		
166	0.4	227	0.4		
167	1.2	228	0.4		
168	3.8	238	0.6		

TABLE II(a) cont'd.

COMPOUND (VII)

M/e	%Ab	M/e	%Ab	M/e	%Ab	M/e	%Ab
39	5.1	63	8.7	91	1.3	136	64.3
40	0.4	64	3.5	92	0.9	137	18.1
41	0.5	65	7.2	93	0.6	138	4.4
42	0.2	66	1.1	102	0.2	139	1.1
43	0.6	67	0.3	103	0.4	146	1.4
44	1.2	69	2.8	104	0.4	147	1.5
45	1.7	70	0.5	105	0.6	148	29.7
49	0.2	71	0.5	106	0.2	149	100.0
50	2.3	74	0.8	107	0.9	150	9.3
51	2.0	75	1.1	108	23.1	151	1.1
52	1.0	76	2.4	109	10.3	163	0.6
53	1.0	77	1.5	110	2.6	164	0.9
54	0.2	78	0.5	111	0.6	165	0.4
55	0.2	79	0.4	118	3.0	168	0.3
57	0.2	81	0.6	119	0.5	171	0.5
58	2.1	82	3.3	120	0.6	184	0.5
59	0.2	83	0.4	121	3.7	216	0.7
60	0.2	84	0.3	122	3.0	252	0.3
61	0.5	89	0.4	123	0.3	253	0.4
62	2.2	90	3.3	135	34.2	257	0.3

TABLE II(a) cont'd.

COMPOUND (VII) cont'd.

M/e	%Ab
283	1.1
284	1.1
285	44.2p
286	8.0
287	2.9
288	0.4

TABLE II(a) cont'd.

COMPOUND (VIII)

M/e	%Ab	M/e	%Ab	M/e	%Ab	M/e	%Ab
38	0.8	79	1.7	133	2.3	283	1.0
39	3.3	83	2.3	134	100.0	284	28.8p
41	0.8	90	0.9	135	11.6	285	5.7
42	4.1	91	2.4	136	9.6	286	1.8
44	4.3	92	1.4	137	7.4	287	0.3
45	1.1	102	1.3	138	0.6		
50	2.2	103	2.0	142	1.1		
51	3.6	104	3.8	145	5.7		
52	1.0	105	4.0	146	11.5		
58	1.3	106	1.2	147	51.0		
63	3.2	108	8.7	148	97.7		
64	1.3	109	7.1	149	10.7		
65	4.3	110	1.3	150	1.2		
66	0.9	118	1.8	164	0.6		
69	5.8	119	1.4	165	1.0		
74	0.8	120	4.4	175	1.1		
75	1.3	121	1.1	184	0.4		
76	2.7	130	1.0	240	0.5		
77	7.7	131	6.0	252	0.7		
78	2.3	132	4.2	282	1.4		

TABLE II(a) cont'd.

COMPOUND (XII)

M/e	%Ab	M/e	%Ab	M/e	%Ab
38	0.6	90	0.6	167	0.5
39	3.6	91	7.1	176	0.6
41	0.8	92	1.9	241	0.4
44	0.5	103	0.5	253	0.3
45	1.0	105	0.5	255	0.6p
50	1.3	108	10.8	256	0.1
51	2.0	109	9.8		
52	0.5	110	1.2		
58	1.2	111	0.5		
62	0.5	117	0.6		
63	2.4	118	0.6		
64	0.8	119	0.5		
65	2.3	136	17.5		
66	0.6	137	20.1		
69	5.0	138	2.5		
75	0.7	139	1.0		
76	1.2	149	1.2		
77	1.4	164	100.0		
82	1.9	165	10.0		
89	0.9	166	5.1		

TABLE II(a) cont'd.

COMPOUND (XIII)

M/e	%Ab	M/e	%Ab	M/e	%Ab
37	1.0	76	2.7	152	0.6
38	2.7	77	13.0	223	1.5
39	7.5	78	6.8	224	1.4
40	1.2	79	1.0	225	46.6p
44	3.0	91	1.2	226	9.8
47	0.5	92	18.9	227	1.2
49	0.5	93	6.9		
50	4.9	94	8.4		
51	9.3	95	1.3		
52	3.2	103	2.5		
53	1.5	104	23.1		
55	0.7	105	17.8		
61	0.5	106	2.9		
62	1.3	119	0.8		
63	7.1	120	44.9		
64	8.8	121	100.0		
65	9.1	122	13.1		
66	2.1	123	1.1		
74	1.4	138	0.6		
75	1.3	148	1.4		

TABLE II(a) cont'd.

COMPOUND (XIV)

M/e	%Ab	M/e	%Ab	M/e	%Ab
37	0.9	78	2.0	133	1.4
38	2.7	79	0.9	134	43.6
39	7.5	90	1.9	135	41.5
40	0.9	91	3.2	136	4.6
41	0.5	92	16.0	137	1.1
44	8.1	93	7.1	138	1.4
50	2.6	94	6.4	148	0.9
51	3.0	95	0.6	162	0.5
52	0.9	102	0.6	224	0.8
53	1.5	103	1.4	240	1.4
55	0.7	104	1.1	253	4.8
62	1.5	105	0.5	254	3.8
63	7.2	107	0.9	255	45.3p
64	9.4	108	2.2	256	8.2
65	12.1	118	0.6	257	1.2
66	1.8	119	4.8		
74	0.9	120	35.5		
75	1.0	121	100.0		
76	1.7	122	8.7		
77	4.9	123	0.7		

TABLE II(a) cont'd.

COMPOUND (XV)

M/e	%Ab	M/e	%Ab	M/e	%Ab	M/e	%Ab
37	2.3	66	6.5	106	0.5	164	0.6
38	3.4	67	0.9	107	3.2	176	0.5
39	7.8	74	1.0	108	0.7	177	1.4
40	3.7	75	1.8	117	1.1	178	36.0
41	0.7	76	4.1	118	2.2	179	4.2
43	0.8	77	3.2	119	2.6	180	0.9
44	58.9	78	1.3	120	19.6	193	100.0
45	0.8	79	1.3	121	3.9	194	11.6
47	2.0	89	0.6	122	1.0	195	2.8
49	0.7	90	3.2	132	2.6	196	0.6
50	3.7	91	1.8	133	3.6	226	0.7
51	3.1	92	7.9	134	0.6	313	19.2
52	1.4	93	1.9	135	11.0	314	3.8
53	3.2	94	36.5	136	1.1	315	0.7p
55	2.9	95	5.6	147	0.9		
61	0.9	101	0.5	148	0.5		
62	2.0	102	1.7	150	10.0		
63	5.3	103	2.1	151	1.0		
64	11.7	104	2.1	162	0.8		
65	8.7	105	1.4	163	3.1		

TABLE II(a) cont'd.

COMPOUND (XVII)

M/e	%Ab	M/e	%Ab	M/e	%Ab	M/e	%Ab
37	1.9	63	7.3	101	0.5	126	0.6
38	4.2	64	9.9	102	2.0	127	0.8
39	5.8	65	4.4	103	1.1	128	0.6
40	1.7	66	2.7	104	1.0	133	0.4
41	0.9	67	0.6	105	25.0	134	1.2
42	0.5	73	1.0	106	3.8	135	0.4
43	2.4	74	3.1	107	0.7	136	1.7
44	34.1	75	4.9	110	0.7	137	1.9
45	0.9	76	3.2	111	1.7	138	3.9
46	0.4	77	17.1	112	0.7	139	1.3
47	1.1	78	4.7	113	0.6	140	1.1
49	0.9	79	0.8	117	0.5	148	0.6
50	2.4	90	1.5	118	1.0	150	0.6
51	8.9	91	3.3	119	4.9	152	4.1
52	2.8	92	21.6	120	100.0	153	1.8
53	1.4	93	2.3	121	18.2	154	3.1
55	1.2	94	40.5	122	20.5	155	1.3
58	0.7	95	2.8	123	4.6	156	0.7
61	1.1	99	1.1	124	1.2	182	1.8
62	1.9	100	2.2	125	0.5	183	1.2

TABLE II(a) cont'd.

COMPOUND (XVII) cont'd.

M/e	%Ab
184	3.6
185	1.2
186	1.1
238	0.4
239	0.3
268	0.6
272	2.3
273	0.8
274	1.3
275	0.4
302	1.6
303	0.3
304	1.1p
305	0.2
306	0.2

CHAPTER III

ANALYSIS OF MIXTURES

It frequently happens that samples submitted for mass spectrometry prove to be mixtures. Mixtures are usually easily identified as such by their mass spectra if the peaks of highest $^{m}/e$ values differ by a number of units which does not correspond to an easily eliminated fragment. In organic chemistry, for example, a mass difference of ten or six between the peak of highest $^{m}/e$ value and the next highest (excluding "isotope peaks") would be indicative of a mixture. (M - 2) peaks are usually evidence of the presence of more than one substance[48] but care must be taken, as the mixture may be an artefact due to thermal dehydrogenation of a pure compound (see chapter II, p. 27). Two fragment ions from an undetected parent molecule-ion could be mistaken for parent molecule-ions of two different substances, e.g., the (M - 15) and (M - 18) peaks of an alcohol. Accurate mass measurements and a partial interpretation of the spectrum[64] would resolve this difficulty.

The use of metastable peaks, coupled with accurate mass measurements will often establish the presence of unrelated fragmentation pathways and hence the presence of a mixture in the mass spectrometer

sample system.

Sometimes a more laborious approach is necessary. Normally this involves attempted separation of the components of the mixture. Even a partial separation may be extremely useful as re-determination of the mass spectra of the separated fractions will give spectra in which the relative peak heights have changed. Peaks may be assigned to one component or the other and even this empirical approach may lead to identification[65].

Fractionation may be carried out in the mass spectrometer sample system itself. The high vacuum of the mass spectrometer enables the operator to carry out very efficient fractional distillations. These can be done at very low temperatures which, according to the Clausius-Clapeyron equation[65], gives greater efficiency of separation. An elegant apparatus for this purpose has been designed by Bokhoven and Theeuwen[66].

The direct insertion probe provides a very simple yet efficient method for fractionation of mixtures: an example of this is the tentative identification of a contaminant in a supposedly pure sample of a naturally occurring compound. Whilst monitoring the spectrum by means of the oscilloscope display certain peaks were seen to be diminishing in height with respect to the main part of

the spectrum. Two spectra were recorded with an interval of a few minutes between them. This enabled the $^{m}/e$ values of the peaks in question to be established as 266, 225, 197 and 169. On reintroduction of the sample accurate mass measurements gave the formulae of these ions as $C_{17}H_{14}O_3$, $C_{14}H_9O_3$, $C_{13}H_9O_2$ and $C_{12}H_9O$ respectively. From the formula of $^{m}/e$ 266 ($C_{17}H_{14}O_3$, eleven "double bond equivalents") and the successive losses of carbon monoxide, the impurity was probably a substituted xanthone[67]. This compound has not been isolated but others from the same source (Mammeia americana) have been identified as xanthones[68].

If two different mixtures of the same two compounds are available a simple, though laborious arithmetical treatment will give the complete spectrum of each. This was first described in detail by Meyerson[69] who obtained, from mixtures of cyclohexane and benzene, "derived" spectra which were nearly identical to the spectra of the pure hydrocarbons. The method depends on there being two peaks in the mixture spectrum one of which is due solely to the first component and the other to the second. The ratio of the heights in the two mixture spectra of a peak belonging entirely to the cracking pattern of one compound is found. All of the peak heights in the appropriate spectrum are multiplied by this ratio. Subtraction

of one spectrum from the other then gives the mass spectrum of the second component. A similar procedure gives the spectrum of the first compound. If only one "pure" peak is available then the spectrum of the other component only may be obtained. Suitable peaks for the analysis may be made by examination of the mixture spectra, e.g., in a cyclohexane/benzene mixture the highest $^{m}/e$ value is 84; the peak at $^{m}/e$ 78 must then be due to a different component. Even if a choice of peaks cannot be made from examination of the spectra the method can still be applied. If at any mass number m in the mixture spectrum the height per unit of pressure of the peak due to the first component is h_1 and that due to the second is h_2 and the pressures of the two substances in the ion source are p_1 and p_2 respectively, then

$$H_1 = h_1p_1 + h_2p_2 \qquad (1)$$

where H_1 is the peak height in the mixture spectrum. Suppose p_1 changes by an amount Δp_1, p_2 remaining constant. Then

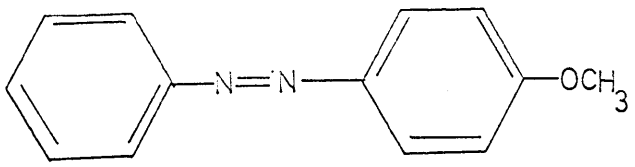
$$H_2 = h_1(p_1 + \Delta p_1) + h_2p_2 \qquad (2)$$

where H_2 is the peak height at mass m in this second mixture. From (1) and (2)

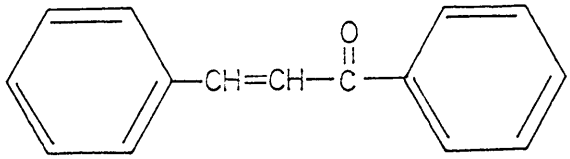
$$\frac{H_2}{H_1} = 1 + \frac{h_1 \Delta p_1}{h_1 p_1 + h_2 p_2} \qquad (3)$$

For an increase in the contribution of the first component to the mixture the ratio of the peak heights at mass m will thus be greater the smaller the value of h_2. Ideally, if the ratios of all the peak heights in one mixture spectrum to the corresponding ones in the other are calculated, the greatest value found will give the peak(s) due solely to the first compound. Conversely, the smallest ratio will give the peak(s) derived from the second compound. An advantage of this method is that no initial interpretation of the mixture spectra is required. The "derived" spectra can thus be obtained by making use of an electronic computer.

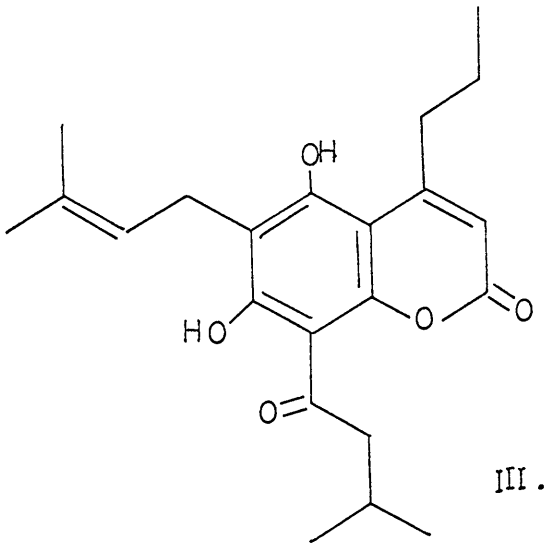
An illustration of the above "peak ratio" method and a comparison of the derived spectra obtained with those of the pure compounds is given in Table IIIa for a mixture of solid samples. The compounds used were p-methoxyazobenzene and benzylideneacetophenone (Fig. IIIa, I and II resp.). These were chosen simply because



I.



II.



III.

Fig. IIIa.

TABLE III(a)

m/e	A	B	C	D	I	II
50	249.6	358.9	15.4	17.8	16.0	18.8
51	755.5	1140.7	42.3	58.2	47.1	58.8
62	48.2	58.2	3.9	2.5	4.2	2.7
63	235.2	223.1	23.9	7.3	24.1	7.8
64	273.6	179.5	34.3	1.9	34.1	1.7
74	65.0	108.0	2.8	5.8	3.0	6.3
75	72.5	122.5	3.0	6.7	3.0	6.9
76	129.6	215.3	5.7	11.6	6.0	12.0
77	1504.0	2077.0	100.0	100.0	100.0	100.0
78	148.8	189.2	11.2	8.6	10.9	8.5
79	41.5	30.5	4.9	0.6	4.4	0.6
90	15.3	28.4	0.0	1.6	0.0	1.4
91	15.8	14.2	1.7	0.4	1.8	0.5
92	296.6	179.5	38.4	0.9	27.3	1.0
93	27.0	17.5	3.4	0.0	3.0	0.0
101	12.3	25.5	0.0	1.5	0.0	1.5
102	101.0	212.4	0.8	12.7	0.0	13.8
103	249.6	550.0	0.0	33.4	0.0	33.2
104	25.5	56.0	0.0	3.4	0.0	3.2
105	191.0	404.5	1.3	24.2	2.2	23.2

TABLE III(a) cont'd.

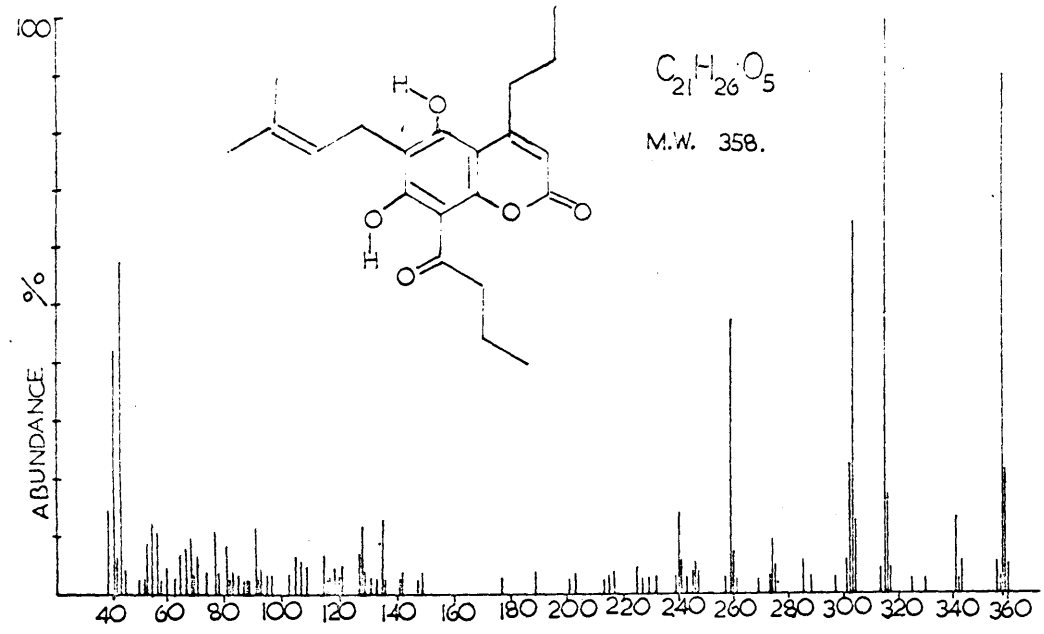
m/e	A	B	C	D	I	II
107	521.3	315.3	67.5	1.5	58.4	1.6
108	49.8	30.7	6.4	0.0	5.3	0.0
115	80.4	58.5	9.6	1.0	9.4	1.3
130	50.0	110.5	0.0	6.7	0.0	6.4
131	178.6	392.9	0.0	23.9	0.0	23.3
132	18.2	39.8	0.0	2.4	0.0	2.3
135	188.2	114.3	24.3	0.6	19.3	0.6
141	36.1	21.9	4.7	0.0	4.1	0.0
151	11.0	17.5	0.1	0.9	0.0	1.1
152	22.5	39.5	0.8	2.2	0.7	2.4
165	59.5	131.0	0.0	8.0	0.0	8.1
178	54.5	116.5	0.0	7.0	0.0	7.4
179	108.5	232.8	0.0	14.0	0.0	14.7
180	35.5	75.0	0.0	44.9	0.0	48.3
207	326.4	695.5	1.8	41.8	0.0	43.2
208	255.4	545.1	1.4	32.8	0.0	34.2
209	40.5	85.5	0.0	5.1	0.0	5.2
210	4.8	9.0	0.0	0.5	0.0	0.6
212	206.0	115.5	27.4	0.0	22.3	0.6
213	33.5	19.0	4.4	0.0	3.5	0.0

they were available as very pure samples and had similar molecular weights. The peak heights of mixture "B" divided by those of mixture "A" gave 2.20 as the highest ratio at $^{m}/e$ 103 and the lowest ratio as 0.56 at $^{m}/e$ 212. The derived spectra obtained using these ratios are shown in columns C and D. The fact that the largest ratio did not occur at $^{m}/e$ 208, the parent molecule ion of compound II - and obviously not having a contribution from I, resulted in a small residual contribution in the derived spectrum C from compound II. The smallest ratio, 0.56, did occur at the mass of the parent molecule ion of I with the result that subtraction of the spectrum of this compound from the mixture spectrum was complete and the derived spectrum D is almost identical to that of the pure compound, II. All recorded abundances below 0.5% of the base peak ($^{m}/e$ 77 in all spectra) have been made zero in Table IIIa. The small contribution of compound II to the derived spectrum of compound I is not serious enough to interfere with subsequent analysis of the spectrum. Where no obvious choice of peaks for analysis exists it can be concluded that this ratio method will produce a satisfactory result, provided of course that the minimum requirement of one "pure" peak for each component is present in the mixture spectrum.

Subtraction of the correct quantities from the peak heights in any mixture spectrum should give a "derived" spectrum in which a number of peak heights are zero or nearly so and no large negative values are present. Simple application of the above subtractive technique may fail in the first instance if a mixture of two homologues is encountered. Here the (M - 15) peak of the higher molecular weight compound will have its "isotope peak" at the same mass number as the parent molecule-ion of the other component of the mixture, (M - 29) from one is the same as (M - 15) from the other, etc. Hence only one "pure" peak, that due to the parent molecule-ion of the higher homologue, may be present in the mixture spectrum so that the derived spectrum of the other component only may be obtained. If, however, the high molecular weight substance has no (M - 1) peak in its spectrum, it may be reasonably assumed that this is true also of its lower homologue. The (M - 15) peak of the first compound is thus also a "pure" peak. Knowledge of its formula[70] will enable the abundance of its isotopic peak to be determined and subtraction of this from the peak at (M - 14) will give the contribution of the parent molecule-ion of the lower homologue to the mixture spectrum. Thus the spectra of both components of the mixture may be derived.

Such a situation arose during a recent investigation of the compounds obtained from Mammeia americana[68]. A substance was isolated which had a melting point and spectroscopic properties identical with those of mammein (III, Fig. IIIa), a compound previously isolated and characterized by Djerassi, Finnegan, Gilbert and Eisenbraun[71]. The mass spectrum of this substance gave a molecular weight equal to that of mammein (372) and accurate mass measurement proved that it had the same formula, $C_{22}H_{28}O_5$. However the spectrum also showed the presence of about 30% of a lower homologue (mol. wt. 358). The best separation obtainable by thin layer chromatography resulted in mixtures containing different relative amounts of the two substances. The peak height at $^m/_e$ 358 in two mixture spectra was corrected for the heavy isotope contribution[70] of the (M - 15) ion of the larger molecule and application of the subtractive method gave the derived spectra of the two substances. These spectra and the structures assigned to the two components (see chapter IV) are given in Fig. IIIb.

The previous workers[71] had reported that iso-valeric acid (from the iso-valeryl side chain) and n-butyric acid (from C - 4 together with its n-propyl substituent) were two of the hydrolysis products of mammein. A more likely explanation seemed to be that


100
%
ABUNDANCE
$C_{21}H_{26}O_5$
M.W. 358.
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360

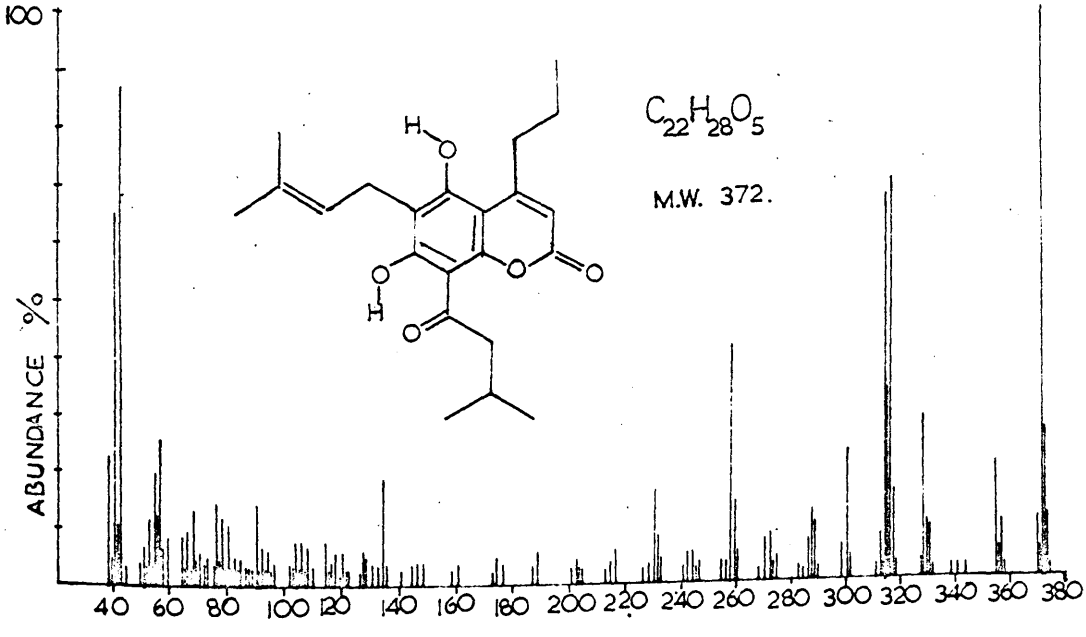

100
%
ABUNDANCE
$C_{22}H_{28}O_5$
M.W. 372.
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380

Fig. IIIb.

the sample of mammein used in their hydrolysis experiment also included some of its lower homologue, hydrolysis of which would yield n-butyric acid from the n-butyrl side chain. A sample of mammein, kindly supplied by Dr. R.A. Finnegan, was examined in the mass spectrometer. As expected it was also found to contain about 30% of the compound of molecular weight 358.

GAS CHROMATOGRAPHY - MASS SPECTROMETRY

Gas chromatography is one of the most efficient and versatile methods available to the organic chemist for separation of complex mixtures. The chromatograms obtained can also be used for identification of the components of mixtures by making use of their retention times on the column. Experimental methods of obtaining structural information solely from retention times, however, are rather involved and the results may be inconclusive[72]. The technique, then, is not entirely satisfactory for obtaining qualitative identifications, though it is superior to all others except mass spectrometry for microgram quantities. Compounds emerging from the column are therefore best examined by some other analytical method if positive identifications are to be obtained. This necessitates

the collection of fractions[73] in suitable amounts for such techniques as ultra-violet, infrared, nuclear magnetic resonance, or mass spectrometry.

If the analytical problem involves identification of known compounds only, infrared and mass spectrometry are the most suitable techniques. The infrared and mass spectra of individual compounds are highly characteristic and provide the most reliable "fingerprints" of any given structure. For the identification of fractions collected from a gas chromatograph, mass spectrometry is usually preferred, since infrared spectra on samples of less than about 50 µg. are not highly characteristic. This requires only a minute amount of material if a direct evaporation sample system is used. Difficulties experienced in collecting small fractions on account of the volatility of the sample or because of aerosol formation can be overcome by using some of the column packing to trap the sample[74]. Transfer of the fraction presents no difficulties as the trap can be attached directly to the mass spectrometer probe. Heating the probe produces a sufficient vapour pressure of the sample to provide a mass spectrum without serious background from the stationary phase in the trapping device. Good quality high resolution mass spectra can be obtained from 0.1 µg. of sample by this method[74].

Trapping of G.C. fractions and subsequent identification, however, may involve many hours of labour. Further, the method - to be completely successful - depends to a great extent on obtaining good resolution of the mixture on the G.C. column - and this is not always possible. For routine identification of mixtures of known constituents, then, direct coupling of the gas chromatograph to the mass spectrometer seems preferable. This enables mass spectra of the mixture components to be obtained as they emerge from the column. If an unresolved mixture is encountered, several mass spectra taken during its elution time will identify it as such, and examination of the different mixture spectra will enable certain mass peaks to be assigned to one component or the other; this may give qualitative and quantitative information (see below). Another advantage of direct coupling of the gas chromatograph to the mass spectrometer is that such hazards as chemical change of condensed samples, e.g. by oxidation, are avoided. One common objection to this technique is that the mass spectrometer in such a combination is not then available for other purposes[76]. This objection, at first glance a serious one, may not be considered so when it is realized that complete mass spectra of the components of, say, a twenty-component mixture may be obtained in the same time that is

taken for a single pure compound by conventional mass spectrometry.

Attachment of a gas chromatograph to a mass spectrometer presents some difficulties in instrumentation. For the correct functioning of the mass spectrometer, the pressure inside the source and analyser must not exceed about 10^{-5} torr. At this pressure, the pumping system can deal with an inflow of gas of about 0.1 ml. atmospheres per minute. The effluent from the column is about 50 ml. atmospheres per minute. Earlier attempts to circumvent this difficulty[77,78] consisted in splitting the effluent of the gas chromatograph so that less than one per cent entered the mass spectrometer. This of course meant that the sensitivity was greatly reduced, as 99% of the sample was "discarded" before it could enter the ion source. Some workers preferred to use capillary-column gas-chromatographs which have a gas flow of about 0.5 - 2 ml. atmospheres per minute and so can be led straight into the mass spectrometer[79]. These columns have also a much higher efficiency of separation than packed columns but are limited in the amount of material which can be injected and thus far have also proved to be of limited use for high molecular weight compounds.

A more sophisticated approach to the problem of overcoming the difficulties due to different gas flows in the two instruments

has been the development of "molecule separators" by Ryhage[80] and Biemann[81]. These work on the principle that if helium, which has a much greater rate of diffusion than sample molecules, be used as a carrier gas it may be preferentially removed before entry of the column effluent into the mass spectrometer. Ryhage[80] using his molecular beam type of separator claims that he obtains ion currents one hundred times greater than without it; when the same conditions of gas flow into the mass spectrometer are maintained and the same amount of material is injected onto the column.

As a compound emerges from the gas chromatograph its vapour pressure inside the mass spectrometer ion source rapidly increases to a maximum value and then quickly decreases as the sample is pumped away. Unless the desired mass range can be scanned very quickly with respect to the elution time of the gas chromatograph peak, spectra will be "biased" according to whether the pressure is increasing or decreasing during the mass scan. For this reason, time-of-flight instruments capable of producing thousands of complete mass spectra per second were at first the preferred choice[77,79]. Most laboratories, however, were equipped with some form of magnetic dispersion instrument. These generally have higher resolving powers and sensitivity than the time-of-flight mass spectrometers.

In any case it was soon apparent that the limitation of scanning speed was in the method of recording the spectra[82] and even this can be overcome as instruments capable of rapidly scanning a wide mass range with a resolving power of many thousands are now commercially available[18]. Simultaneous collection of all the ion currents by a photographic plate circumvents the need to scan the mass spectrum: high resolution spectra may be obtained from very small samples using this method[83].

Mass spectrometers designed primarily for identification of gas-liquid-chromatograph effluents have recently become commercially available. The following section describes the use of one of these in the solution of a problem in the chemistry of diterpene hydrocarbons[84].

THE ACID-CATALYSED REARRANGEMENT OF DITERPENE HYDROCARBONS

A previous investigation[85] of the products formed by the action of dry hydrogen chloride on stachene had been only partly successful owing to the difficulty of obtaining them in amounts sufficient for conventional methods of analysis. Identifications relied mainly on gas chromatographic retention times. Of the possible products

from stachene the pairs of isomers isokaurene/isoatisirene and kaurene/atisirene were found to have identical retention times on a variety of columns. Positive identification of these compounds from gas-liquid chromatography was therefore impossible as also was an estimation of the amounts of each present in the reaction mixtures. The recent acquisition of a combined gas-chromatograph-mass-spectrometer prompted a new attack on this problem.

The previous work had shown that the products from the treatment of a diterpene hydrocarbon with hydrogen chloride in chloroform would be a set of isomeric diterpene hydrocarbons. It was considered that it would be futile to attempt to assign structures to these from their cracking patterns. The first task, therefore, was to obtain a set of pure reference compounds and to run their mass spectra under the same conditions as were to be used in the subsequent work. A few of the spectra, together with the structures of the compounds, are given in figures IIIc-g.

The samples were introduced to the mass spectrometer by means of a 1% S.E. 30 column at 150°C. This gave suitably short retention times. S.E. 30 was chosen as the stationary phase as, at 150°, column bleed is negligible and all of the compounds encountered which could be separated by gas-liquid-chromatography were completely

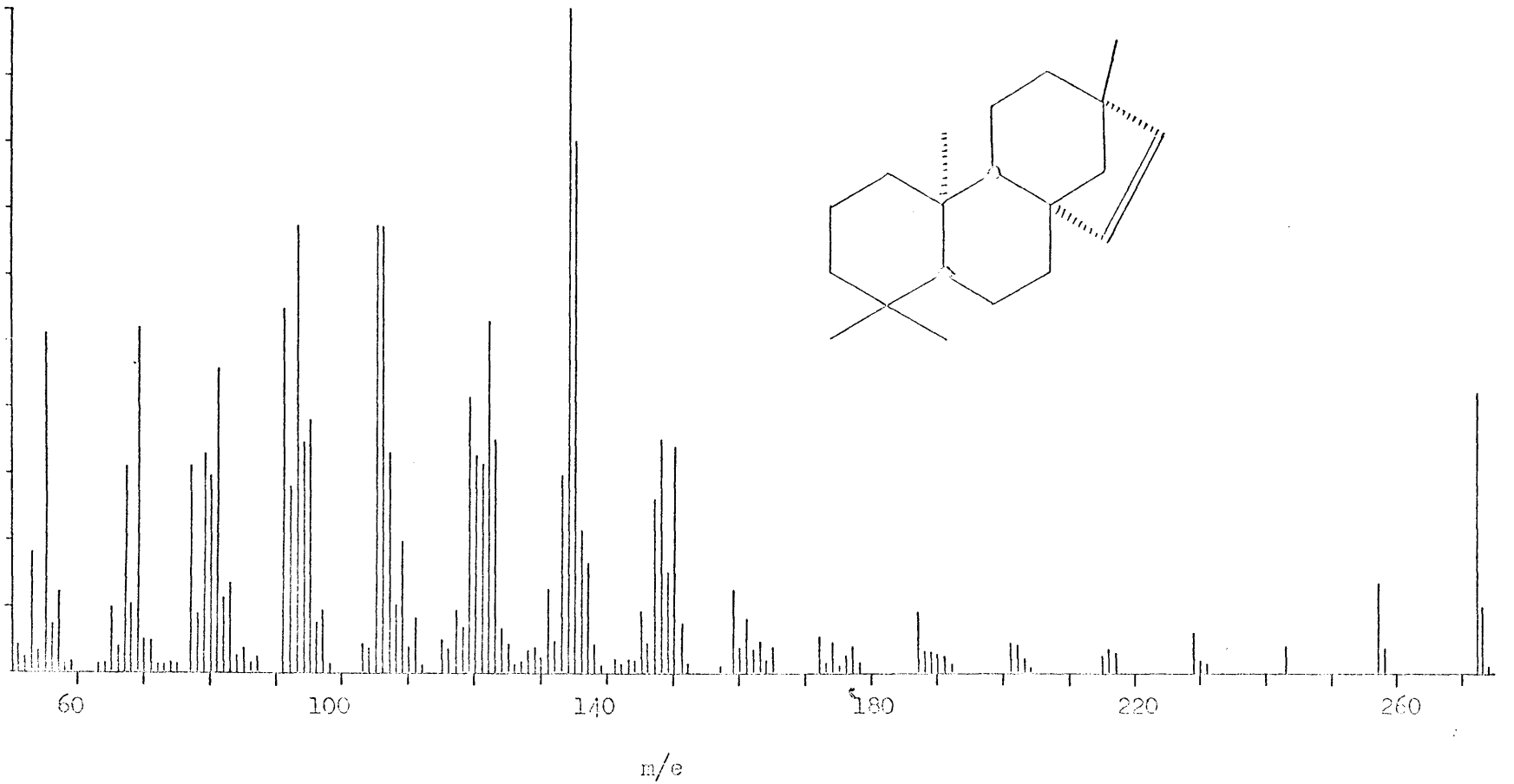


Fig. IIIc. Mass spectrum of stachene.

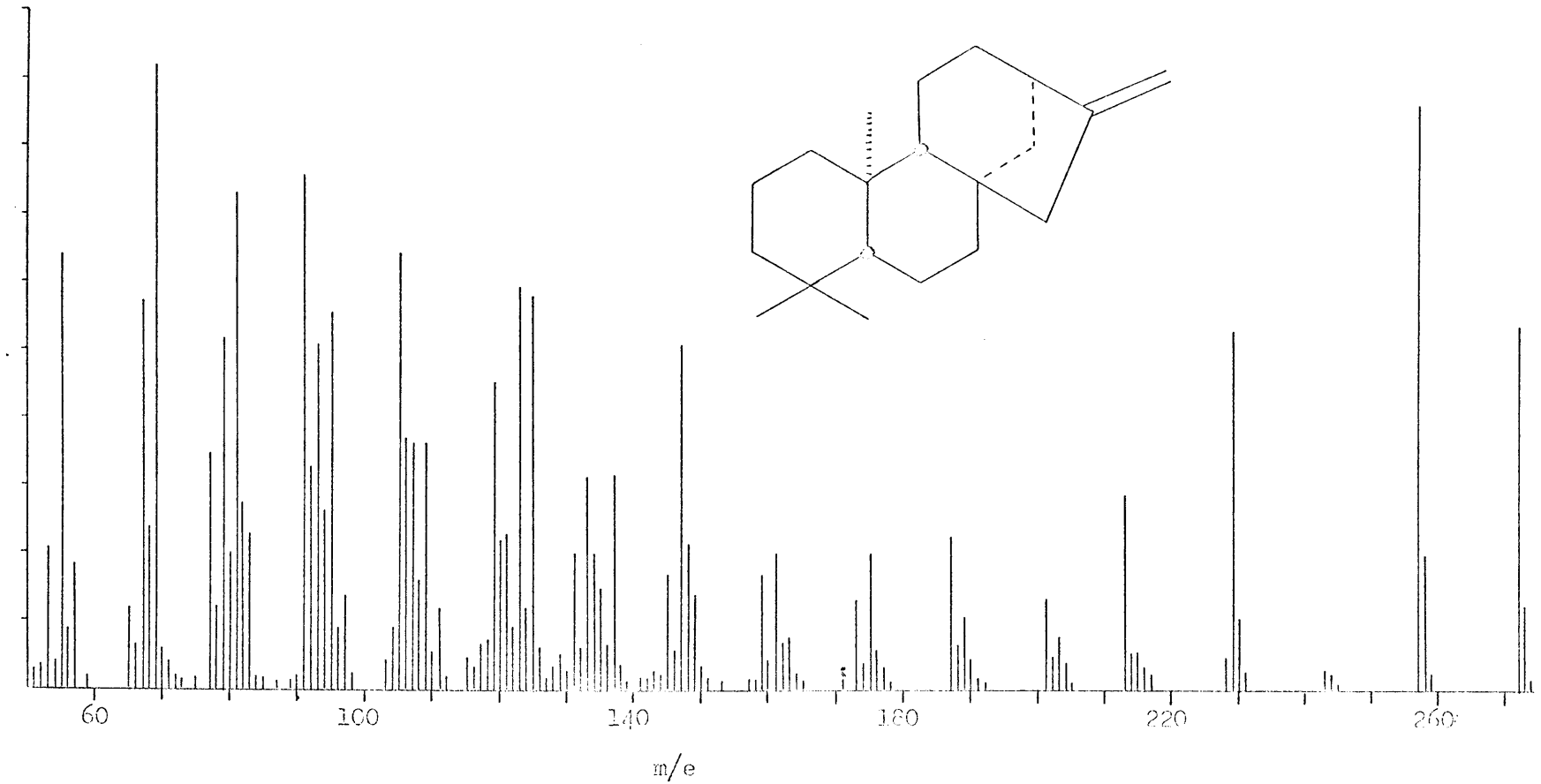


Fig. IIId. Mass spectrum of kaurene.

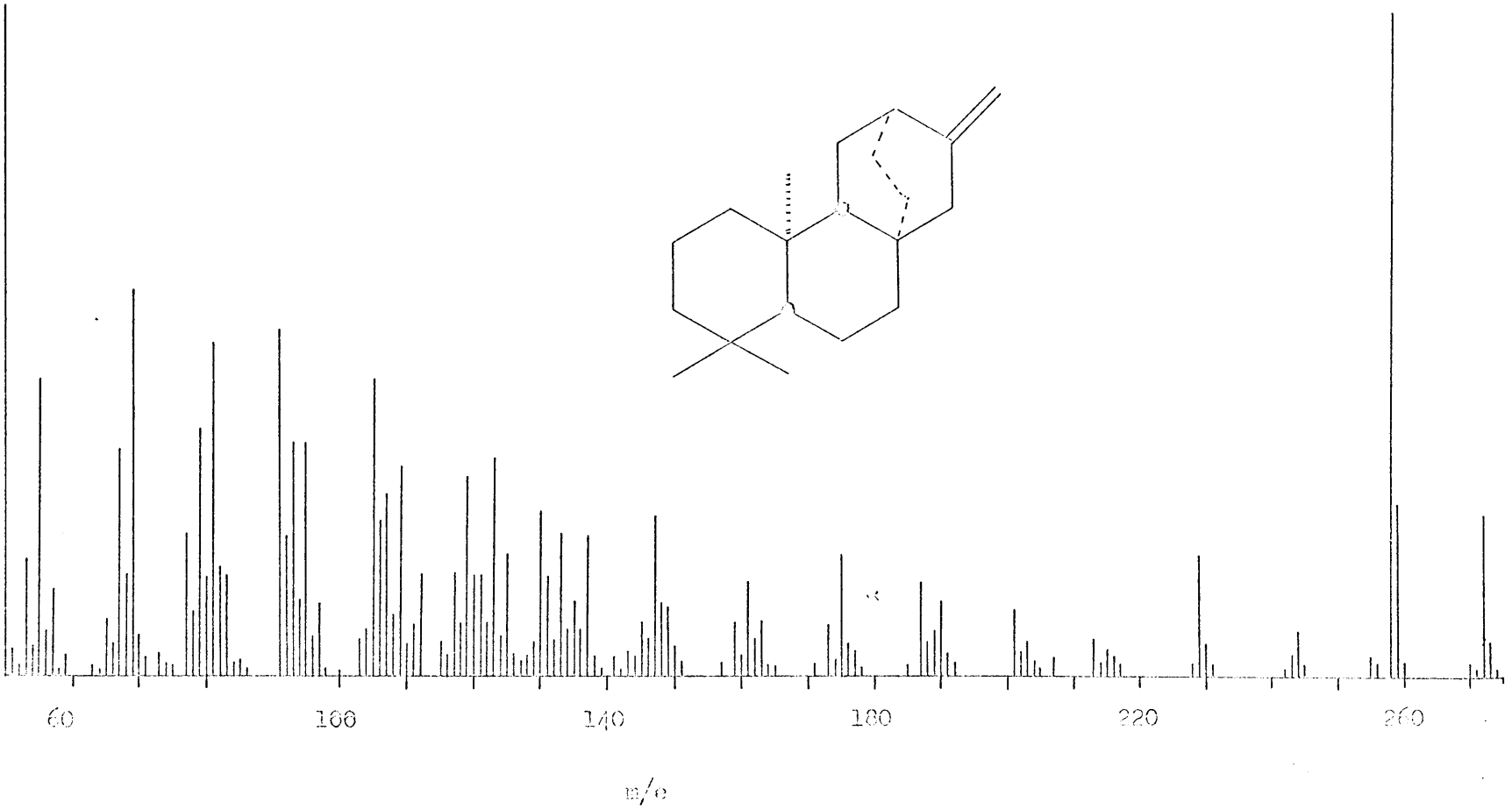


Fig. 11e. Mass spectr[illegible]

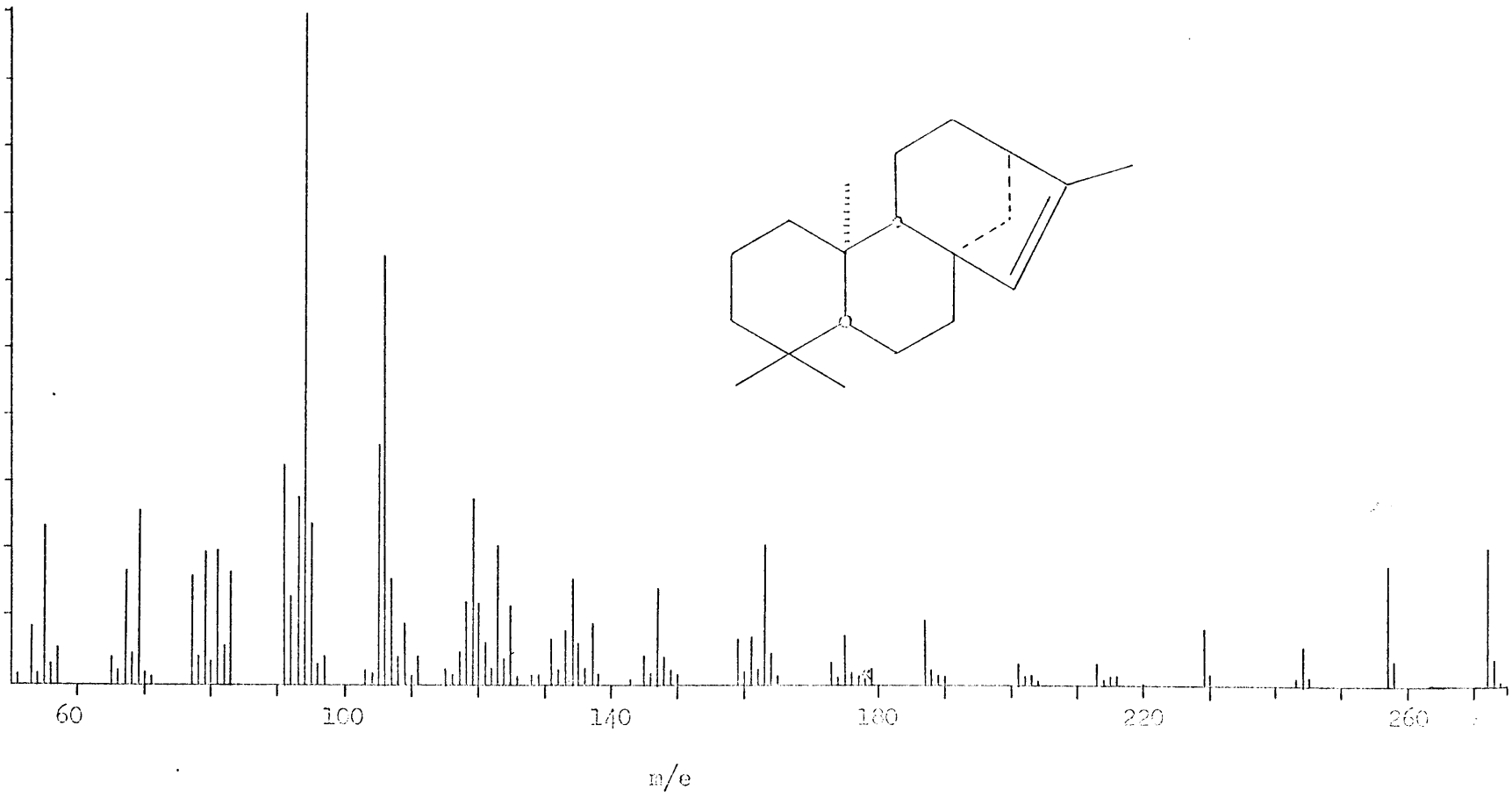


Fig. IIIf. Mass spectrum of isokaurene.

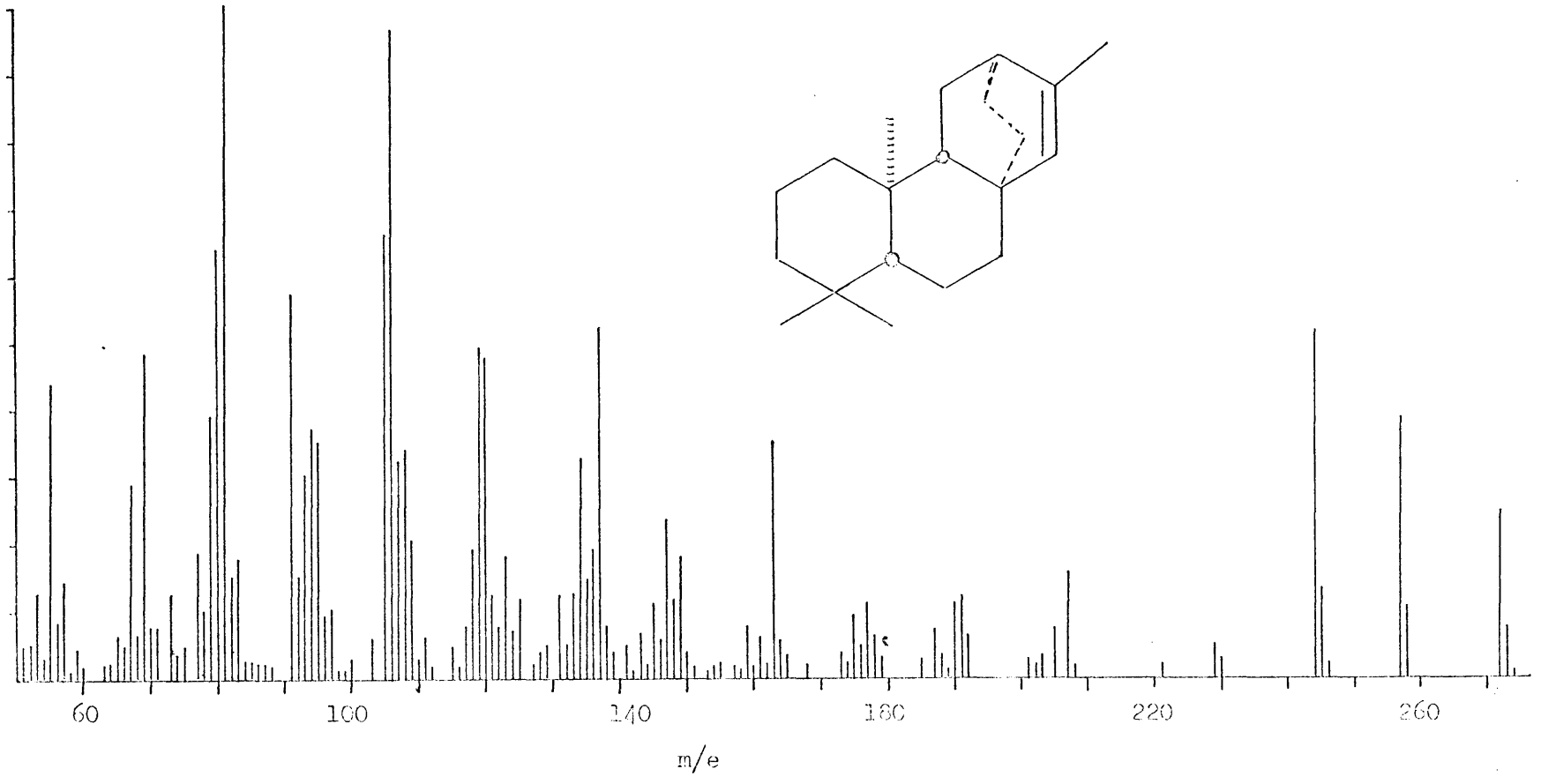


Fig. IIIg. Mass spectrum of iconbisirene.

resolved on this phase. Fig. IIIh shows the reproducibility of results obtained when the extremes of pressure change are encountered in the source during the mass scan. The spectra were obtained by scanning from mass 12 to 300 in about 1.5 seconds. Scan A, taken on the up-slope of the gas-liquid-chromatograph peak shows a gradual increase compared with scan B in the relative abundances of ions of $^{m}/e$ values greater than $^{m}/e$ 109 (the base peak). This increase is rather irregular, especially for smaller peaks, due to statistical variations in the collected ion-currents at the high scanning speed used[82]. Reference spectra were therefore obtained by scanning at the top of the gas-liquid-chromatograph peak, i.e., when the pressure in the ion source is virtually constant during the mass scan.

The results obtained from treatment of various diterpene hydrocarbons with dry hydrogen chloride in chloroform are given in Table IIIb. Identification of reaction products was based on their mass spectra and on gas-liquid-chromatograph retention times. Estimation of the quantitative composition of the reaction mixtures was by peak area in the first instance. Where components could not be separated by gas-liquid-chromatography the relative amounts of these were calculated from the mass spectra of the mixtures as

Table IIIb

Product composition after acid treatment.

Hydrocarbon	Time of isomerisation*		Product composition (%; approx.)		
	a	b		a	b
Stachene (II)	4 days	21 days	Stachene (II)	44%	35%
			Kaurene (III)	11	13
			Isokaurene (IV)	33	38
			Isoatisirene (V)	11	13
			Atisirene (VIII)	1	1
Kaurene (III)	2 hr.	14 days	Kaurene (III)	25	23
			Isokaurene (IV)	75	71
			Isoatisirene (V)	0	5
			Atisirene (VIII)	0	1
Isoatisirene (V)	2 hr.	14 days	Kaurene (III)	0	-
			Isokaurene (IV)	0	0.5
			Isoatisirene (V)	92	91
			Atisirene (VIII)	8	8
Trachylobane (VI)	15 min.	14 days	Kaurene (III)	1	1
			Isokaurene (IV)	3	3
			Isoatisirene (V)	90	90
			Atisirene (VIII)	6	6

* Only two representative experiments are quoted.

m/e	A	B
109	100.0	100.0
110	11.1	11.0
131	16.3	14.3
133	44.9	41.7
135	13.2	12.3
137	8.2	7.3
187	27.2	26.4
188	7.6	7.8
189	11.2	10.3
190	9.9	8.1
215	9.7	8.9
216	6.8	6.8
229	16.3	15.6
243	22.1	22.0
244	8.4	7.1
257	71.1	64.0
258	16.8	15.1
259	3.2	2.7
272	60.1	53.3
273	15.1	13.2

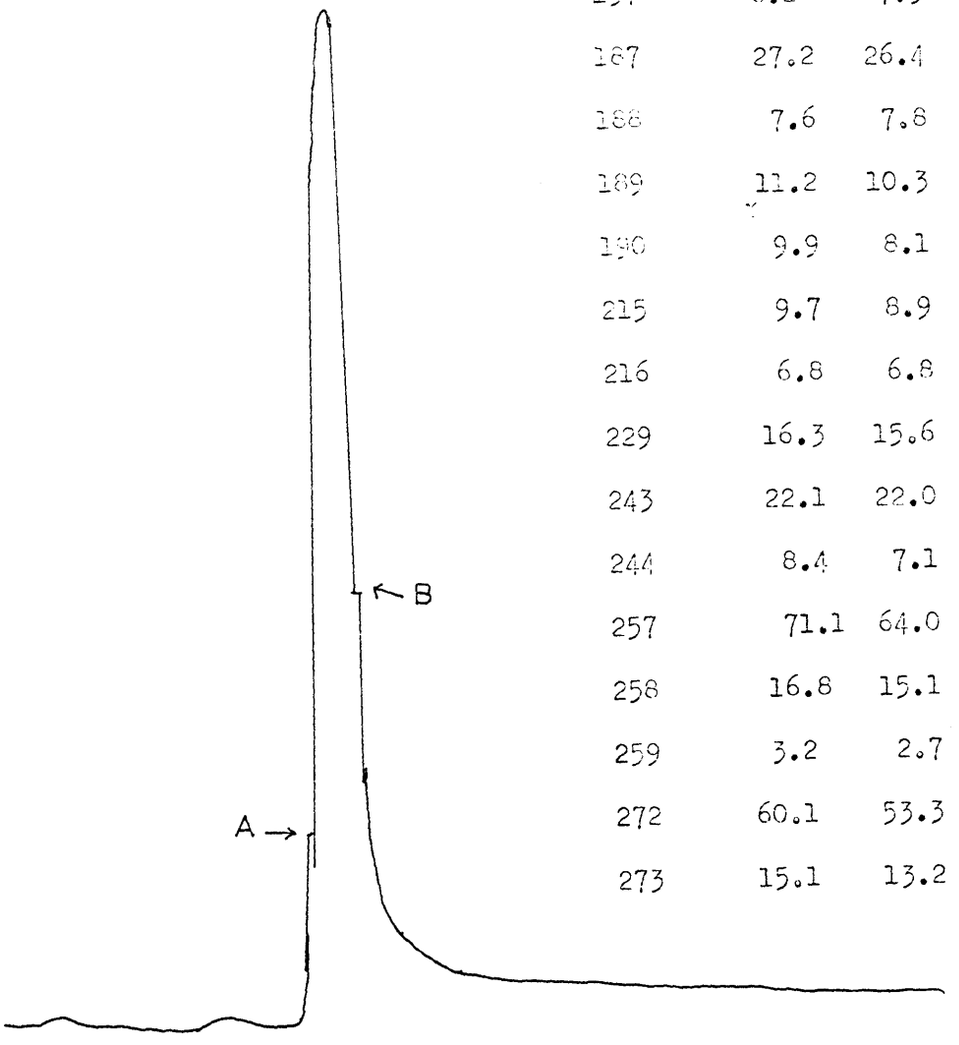


Fig. IIIh.

described in detail in the following section.

ESTIMATION OF THE COMPOSITION OF INSEPARABLE MIXTURES FROM "DERIVED" GAS-LIQUID CHROMATOGRAMS[86].

Prolonged (15 hours) treatment[84] of stachene (Fig. IIIc) with hydrochloric acid in chloroform gave a mixture whose gas-liquid chromatogram had three well-resolved peaks (Fig. IIIi). The peak A was shown by gas-liquid chromatography-mass spectrometry to be unchanged stachene. This technique, coupled with thin layer chromatography on silver nitrate plates, demonstrated that peak B in fact derives from a mixture of isoatisirene and isokaurene. Isoatisirene is the only tetracyclic diterpene hydrocarbon examined which has a very abundant (M - 28) peak in its mass spectrum (Fig. IIIg). Its presence in a mixture with isokaurene is therefore easily detected.

The relative amounts of isokaurene and isoatisirene were determined by a method similar to that used by Lindeman and Annis[75]. During a gas-liquid-chromatographic run on a complex hydrocarbon mixture these workers had recorded many mass spectra. Certain peaks characteristic of individual substances in the mixture were chosen and their abundances plotted against time. A "derived"

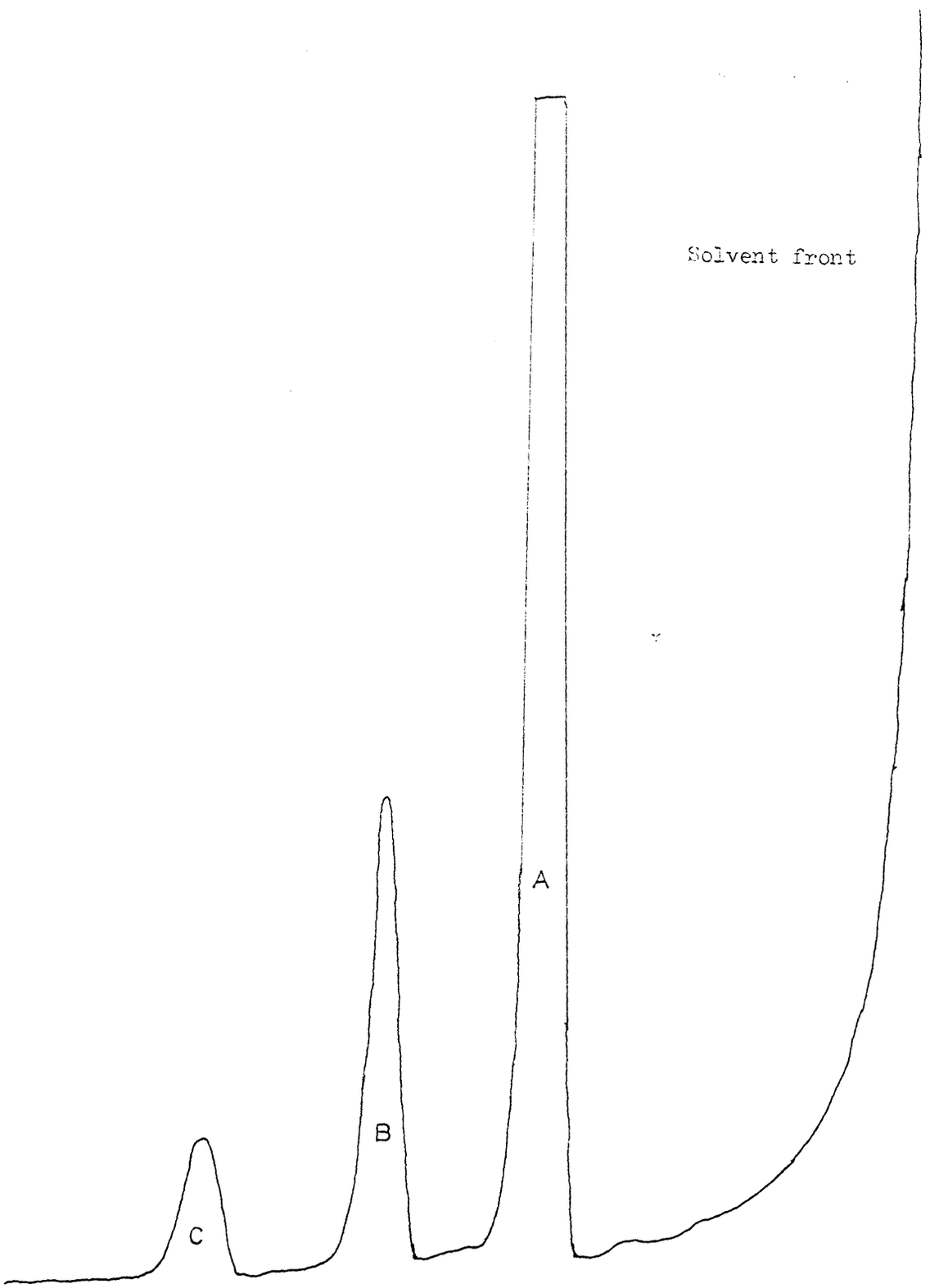


Fig. IIIi.

chromatogram was thus constructed which had a separate peak for each of the components, even though the experimentally obtained chromatogram showed poor resolution of the mixture. Using mass spectral sensitivity data a complete quantitative analysis of the mixture could be obtained from the "derived" chromatogram.

In the present analysis two difficulties had to be overcome. Firstly, the mass spectra of isokaurene and isoatisirene overlap at every mass number: simultaneous equations had thus to be used for quantitative analysis of the mixtures[87]. Secondly, the mass spectrometer was equipped only with a G.L.C. introduction system. Measurement of the relative sensitivities of peaks in the isokaurene and isoatisirene spectra was thus impracticable[87]. It has been shown, however, by Otvos and Stevenson[88] that hydrocarbon isomers have the same total ion current, ΣI, per mole of sample vapour introduced into the ion source of the mass spectrometer. This may be used in the quantitative analysis of mixtures of isomers as follows:-

$^{m}/_{e}$ 123 in the mass spectrum of isokaurene is 2.15% of ΣI whereas in the spectrum of isoatisirene this ion is 0.90% ΣI. This means that in the mass spectrum of a mixture of equal parts of isokaurene and isoatisirene the relative contributions of these

to the mixture peak height at $^{m}/e$ 123 are 2.15 and 0.90 respectively, i.e.,

$$H_{123} = 2.15C_K + 0.90C_A \qquad (1)$$

where H_{123} is the mixture peak height at $^{m}/e$ 123 (measured in arbitrary units) and C_K and C_A are the respective relative concentrations of isokaurene and isoatisirene in the mixture. Similarly at $^{m}/e$ 137,

$$H_{137} = 0.95C_K + 2.63C_A \qquad (2)$$

Solution of equations (1) and (2) and the evaluation of $100C_K/(C_K + C_A)$ gives the percentage of isokaurene in the mixture.

Solution of equations (1) and (2) for several mass spectra taken at various points on the gas-liquid-chromatograph peak B, Fig. IIIi, gave results ranging from 52% isokaurene at the front of the peak to 92% at the tail. Use of several other pairs of peaks and of summations of peaks in the spectra gave results agreeing within 3% of the values found from the $^{m}/e$ 123 and $^{m}/e$ 137 peak heights. The unresolved peak B therefore had a greater

concentration of isokaurene at the tail than at the front and any one mass spectrum gave only the concentration of the mixture present in the ion source during the scan time.

The method of estimating the overall composition of the mixture and an indication of the accuracy attainable is described below for a synthetic mixture of isokaurene and isoatisirene.

$^{m}/e$ 123 and $^{m}/e$ 137 were chosen as suitable peaks for the analysis as one of them ($^{m}/e$ 123) is much more abundant in the spectrum of isokaurene than it is in the spectrum of isoatisirene and is thus sensitive to changes in the isokaurene content of the mixture[87]. Similarly $^{m}/e$ 137 is sensitive to the isoatisirene concentration. Solution of many simultaneous equations such as (1) and (2) would be extremely tedious. This can be avoided as follows:-

In an equimolar mixture of isoatisirene and isokaurene the peak heights of $^{m}/e$ 123 and $^{m}/e$ 137 are in the ratio

(2.15 + 0.90) : (0.95 + 2.63), see equations (1) and (2). Similarly for a mixture containing 75% isokaurene,

$$^{m}/e\ 123 : {}^{m}/e\ 137 = (3 \times 2.15 + 0.90) : (3 \times 0.95 + 2.63)$$

A few such simple calculations enables the graph of Fig. IIIj

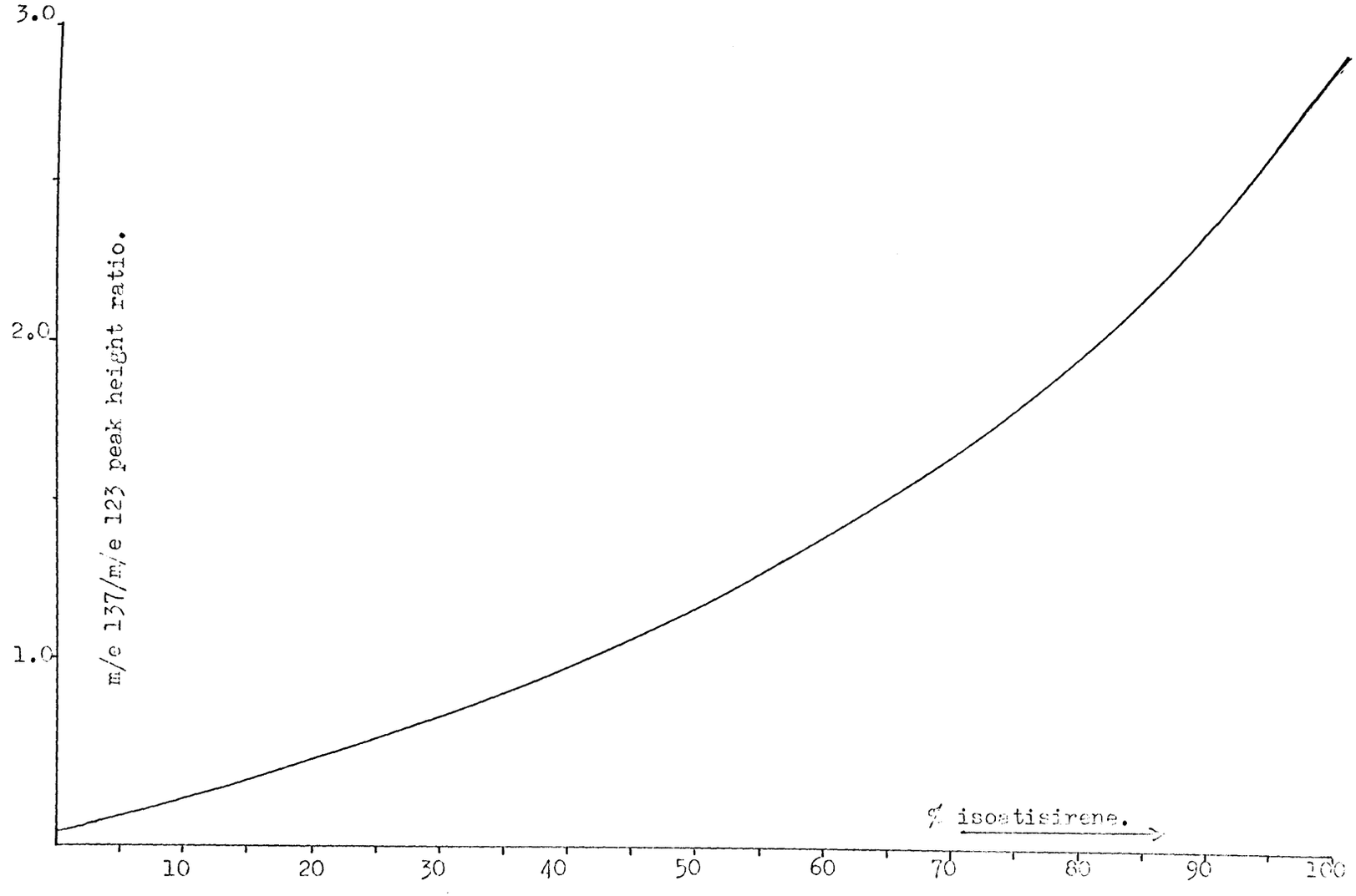

3.0
2.0
1.0
m/e 137/m/e 123 peak height ratio.
% isostisirene.
10
20
30
40
50
60
70
80
90
100

Fig.IIIj.

to be drawn. If the ratio of $^{m}/_{e}$ 137/$^{m}/_{e}$ 123 in any mixture spectrum is measured then the composition can be read directly from the graph.

There were two further reasons for choosing suitable peaks which are close together in $^{m}/_{e}$ value. This mass range can be scanned in a fraction of a second; thus pressure changes between recording of the two analytical peaks are negligible. When the magnet scan button of the mass spectrometer is pressed, the signal to the total ion current (G.L.C.) recorder is interrupted; the pen thus draws a horizontal line (Fig. IIIh). At the end of the scan the pen "kicks", drawing a vertical line through the G.L.C. peak. For a very short scan only the vertical line can be seen; thus an accurate measure is given of the time at which the scan is taken.

A mixture was made up from a solution of isoatisirene and a solution containing isokaurene and stachene. Stachene is well separated from the other two compounds on a S.E. 30 column and served as a marker compound from which the amount of isokaurene in the mixture could be calculated, the stachene/isokaurene solution being run separately and the relative areas of the two G.L.C. peaks measured. The column was run at 120^{o}C to give

broad G.L.C. Peaks, twenty scans over the mass range $^{m}/e$ 123 to $^{m}/e$ 137 were taken on the isokaurene/isoatisirene peak and the ratios of $^{m}/e$ 137 to $^{m}/e$ 123 peak heights were calculated. The composition of the mixtures were read from the graph, Fig. IIIj and the ordinates through the scan positions (Fig. IIIk) divided up in the ratios of the isokaurene compositions. The smooth curve A (Fig. IIIk) was drawn through the points obtained. A similar procedure gave curve B for isoatisirene. Measurement of the areas enclosed by A and B and the base-line gave the composition of the mixture as 32.7% isokaurene. The mixture was re-run on an analytical G.L.C. Measurement of the areas of the isokaurene/isoatisirene peak and of the stachene reference peak gave the composition as 31.3% isokaurene.

Thus the relative amounts of isokaurene and isoatisirene in a mixture can be determined with reasonable accuracy by gas-chromatography-mass-spectrometry.

In a separate experiment[84] it was found that, on treatment of isoatisirene with hydrochloric acid, equilibrium with atisirene was rapidly established. The equilibrium mixture consisted of 92% isoatisirene and 8% atisirene. It could, therefore, be safely assumed that in any of the isomerization products in which

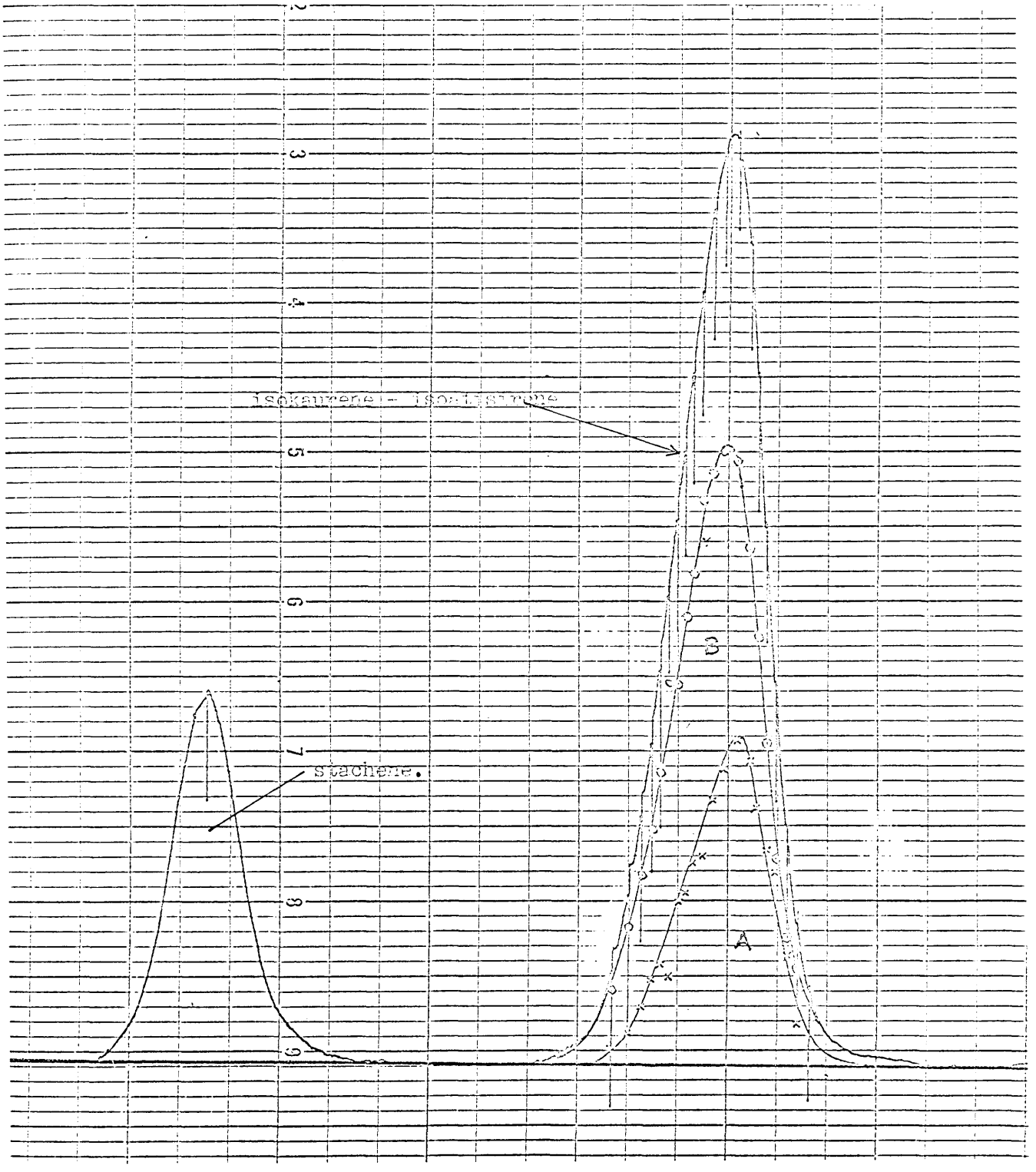


Fig. IIIk.

isoatisirene was found there would also be the equilibrium amount of atisirene. The areas of peaks A, B and C in the gas-liquid chromatogram (Fig.IIIi) of the products from the isomerization of stachene gave the composition of the mixture as stachene: (isokaurene + isoatisirene): (atisirene + kaurene). Mass spectrometric analysis of Peak B, Fig.IIIi, by the method described above, gave its composition and, assuming that isoatisirene and atisirene were present in the equilibrium amounts, the composition of peak C could be calculated. Thus the complete composition of a complex mixture could be determined (Table IIIb) even though some of the components could not be separated from each other.

EXPERIMENTAL

Mass spectra were obtained, at low resolving power, on an A.E.I., Manchester Ltd., M.S.9 instrument using 70 e.V. electrons.

Combined gas-chromatography-mass-spectrometry was done on an L.K.B., Stockholm, 9,000-A gas-chromatograph-mass-spectrometer. This has a Ryhage molecule separator. G.L.C. traces (total ion current records) were obtained using 20 e.V. electrons to avoid ionizing the helium carrier gas. Mass spectra were recorded using 70 e.V. electrons. The G.C. column used was packed with

S.E. 30 (1%) on Gas Chrom. P.

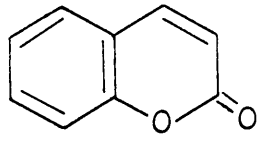
CHAPTER IV.

SOME COMPOUNDS FROM MAMMEIA AMERICANA L.

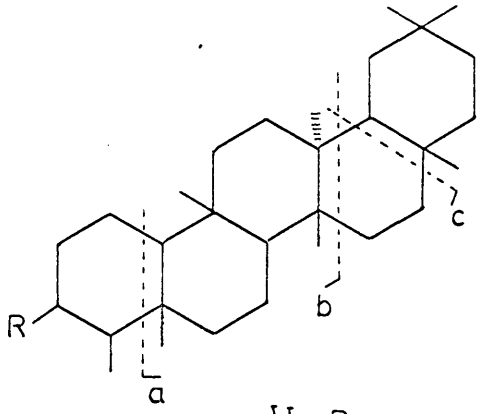
A recent investigation[68,89] of the seeds of Mammeia americana L. has resulted in the isolation and characterization of a large number of compounds. Most of these are derivatives of coumarin (I, Fig. I), a few of which had been obtained and their structures established by previous workers[71,90,91]. Two compounds not related to the others have been identified. These were known compounds but had not been previously found in the extracts from Mammeia. Only a few milligrams of each of these were obtained and without high resolution mass spectrometry their identification would have been very difficult.

A) COMPOUNDS NOT RELATED TO COUMARIN

One of these had a molecular weight of 426 and accurate mass measurement established the formula as $C_{30}H_{50}O$. The compound, therefore, had a total of six double bonds and rings. Its abundant parent molecule-ion suggested a fairly rigid type of structure and the overall appearance of its mass spectrum (Fig. II) was similar to published spectra of pentacyclic triterpenes[92]. In the

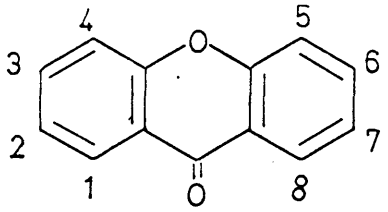


I

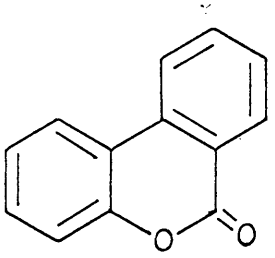


II , R = H

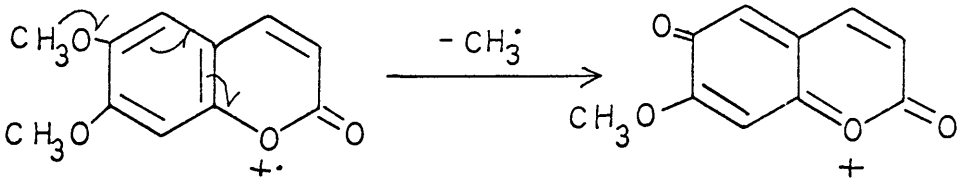
III , R = O



IV



V



VI

VII

Fig. I.

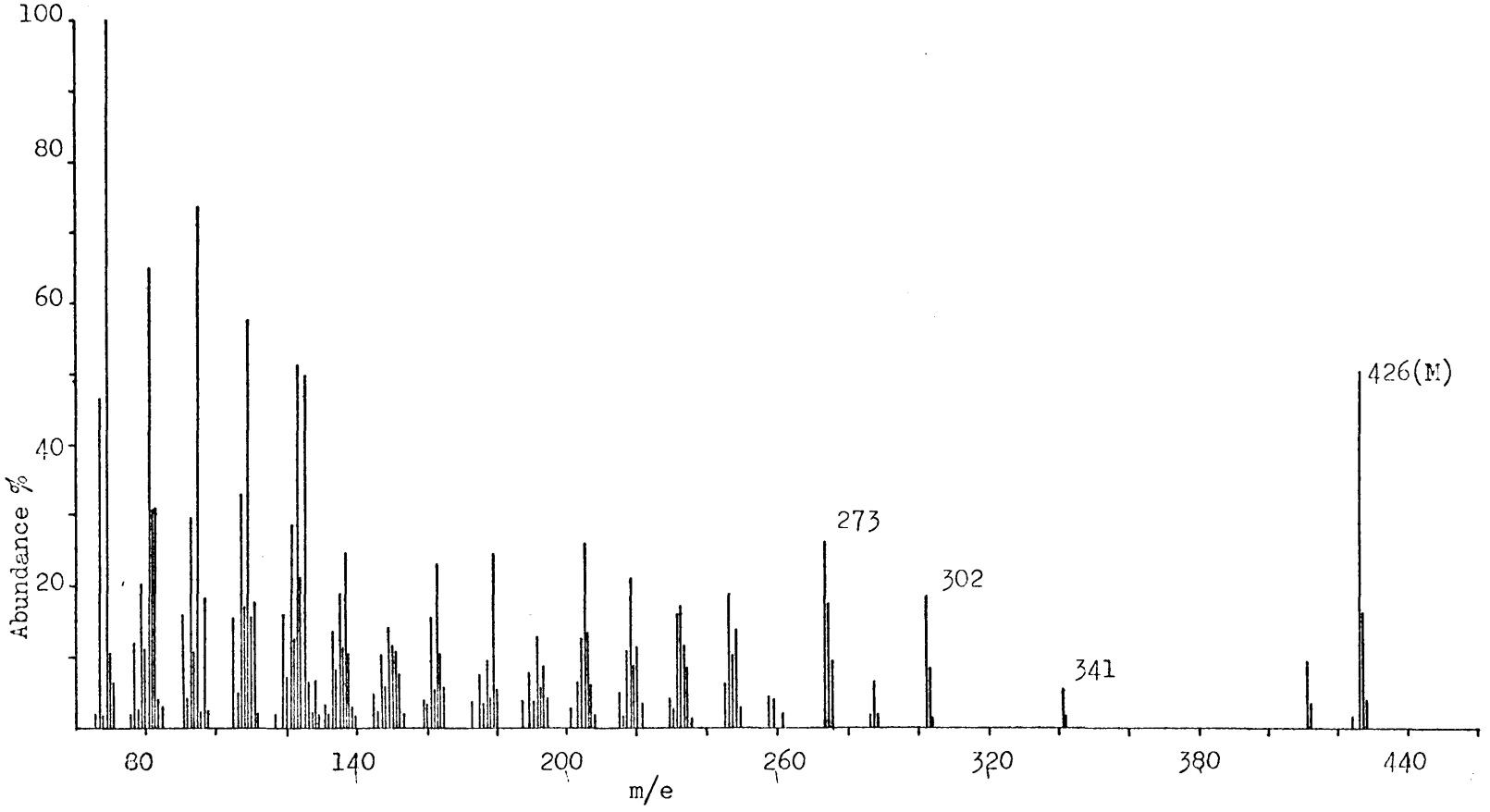
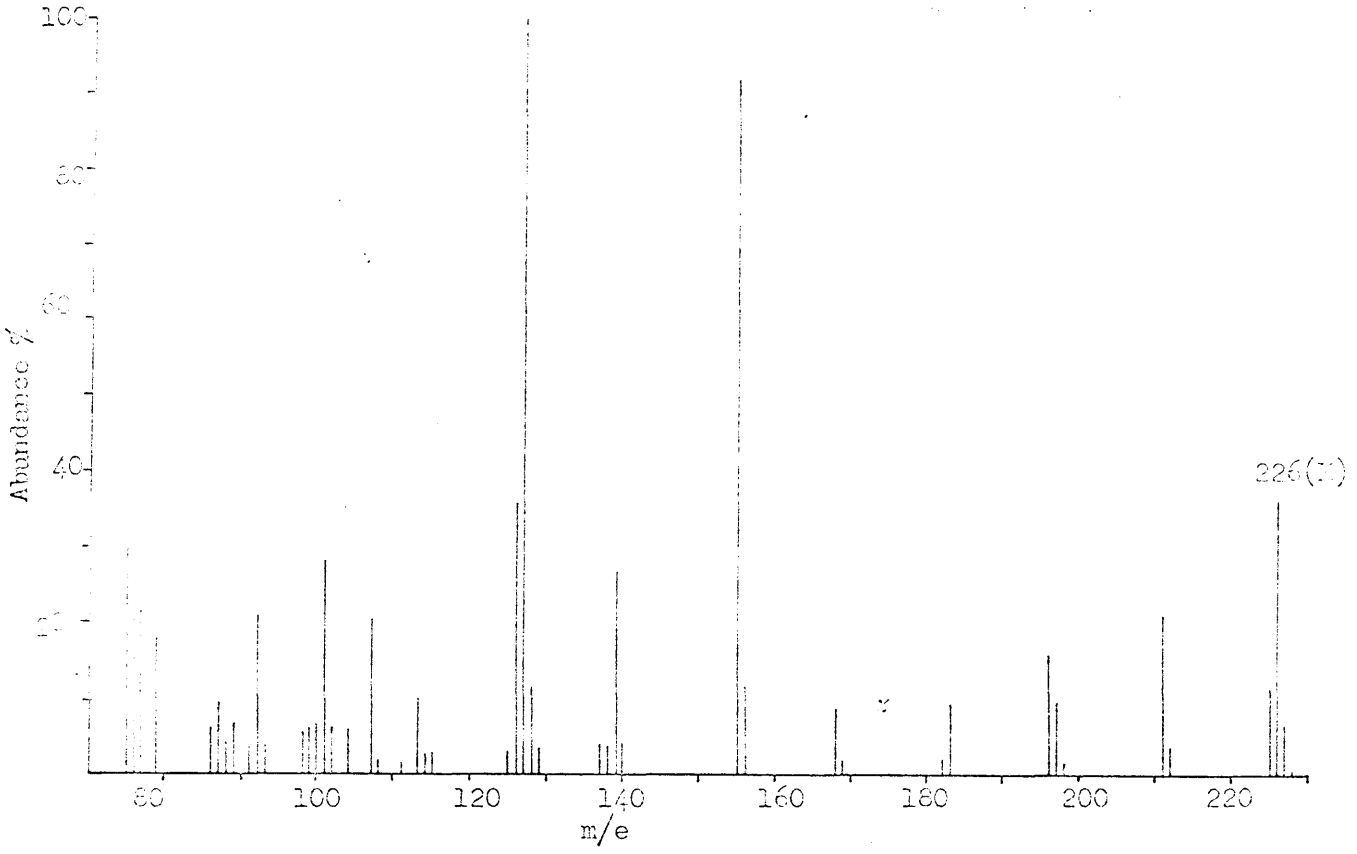


Fig. II. Mass spectrum of triterpene from Mammeia americana L.

investigation of the structure of a derivative of friedelane (II, Fig. I) Shannon[93] had obtained the mass spectra of several related substances. A brief list of fragment ions formed from these compounds showed that friedelan-3-one (III, Fig. I) had peaks in its mass spectrum at $^{m}/_{e}$ 341, 273 and 302 arising from cleavages a, b and c respectively (Fig. I). The formula and mass spectrum of the compound from Mammeia americana suggested that it was in fact friedelan-3-one. Comparison of the infrared and mass spectra with those of an authentic sample (kindly supplied by Dr. R. Stevenson, Brandeis University, Waltham, Mass.) and a mixed melting point determination confirmed the identity of the unknown compound with friedelan-3-one.

The spectrum of the second compound is given in Fig. III. The formula was found to be $C_{14}H_{10}O_3$ by accurate mass measurement. Having ten "double bond equivalents" the compound was most likely a xanthone or a benzocoumarin - xanthones were known to occur in other members of the same plant family (Guttiferae) as Mammeia americana[68]. The parent compounds of these two types (IV and V respectively in Fig. I) have almost identical mass spectra[67]; it was thus impossible to decide to which of these two classes the Mammeia compound belonged. Infrared spectrometry, however, showed



Mass measurements.

Observed	Formula	Calculated
226.0628	$C_{14}H_{10}O_3$	226.0630
225.0549	$C_{14}H_9O_3$	225.0552
196.0525	$C_{13}H_8O_2$	196.0524
183.0447	$C_{12}H_7O_2$	183.0445
155.0492	$C_{11}H_7O$	155.0497

Metastables.

Observed	Transition	Calculated
172.5	225 → 197	172.5
158.8	211 → 183	158.7
144.0	196 → 168	144.0
131.2	183 → 155	131.3
116.5	168 → 140	116.7
104.2	155 → 127	104.1

Fig. III. Mass spectral data of a xanthone from Mammeia americana L.

that it was in fact a xanthone.

The spectra of a number of substituted xanthones have been studied by Clayton[67] and the correlations between their mass spectra and structures proved to be of considerable assistance in identifying the compound discussed here.

The salient feature of the mass spectrum of xanthone[67,94] is the successive elimination of the two oxygen atoms as carbon monoxide followed by loss of a hydrogen atom. Methoxylated xanthones[67] have abundant (M - 1) species in their mass spectra arising from expulsion of hydrogen from the methoxyl function. A rather unusual feature in the spectra of the compounds with methoxyl substituents adjacent to the carbonyl group is the abundant loss of water[67]; a phenomenon common also to anthraquinones with this substitution pattern[95].

Two characteristic fissions of aromatic methyl ethers are also relevant to the discussion of the mass spectrum of Fig. III. These compounds eliminate formaldehyde (30 mass units) by cleavage of the aryloxy bond and transfer of a hydrogen from the methoxyl function to the aromatic ring[36]. Rupture of the alkyl-oxygen bond gives a positive ion which then decomposes by expulsion of carbon monoxide. According to Biemann[96] the latter process

occurs because the ion resulting from loss of a methyl radical has its positive charge localized on an oxygen atom bearing only six electrons: expulsion of a molecule of carbon monoxide results in a stable even-electron ion.

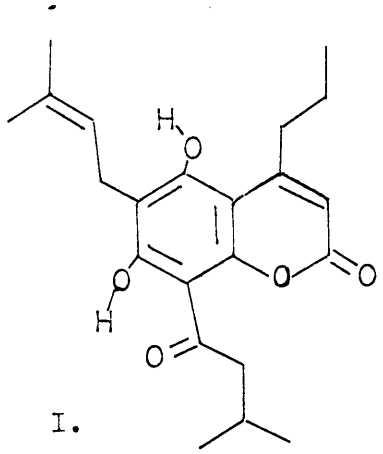
Assignment of metastable transitions and accurate mass measurements revealed that the compound from Mammeia was a methoxy xanthone. Loss of hydrogen from the parent molecule-ion was followed by elimination of carbon monoxide to give $^{m}/e$ 197. In another fragmentation process formaldehyde was eliminated to give $^{m}/e$ 196 (no metastable observed for this); the compound, therefore, had a methoxyl group rather than a methyl and hydroxyl. The ion of $^{m}/e$ 196 corresponds to the parent molecule-ion of xanthone and the ions $^{m}/e$ 168, 140 and 139 are analogous to the pattern given by its fragmentation[94]. A third degradation pathway consisted of expulsion of a methyl radical followed by loss of carbon monoxide, thus confirming the presence of the methoxyl group: the resulting ion , $^{m}/e$ 183, gave the base peak of the spectrum, $^{m}/e$ 127, by the successive eliminations of two carbon monoxide molecules.

From the absence of a (M - 18) peak it was concluded that the compound could not be 1-methoxy xanthone[67], although the

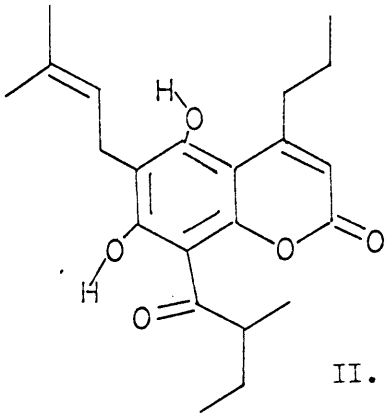
spectrum of this compound has not been published. The (M - 15) ion was found to be about ten times more abundant, as compared to the parent molecule-ion, than in the published spectra of other xanthones. None of these had methoxyl groups in the 2, 4, 5 or 7-positions (IV, Fig. I) from which loss of the methyl radical could give rise to a conjugated oxonium ion. Formation of these ions has been postulated[97] as the driving force for facile cleavage of side chains in pyrones, e.g., coumarins and chromones. Djerassi and Shapiro[98] have shown that the loss of a methyl radical from the parent molecule-ion of 6, 7-dimethyoxy-coumarin (VI, Fig. I) involves only the 6-methoxyl function, giving the oxonium ion VII, Fig. I. It therefore seemed likely that the xanthone isolated from Mammeia americana seeds was either 2- or 4-methoxy xanthone. A sample of synthetic 2-methoxy xanthone was readily available (kindly supplied by Dr. H. Suschitzky, Royal College of Advanced Technology, Salford) and this proved to be identical with the naturally occurring compound.

B) COUMARINS FROM MAMMEIA AMERICANA L.

The isolation of mammein (I, Fig. IV) was reported by Pagan and Morris[99] in 1953. The formula was established (1958) by Djerassi and co-workers as $C_{22}H_{28}O_5$, and some of the functional



I.



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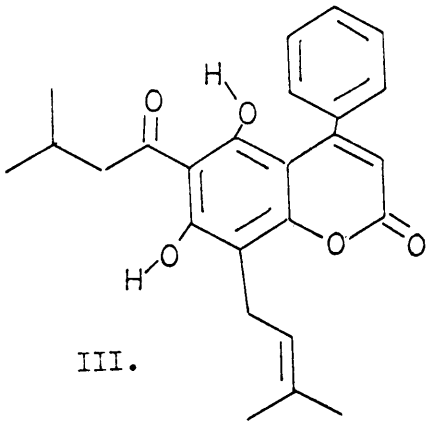
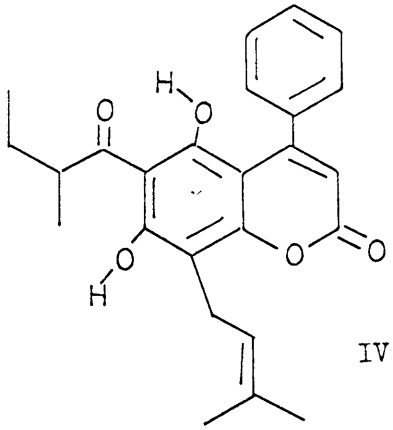
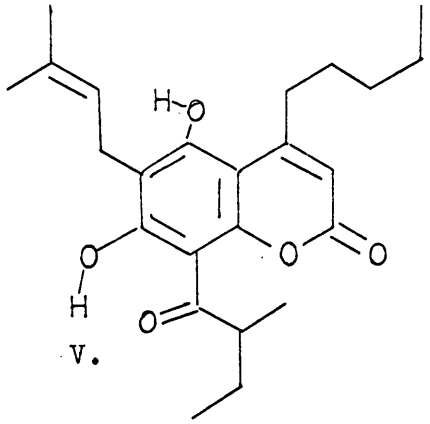


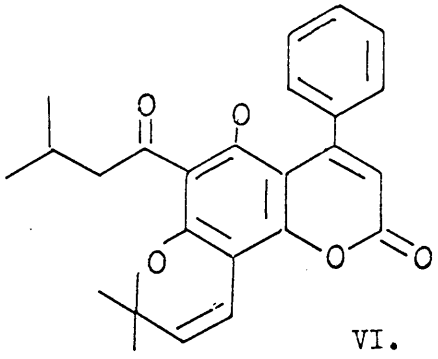
III.



IV.



V.



VI.

Fig. IV. Some coumarins from Mammeia americana L.

groups were characterized. A later communication[71] by the same school described the complete structure elucidation by chemical degradation.

A more recent investigation of the compounds of Mammeia americana[89] resulted in the isolation of a compound which from its melting point and spectroscopic properties appeared to be identical with the mammein of the literature. The mass spectrum of this compound, however, showed that it was in fact a mixture. The main component had a molecular weight of 372 and the formula was found by accurate mass measurement to be identical with that of mammein, i.e. $C_{22}H_{28}O_5$. There was, however, a considerable amount of a compound of molecular weight 358 ($C_{21}H_{26}O_5$) in the sample. Metastable peaks at $^m/e$ 266.5 and 277 showed that the largest peak in the mixture spectrum, $^m/e$ 315, was derived from both components by loss of $C_4H_9\cdot$ and $C_3H_7\cdot$ respectively (the formulae of the radicals eliminated was determined by mass measurement). Further decomposition of $^m/e$ 315 resulted in the elimination of a butene molecule to give the second most abundant ion, $^m/e$ 293. For a detailed mass spectrometric study it was decided to attempt to separate the two compounds. This proved to be extremely difficult and the best separation obtainable by thin layer

chromatography resulted only in mixtures which contained different relative amounts of the two homologues. However if two different mixtures of the same two substances are available then the mass spectra of the individual components may be obtained by a simple arithmetical method, as described in chapter III. The spectra of mammein and its lower homologue obtained by this procedure are illustrated in Fig. V. The nature and positions of the functional groups and side chains of mammein had previously been proved by chemical degradation[71].

A number of simpler coumarins have been studied by mass spectrometry. The spectra are characterized by the successive losses of the oxygens as carbon monoxide[94,100]. In coumarin itself (I, Fig. I) loss of carbon monoxide gives the base peak of the spectrum at $^{m}/e$ 118. In compounds with methoxyl or larger side chains elimination of carbon monoxide is less important; β-cleavage of the side chains gives rise to the most abundant fragment ions in the spectra.

To explain the cleavage of γ,γ-dimethylallyl (isopentenyl) side chains of anthraquinonoid pigments, Shannon[101] has proposed the mechanisms a - e, Fig. VI. In the spectrum of mammein (Fig. V.) fragment ions whose formation is explicable by mechanisms

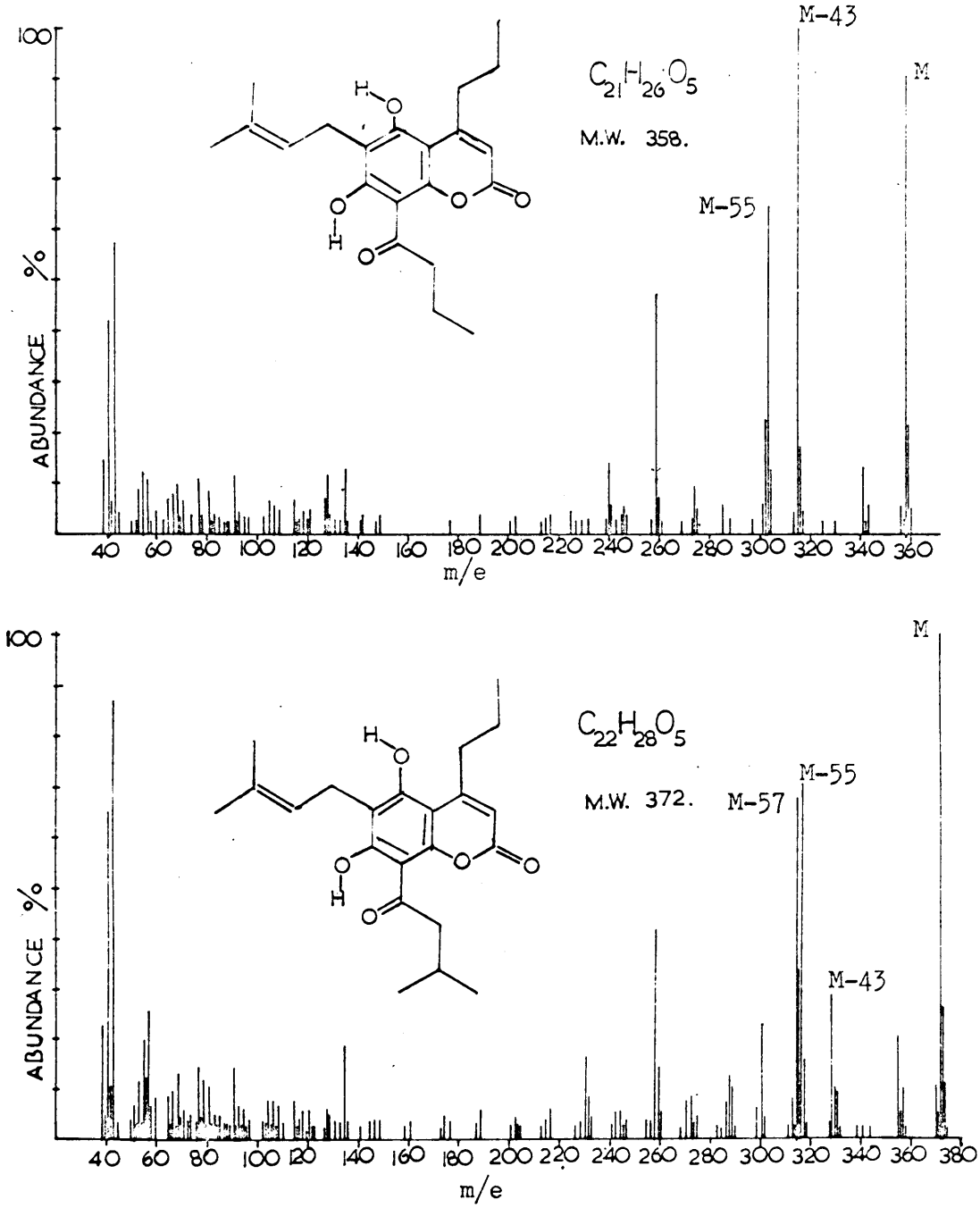


Fig. V. Mass spectra of mammein and its lower homologue.

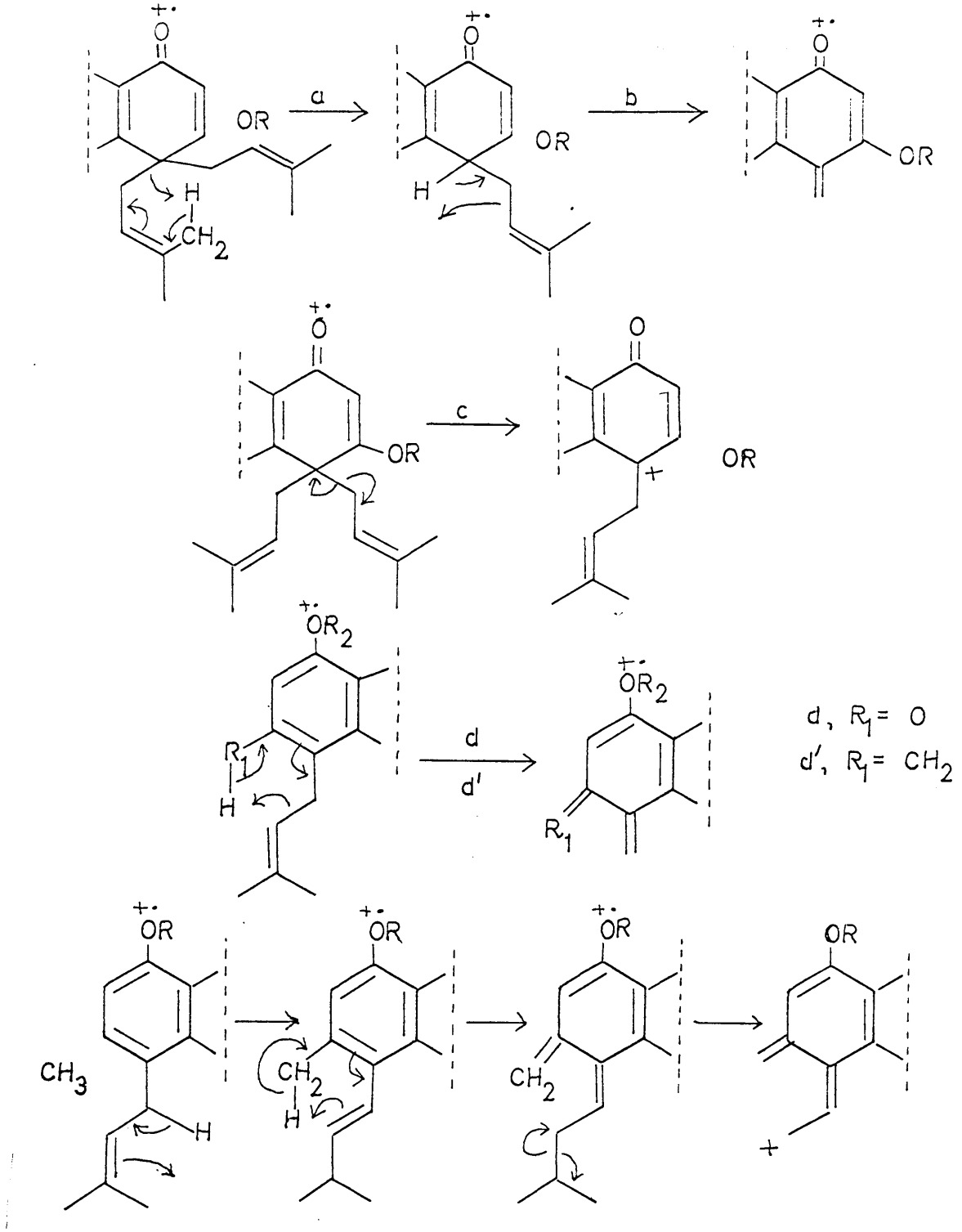


Fig. VI.

d and e are prominent, whereas loss of the entire isopentenyl side-chain as in a and c is not observed.

The initial step in one of the principal degradation pathways of mammein is loss of a butyl radical from the isovaleryl side chain, which is followed by elimination of a butene molecule. This latter fission is readily explained by mechanism d, Fig. VI. Subsequent decomposition occurs with the expulsion of a molecule of carbon monoxide to give $^{m}/e$ 231; a sequence of events shown by the presence in the spectrum of the appropriate metastable peaks; and the formulae of the fragments eliminated were proved by accurate mass measurements. A series of small peaks 28 mass units apart which begins at $^{m}/e$ 203 and ends at $^{m}/e$ 91 corresponds to successive losses of the remaining oxygens as carbon monoxide and of a two-carbon fragment from the n-propyl side chain, although no metastables are observed for this sequence.

In another fragmentation process loss of a butenyl radical occurs by β-fission of the isopentenyl side chain, as is found in the spectra of osthol[94,100] (I, Fig. IX) and dihydro osthol[94] (II, Fig. IX). Formation of a substituted tropylium ion has been proposed as the driving force for this reaction[94] (III, Fig. IX). Rather unexpectedly $^{m}/e$ 317, formed by fission of the

isopentenyl side chain of mammein, does not then decompose by loss of the isovaleryl substituent. To preserve the even-electron character of the ion formed in such a fragmentation transfer of a hydrogen radical would be necessary, and presumably this is not a favoured process. In contrast, decomposition of $^{m}/e$ 315 (formed by loss of $C_4H_9\cdot$ from the isovaleryl side chain of the parent molecule-ion) occurs by loss of a butene molecule to give the abundant even-electron fragment ion $^{m}/e$ 259, as described in the preceding paragraph.

The parent molecule-ion of mammein also fragments by elimination of a propyl radical which, from the formula of the compound (I, Fig. IV) could conceivably be lost by fission of any of the three substituents on C - 4, C - 6 and C - 8. The C - 8 acyl substituent can be discounted as the lower homologue has no analogous (M - 29) ion in its mass spectrum and α-fission of the 4-n-propyl group also seems unlikely. It is, therefore, considered that the loss of 43 mass units comes from the isopentenyl side chain. This is in agreement with the spectra of other isopentenylated compounds such as pencenin (IV, Fig. IX), where (M - 43) ions have been found to be derived exclusively by elimination of a propyl radical[102]. Shannon[101] has proposed the mechanism

e, Fig. VI for this reaction in anthraquinonoid pigments with isopentenyl chains.

The lower homologue of mammein fragments in an analogous fashion: the only significant difference in the spectra (Fig. V) is that this compound eliminates a propyl radical followed by loss of butene instead of butyl and then butene as is observed for mammein. The C - 8 substituent is therefore butyryl as opposed to isovaleryl; normal butyryl seemed the most likely type of acyl side chain as this was known to exist in other naturally occurring compounds[68]. Nuclear magnetic resonance data on the purest available sample confirmed that it was indeed the 8-n-butyryl coumarin[103].

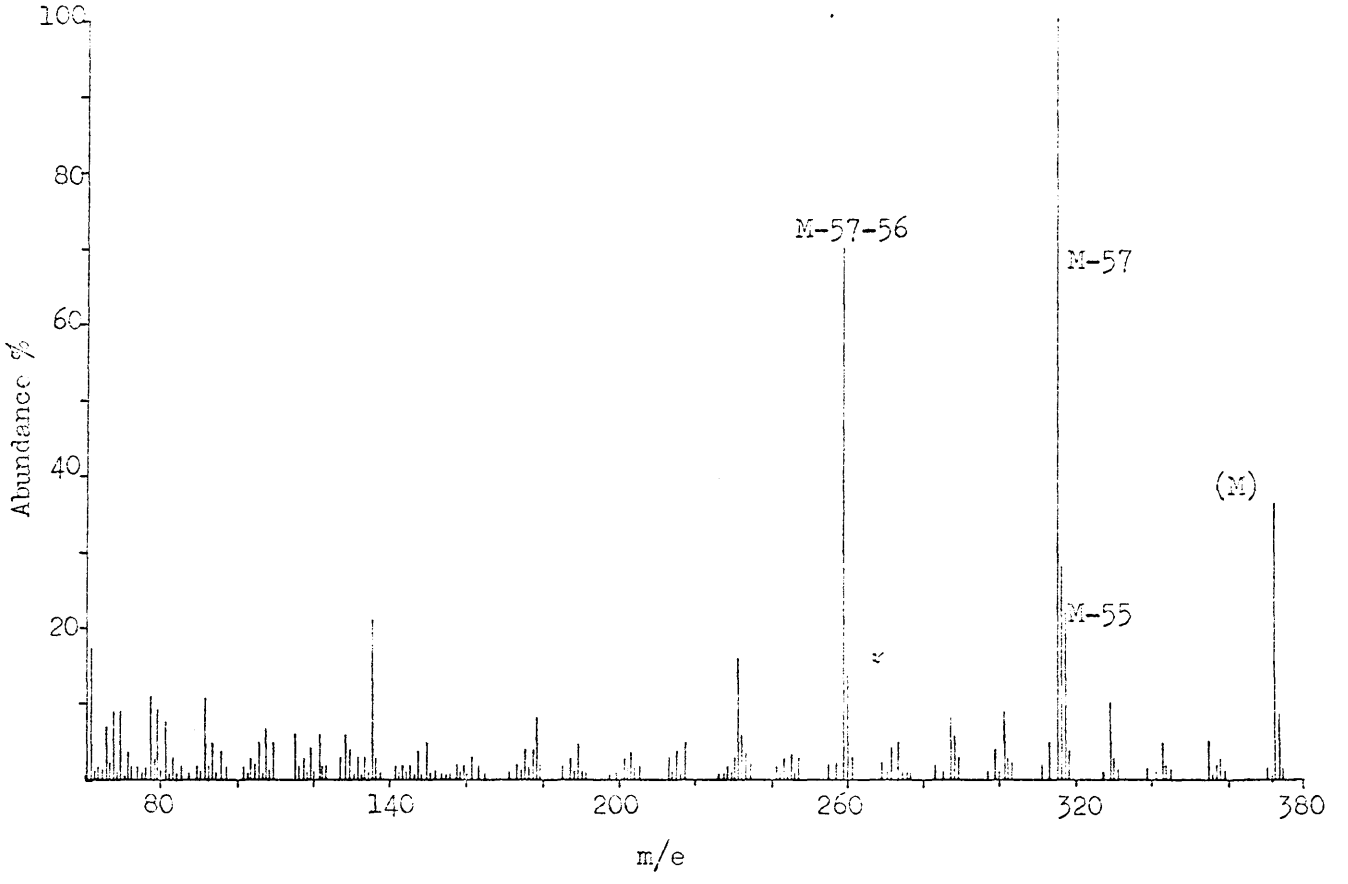
The evidence put forward by Djerassi and co-workers[71] for the structure of mammein was based mainly on the products formed by alkaline hydrolysis. Amongst these was isovaleric acid, which proved the nature of the C - 8 acyl substituent. n-butyric acid was also found, the plausible suggestion being made that it was derived from C - 4 together with the n-propyl substituent. However, the above mass spectroscopic evidence of the existence of a lower homologue and the difficulty experienced in separating it from mammein now suggested that the sample used in the previous

degradative work was, in fact, a mixture and that the n-butyric acid found was derived from the acyl group of the smaller molecule. A specimen of mammein, kindly supplied by Dr. R.A. Finnegan (University of New York, Buffalo) did in fact contain about 30% (mass spectrometry) of the lower homologue.

4-n-Propyl-5, 7-dihydroxy-6-isopentenyl-8-α-methylbutyryl coumarin

Several coumarins isolated from Mammĕia americana L. have been shown to have α-methylbutyryl side chains rather than isovaleryl like mammein itself. This ("anteiso") type of chain has been found in other compounds from the Guttiferae[104,105]. Two groups of compounds have been isolated from Mammeia americana. These are the 4-n-alkyl coumarins and the 4-phenyl coumarins. Compounds previously reported from each of these two groups[71,91,90] had been found to have the "iso" type of side chain. In the recent investigation, however, as many compounds with anteiso acyl chains have been found as those with iso.

Comparison of the mass spectrum of mammein (Fig. V) with that (Fig. VII) of its nearest relative from the anteiso series shows the impossibility of distinguishing with certainty between



Mass measurements.

Measured	Formula	Calculated
372.1936	$C_{22}H_{28}O_5$	372.1937
329.1364	$C_{19}H_{21}O_5$	329.1389
315.1227	$C_{18}H_{19}O_5$	315.1232
259.0611	$C_{14}H_{11}O_5$	259.0606
231.0642	$C_{13}H_{11}O_4$	231.0657
135.0801	$C_9H_{11}O$	135.0810

Metastables.

Observed	Transition	Calculated
266.6	372 → 315	266.7
213	315 → 259	212.9
206	259 → 231	206.1
181.8	259 → 217	181.8

Fig. VII. Mass spectral data of 4-n-propyl-5,7-dihydroxy-6-isopentenyl-8--α-methylbutyryl coumarin.

the two compound types by mass spectrometry. The "McLafferty" rearrangements[106] of the different keto side chains might be expected to provide a means of differentiation; the α-methyl butyryl side chain would eliminate ethylene by this reaction (V, Fig. IX) whereas the isovaleryl would lose propene (VI, Fig. IX). Both types of compound, however, have (M - 42) and (M - 28) peaks in their spectra. These are of very low abundance and not, therefore, useful for structure determination. Further, the (M - 28) peak probably derives partly from loss of carbon monoxide and from elimination of ethylene from the C - 4 propyl substituent. In the 4-phenyl compounds this ion is less than 0.5% of the base peak.

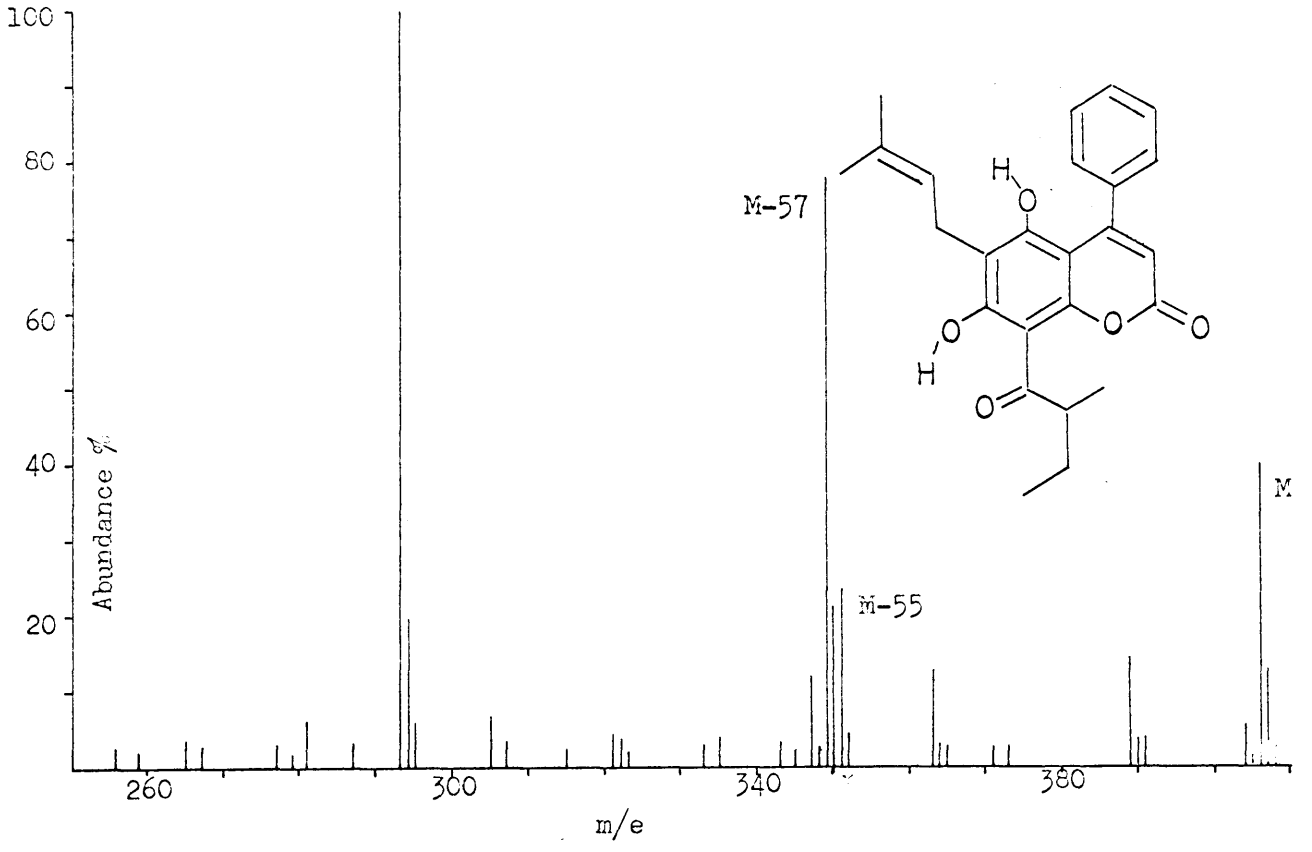
The one significant difference between the spectra of the iso- and the anteiso-acyl substituted coumarins is the abundance of the (M - 55) ion. In mammein (Fig. V) this ion is more abundant than (M - 57), whereas it is much less so in the spectrum of Fig. VII. This observation is supported by the spectra of Figs. VIII and X in which the (M - 55) ions are more abundant compared with (M - 57) in the iso- than in the anteiso-substituted compounds. Branching of the chain adjacent to the carbonyl function increases the probability of fission occurring at this

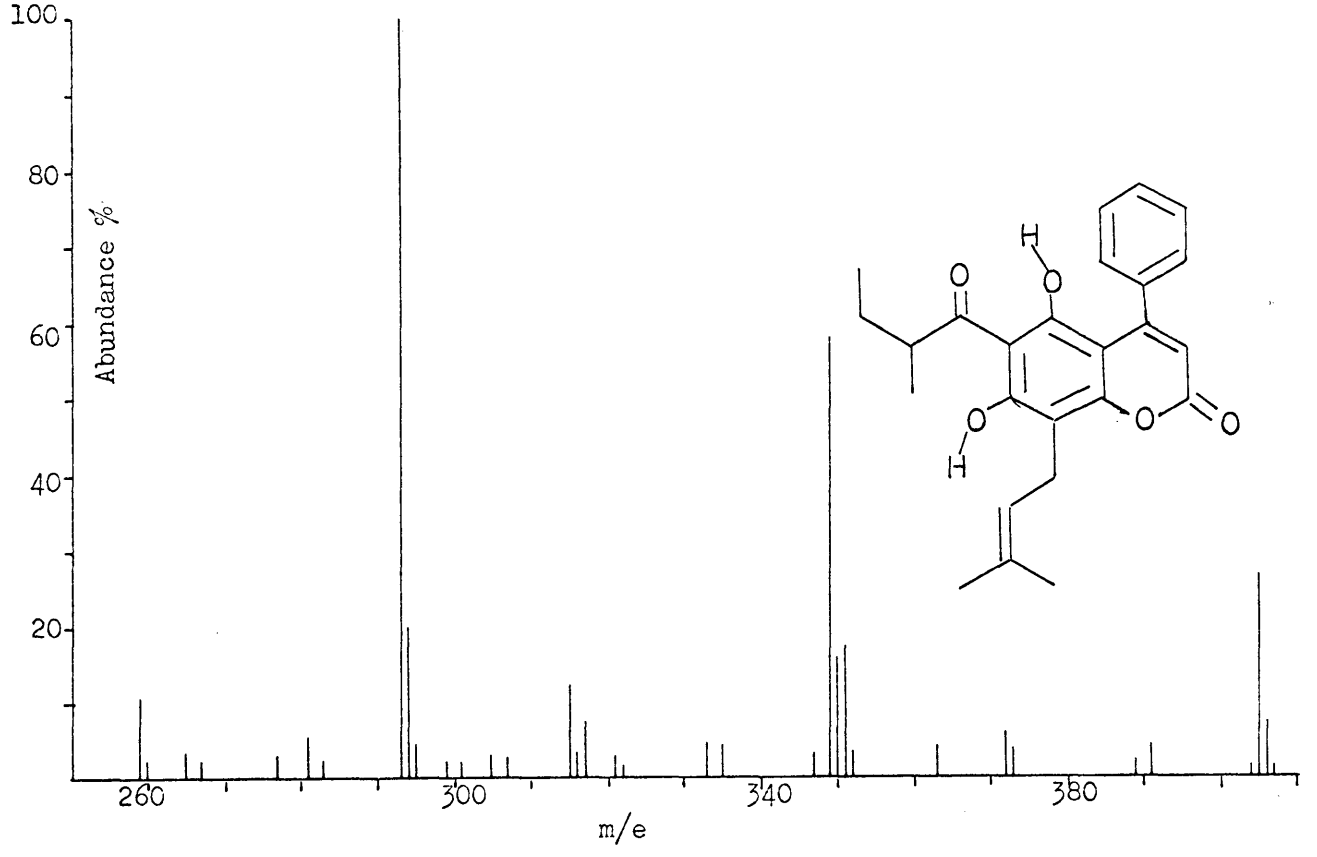
point and thus the competing reaction, β-fission of the isopentenyl substituent, is diminished to some extent.

Comparison of the spectra of coumarins with the side-chain positions reversed.

The coumarins from Mammeia americana have been found to have the acyl substituent in the 6-position and the alkyl chain in the 8-position or vice versa. A comparison of the two spectra of Fig. VIII quite clearly shows that mass spectrometry is unable to distinguish between the two isomers. However the ultraviolet and nuclear magnetic resonance spectra enabled the compounds to be assigned to one of two groups. Compounds were synthesised which had only the two hydroxyls in the 5- and 7-positions and the acyl substituent. These, by ultraviolet and nuclear magnetic resonance spectroscopy, could be correlated with one or the other group of natural compounds. By spectrophotometric control of their Gibbs' reactions[107,108], the synthetic compounds could be classed as 6- or 8- acyl and thus the orientation of the side chains of the naturally occurring compounds was established.

Lack of fission of the C - 4 substituents.


100
80
60
40
20
Abundance %
M-57
M-55
M
260
300
340
380
m/e


100
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60
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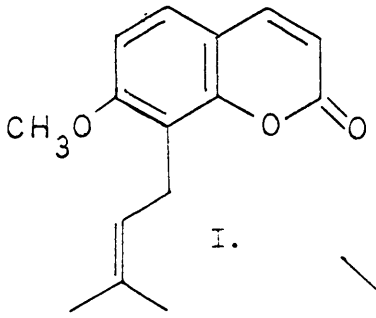
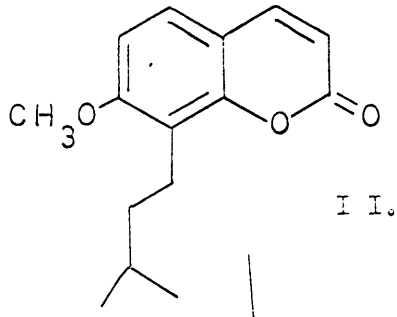
20
Abundance %
260
300
340
380
m/e

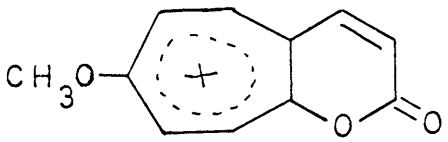
Fig. VIII.



I.



I I.



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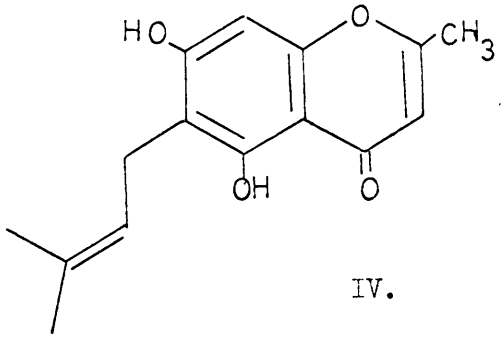
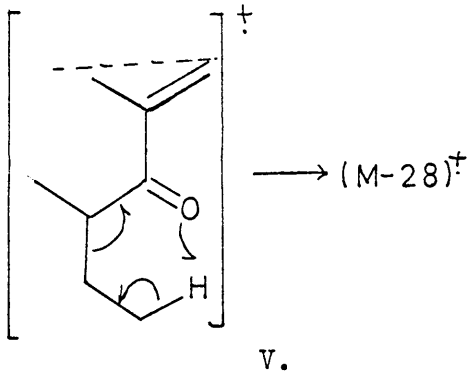
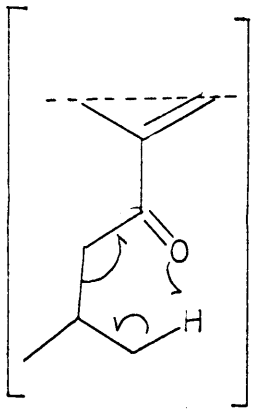


IV.



$\longrightarrow (M-28)^{+\cdot}$

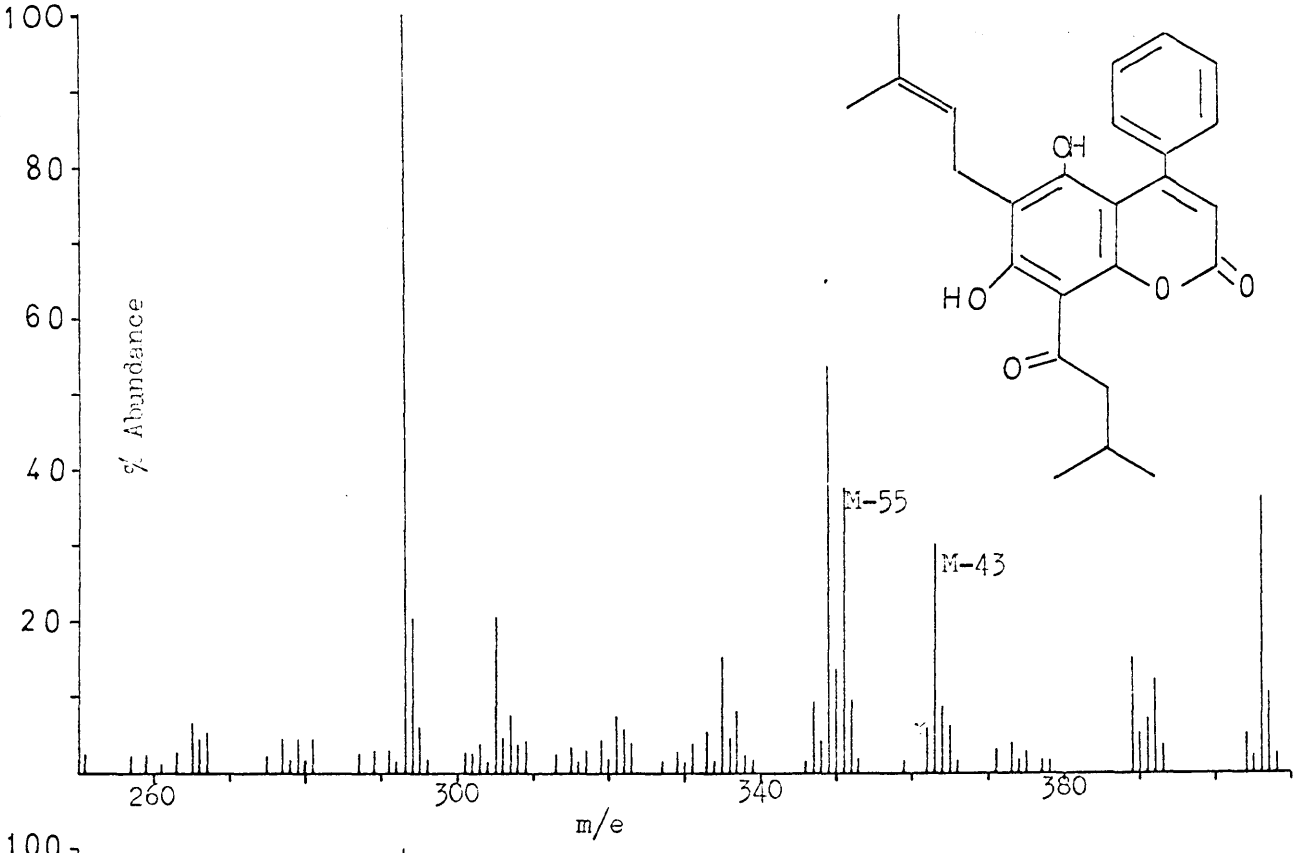
V.



$\longrightarrow (M-42)^{+\cdot}$

VI.

Fig. IX.


100
80
60
40
20
% Abundance
M-55
M-43
260
300
340
380
m/e
OH
HO
O
O
O

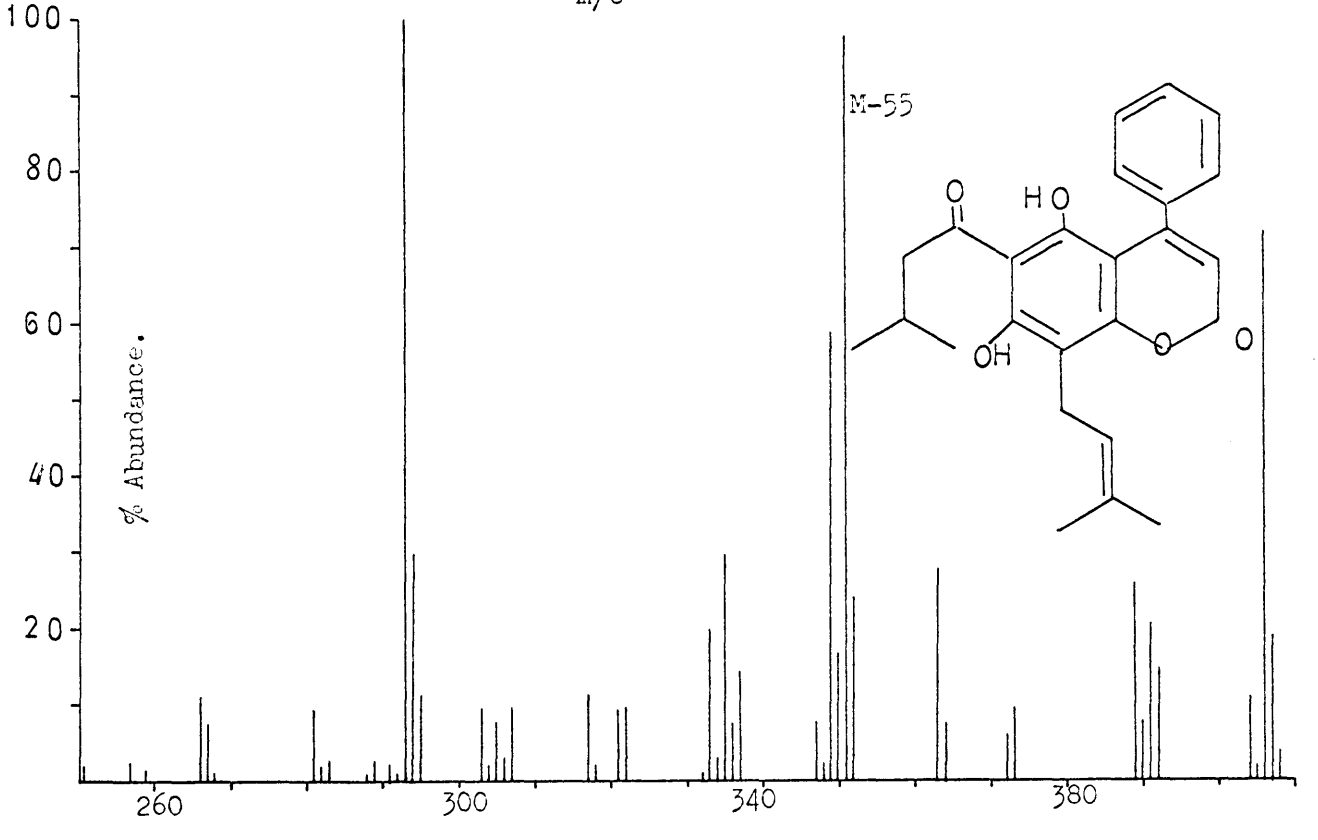

100
80
60
40
20
% Abundance.
M-55
260
300
340
380
O
HO
OH
O
O

Fig. X.

Ions formed by fission of the C - 4 alkyl or aryl substituents are of negligible abundance. In the compounds with n-propyl groups in this position the expected allylic cleavage would give (M - 29) peaks, or (M - 28) peaks by rearrangement of a hydrogen atom. However, as has been previously noted, these peaks are virtually absent in the spectra. A moderately abundant ion occurs at $^{m}/e$ 135 in the spectra of all of the n-propyl substituted coumarins (see, e.g., Figs. V and VII). Accurate mass measurement of this shows it to have the formula $C_9H_{11}O$. The C - 4 substituent must be retained to give the high hydrogen to carbon ratio in this ion. As all but one of the oxygens can be eliminated as carbon monoxide before fission of the C - 4 substituent occurs it is rather difficult to determine its nature. Accurate mass measurement of the parent molecule-ion and interpretation of the spectrum is necessary. For example compound V, Fig. IV was found to have the formula $C_{24}H_{32}O_5$. Its fragmentation pattern consisted of successive eliminations of a butyl radical and a butene molecule, i.e., it had the "usual" five-carbon acyl and hydrocarbon chains. The compound, which had two carbon atoms more than the 4-n-propyl coumarins, could not be a dimethyl ether as these do not eliminate butene (see below, "mammeisin"). Of

the twenty four carbon atoms ten could be assigned to the side chains just mentioned, nine to the coumarin nucleus and the remaining five to the C - 4 substituent. With this knowledge, interpretation of the nuclear magentic resonance spectrum was relatively simple and the compound could be assigned the structure given[103].

Compound V, Fig. IV does have in its mass spectrum an ion of low abundance at $^{m}/e$ 163, i.e., the equivalent of the $^{m}/e$ 135 species in the spectra of the 4-n-propyl coumarins. Likewise the spectra of the 4-phenyl compounds have very small peaks at $^{m}/e$ 169 in their spectra.

Mammeisin (III, Fig. IV).

This compound had previously been isolated and its structure elucidated by Djerassi, Finnegan and Morris[90].

In connexion with the identification of a compound recently isolated from Mammeia americana L. (finally shown to be mammeisin) the diacetate was made. Part of the mass spectrum of this is shown in Fig. XI(a). The additional side chains make available more decomposition pathways to the parent molecule-ion and the spectrum is therefore much more complicated than that of the parent

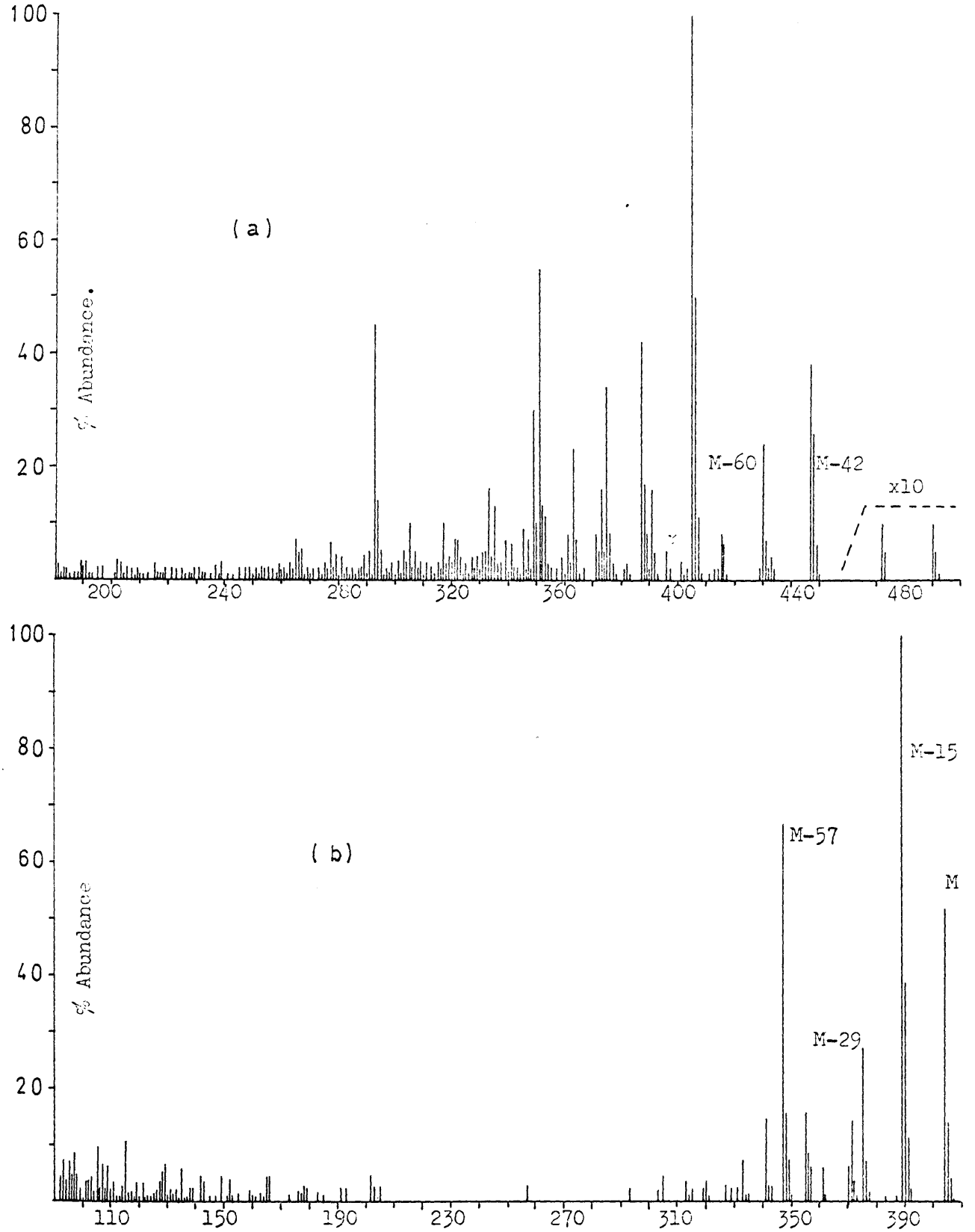
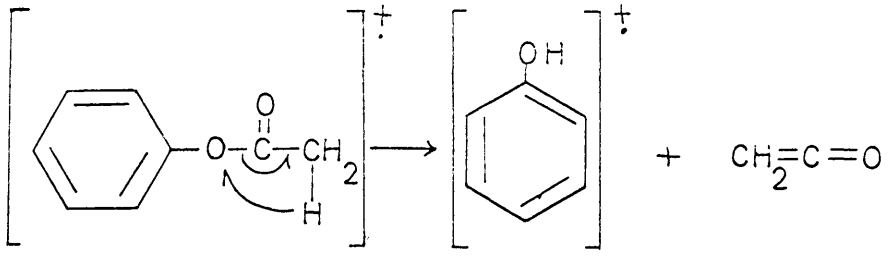


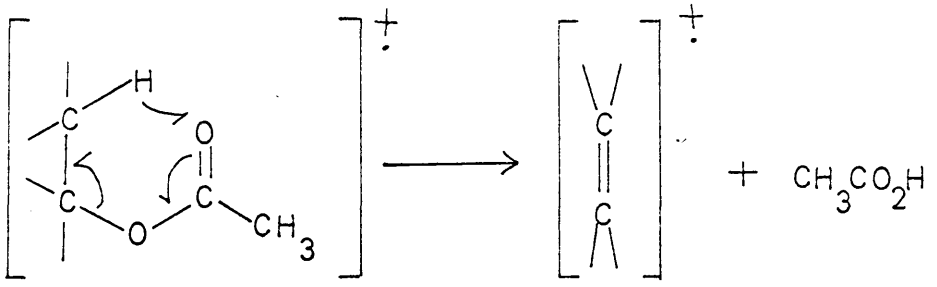
Fig. XI. Mass spectra of (a) mammeisin diacetate (b) mammeigin.

coumarin (Fig. X). Formation of acetates is thus of limited use for structure determination by mass spectrometry but it does show the number of esterifiable hydroxyl functions in a molecule. An interesting point about the spectrum of Fig. XI(a) is that (M - 60) is more abundant than (M - 42). Loss of ketene (42 mass units) is the reaction usually associated with the acetates of phenols[109] (I, Fig. XII) whereas elimination of acetic acid occurs if a γ-hydrogen is available (II, Fig. XII). Biemann has shown[109] by deuterium labelling experiments that acetic acid can also be eliminated by what he calls an "E_1" elimination (III, Fig. XII). It would appear that the acetates of the dihydroxy coumarins also undergo this type of fragmentation, one of the hydrogens of the side chains being removed along with the acetoxy group. The existence of the abundant ion (M - 60) along with (M - 42) makes it rather difficult to decide whether the acetyl group is on a side chain or on one of the phenolic oxygens of naturally occurring acetates (see below, "Insecticide").

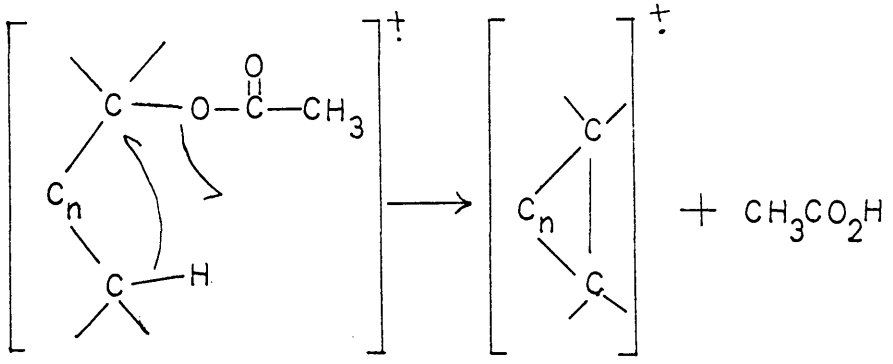
In contrast to the spectrum of the diacetate, that of the dimethyl ether of mammeisin is remarkably simple. The only ions of appreciable abundance are M, (M - 15) and (M - 57), the latter being the base peak of the spectrum. This indicates that the



I.



II.



III.

Fig. XII.

six-membered transition state of reaction d, Fig. VI is necessary for fission of the isopentenyl side chain from the (M - 57) ion.

Mammeigin[91] (VI, Fig. IV).

Accurate mass measurement of the parent molecule-ion of a compound isolated from Mammeia americana proved that it had the formula $C_{25}H_{24}O_5$, i.e., it had one more double bond or ring than the 4-phenyl compounds with "open" C_5 side chains. The base peak of the spectrum (Fig. XIb) was (M - 15). The formula and the abundant (M - 15) ion indicated that the compound had a dimethyl chromene ring[94,110]. As in the spectra of the other compounds there was an abundant (M - 57) ion. The "usual" C_5 acyl chain was therefore present but this ion did not further decompose by the elimination of a molecule of butene; the isopentenyl chain was, therefore, absent and was probably involved in the formation of the suspected chromene ring. The compound was eventually shown to be mammeigin, which had previously been isolated and its structure elucidated.

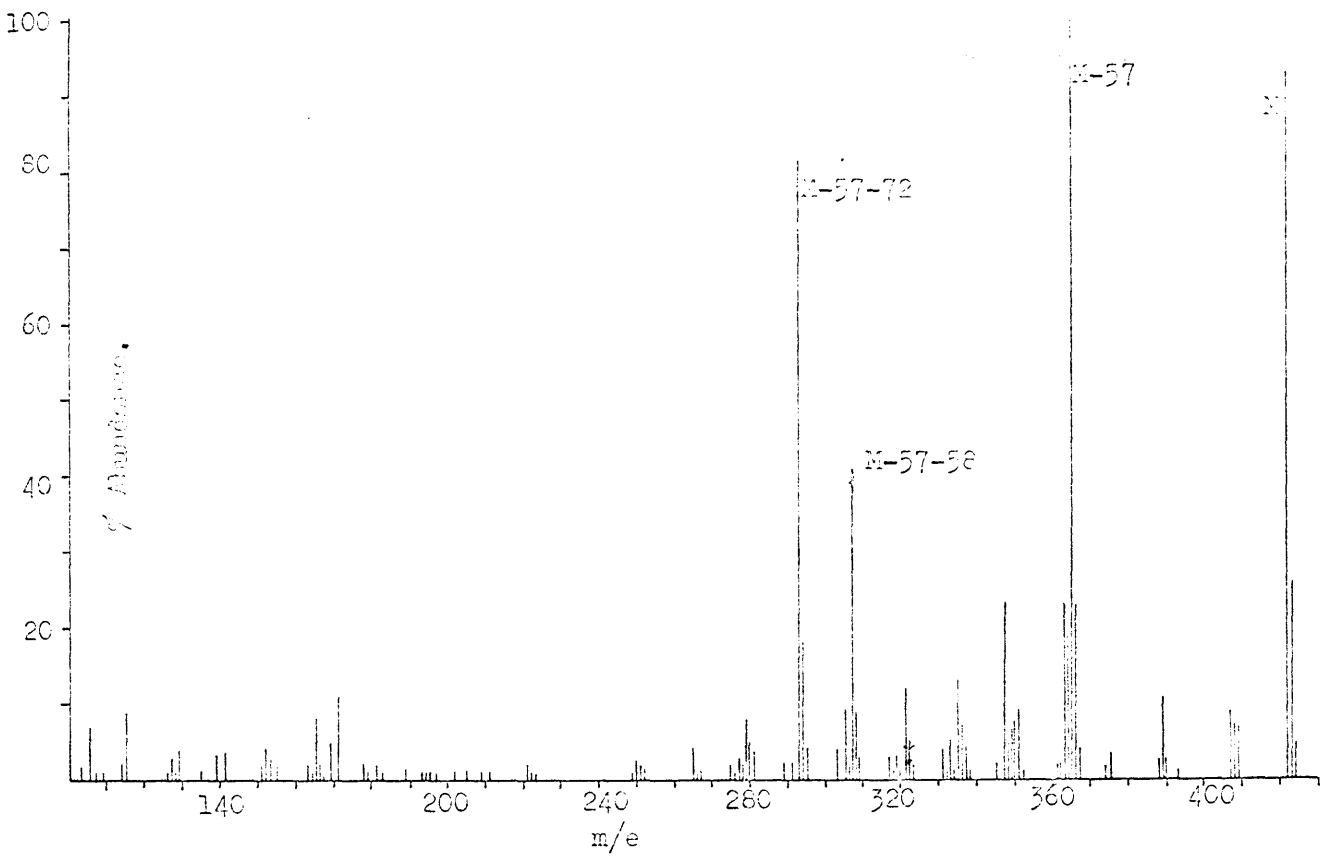
One inexplicable point about the mass spectrum of mammeigin (Fig. XI(b)) is the abundant (M - 29) ion which, by mass measurement, was shown to be due to expulsion of an ethyl radical. This

fragmentation at first suggested that the compound under investigation had the α-methylbutyryl rather than the isovaleryl chain. As none of the other compounds, even those with ethyl groups, have been found to give an abundant (M - 29) peak it is difficult to conceive of its formation from a structure such as that proposed[91] for mammeigin.

Coumarins with Dihydrofuranoid Rings.

The mass spectrum of a compound isolated from Mammeia americana is reproduced in Fig. XIII, which also includes the results of accurate mass measurements of the principal ions and the observed metastable peaks for several fragmentations.

The formula found for the parent molecule-ion by accurate mass measurement, $C_{25}H_{26}O_6$, corresponds to that of the 4-phenyl coumarins discussed previously but has one more oxygen. One of the principal decomposition pathways consists of the initial loss of a butyl radical to give $^m/_e$ 365. The "normal" five-carbon acyl chain is thus present. Further fragmentation of $^m/_e$ 365 gives $^m/_e$ 293 by the expulsion of a species having the formula C_4H_8O. This is analogous to the elimination of butene from the (M - 57) ion of compounds having an isopentenyl side chain. The



Mass measurements.

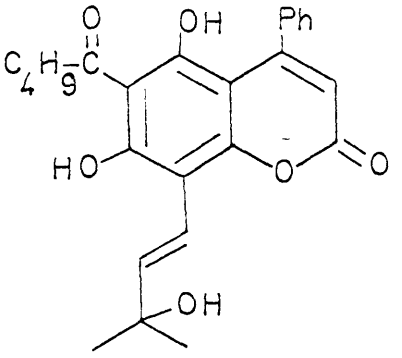
Measured	Formula	Calculated
422.1739	$C_{25}H_{26}O_6$	422.1729
393.1336	$C_{23}H_{21}O_6$	393.1338
389.1380	$C_{24}H_{21}O_5$	389.1389
365.1029	$C_{21}H_{17}O_6$	365.1025
363.1228	$C_{22}H_{19}O_5$	363.1232
347.0919	$C_{21}H_{15}O_5$	347.0919
307.0614	$C_{18}H_{11}O_5$	307.0606
293.0448	$C_{17}H_9O_5$	293.0450

Metastables.

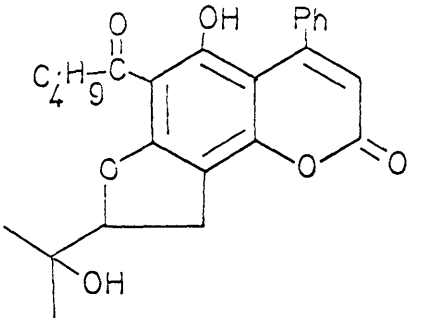
Observed	Transition	Calculated
372	407 → 389	371.8
330	365 → 347	329.9
315.6	422 → 365	315.7
314	422 → 364	314.0
312	422 → 363	312.2
235	365 → 293	235.2
258.5	365 → 307	258.2
270	349 → 307	270.0

Fig. XIII. Mass spectral data of compound II, Fig. XIV

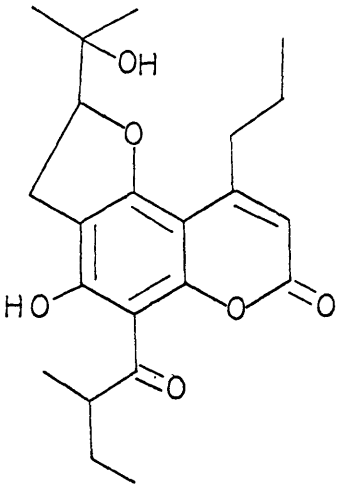
"extra" oxygen, therefore, is present in this part of the molecule; losses of water from the (M - 57) and (M - 15) ions confirm that it occurs as a hydroxyl function. Another decomposition of the parent molecule-ion consists of expulsion of a fragment having the formula C_3H_7O, suggesting that the hydroxyl group is at the branched position of the isopentenyl chain. Compounds with hydroxyisopropyl groups, e.g. endesmol (V, Fig. XIV) have been shown to lose acetone in the mass spectrometer[111]. In the compound under discussion elimination of acetone from the parent molecule-ion and from (M - 57) occurs, thus confirming the presence of the hydroxyisopropyl group. The formula of the compound and the expulsion of an olefinic (C_4H_8O) fragment from (M - 57) indicate that the isoprenoid chain is either part of a ring or has a double bond. A double bond would have to be in the position shown in I, Fig. XIV. Fission of the chain would then require rupture of a double bond. Although this does occur in these compounds, for example to give the (M - 43) ion of mammein, the resultant ion is not very abundant and in any case is formed by a rearrangement which would, in the present case, result in a loss of 73 mass units from (M - 57) and not 72 as is observed. Further, elimination of the hydroxyisopropyl radical from this structure (I, Fig. XIV)



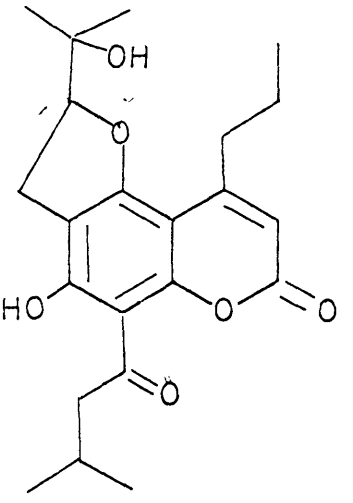
I.



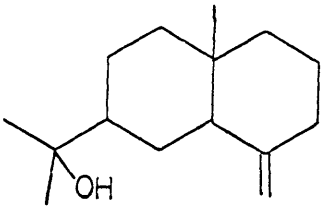
II.



III.



IV.



V.

Fig. XIV.

would require the cleavage of a vinylic bond. A more probable structure from consideration of all of the mass spectrometric data is II, Fig. XIV or the isomer with the acyl group in the 8-position. Loss of a hydroxyisopropyl group from this corresponds to the normal β-fission of ethers. A small loss of an ethyl radical from the parent molecule ion suggests that the acyl side chain is α-methylbutyryl rather than isovaleryl but this has to be treated with some caution (see, e.g., mammeigin).

Structure II, Fig. XIV was suggested for this compound at a time when no other data was available. Since then the nuclear magnetic resonance spectrum has shown that the substance whose mass spectrum is discussed above is in fact a mixture of the two isomers having α-methylbutyryl and isovaleryl side chains[103].

A similar interpretation of its mass spectrum together with other spectroscopic data has shown the presence in the Mammeia extracts of a mixture of the two dihydrofuranoid coumarins III and IV of Fig. XIV.

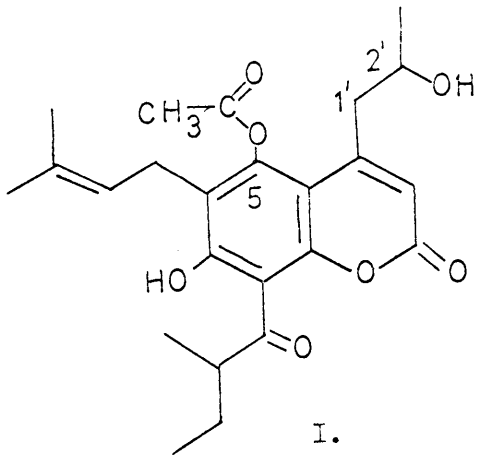
Insecticidal Material.

This compound, which has been discovered to be a very powerful insecticide, is the only one of the Mammeia compounds so far

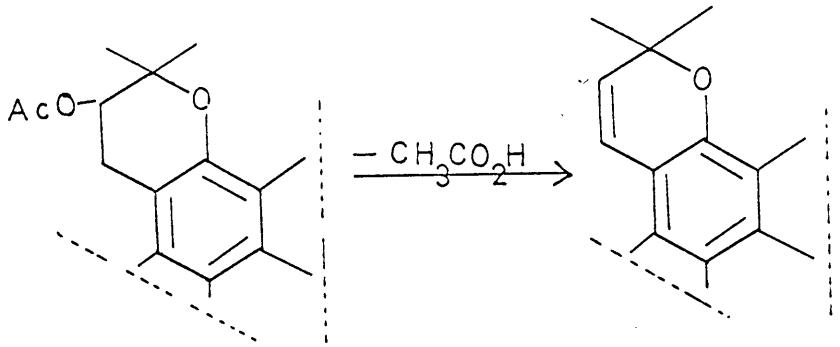
encountered which has been found to be an acetate.

Other spectroscopic techniques have indicated that the insecticidal compound is a 4-alkyl coumarin with an α-methylbutyryl side chain in the 8- position[103]. The tentative structure I, Fig. XV is proposed from consideration of the mass spectra of the parent compound, its hydrolysis product and its diacetate. The latter is useful only in that it shows the presence of two free hydroxyl groups in the naturally occurring compound (see, e.g., mammeisin diacetate).

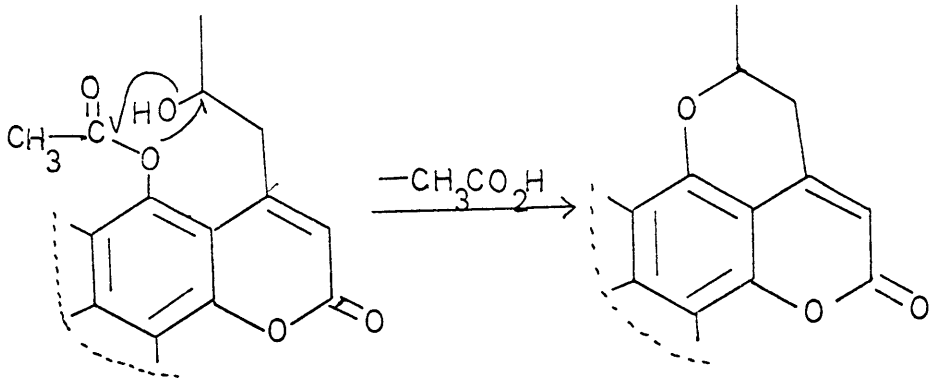
The formula of the insecticidal compound, $C_{24}H_{30}O_7$, corresponds to that of the 4-n-propyl coumarins I and II of Fig. IV with one additional acetoxy group. The mass spectrum (Fig. XVI(a)) has (M - 60) and (M - 42) ions which, as shown by mass measurement and assignment of metastable peaks (Fig. XVII), are due to expulsion of acetic acid and ketene respectively. Peaks at $^m/_e$ 60 and 61 confirm that the compound is an acetate. As noted above elimination of acetic acid, as well as ketene, is observed in the spectrum of mammeisin diacetate. It is, therefore, impossible to decide whether the acetoxy group is linked to a side chain or to the benzenoid ring by the mere presence of (M - 60) and (M - 42) peaks. One interesting point is that the (M - 60) ion shows an abundant



I.



II.



III.

M (M−60)

Fig. XV.

loss of a methyl radical. (M - 15) peaks are not abundant in the spectra of the other coumarins studied, with the exception of mammeigin which has a dimethylchromene ring. This suggests that such a structure is formed by elimination of acetic acid from the parent molecule-ion of the insecticidal material as, e.g., in II, Fig. XV. However the (M - 60) ion decomposes also in the normal fashion of the "open chain" coumarins, by expulsion of a butyl radical from the C - 8 acyl group followed by the rearrangement elimination of butene. Loss of butene does not occur from mammeigin. Compounds with isopentenyl side chains, e.g. mammein, fragment by β-fission of this group. The (M - 60) ion of the insecticidal compound also decomposes by elimination of a butenyl radical to give $^{m}/e$ 315, thus confirming that it has the "open" hydrocarbon chain. This still does not decide whether or not the ester grouping is attached to this part of the parent molecule-ion. However, this can be decided by examination of the spectrum (Fig. XVI(b)) of the hydrolysis product which has a molecular weight of 388, thus confirming that the naturally occurring compound is a mono-acetate. The hydrolysis product fragments in the manner of mammein, i.e., by successive losses of a butyl radical and a butene molecule; it therefore has the "usual" acyl and

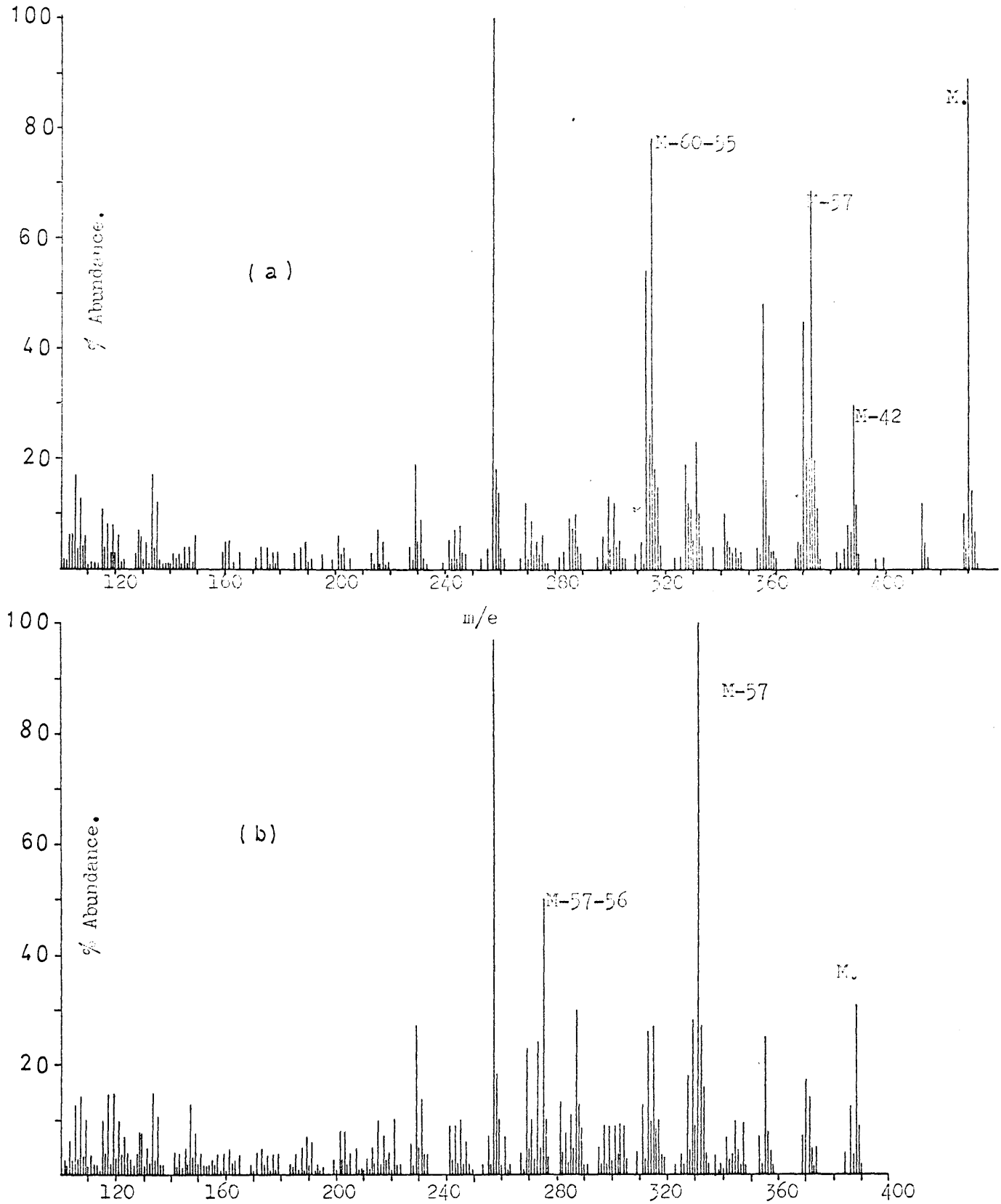


Fig. XVI. mass spectra of (a) insecticidal coumarin (b) its hydrolysis product.

a). Insecticide.

Mass measurements.

Measured	Formula	Calculated
430.2002	$C_{24}H_{30}O_7$	430.1992
388.1888	$C_{22}H_{28}O_6$	388.1886
370.1778	$C_{22}H_{26}O_5$	370.1780
373.1287	$C_{20}H_{21}O_7$	373.1289
313.1076	$C_{18}H_{17}O_5$	313.1078

Metastables.

Observed	Transition	Calculated
350.0	430 → 388	350.1
340.5	370 → 355	340.6
323.5	430 → 373	323.6
319.0	373 → 345	319.1
294.0	373 → 331	293.7
280.0	315 → 297	280.0
268.0	370 → 315	268.2
264.6	370 → 313	264.8
211.0	313 → 257	211.0
204.0	257 → 229	204.1

b). Hydrolysis product.

Mass measurements.

Measured	Formula	Calculated
388.1860	$C_{22}H_{28}O_6$	388.1886
355.1542	$C_{21}H_{23}O_5$	355.1545
3311179	$C_{18}H_{19}O_6$	331.1182
275.0560	$C_{14}H_{11}O_6$	275.0556

Metastables.

Observed	Transition	Calculated
296.0	331 → 313	296.0
282.3	388 → 331	282.4
268.1	370 → 315	268.2
240.0	275 → 257	240.2
228.5	331 → 275	228.5
204.0	257 → 229	204.1

Fig. XVII.

isopentenyl side chains. An abundant loss of water from the resulting ion, $^{m}/e$ 275, of these two decompositions shows that a hydroxyl group is present on the C - 4 propyl chain. Ions at $^{m}/e$ 45 in the spectra of the natural compound and of its hydrolysis product indicate that the hydroxyl group is at the 2^{1}-position (I, Fig. XV). In the insecticidal compound the alcohol could of course be formed by elimination of ketene. The abundance of the (M - 42) ion (Fig. XVI(a)) suggests, however, that the acetyl group is attached to a phenolic rather than the side-chain oxygen. If the ester group is on C - 5 of the coumarin nucleus then the abundant (M - 60) ion can readily be explained by expulsion of acetic acid in the manner shown in III, Fig. XV. The abundant loss of a methyl radical from the chromane ring of the resultant (M - 60) ion is in agreement with the known behaviour of such compounds[94,110].

Although the structure of the insecticidal coumarin has not been finally decided, the one given in I, Fig. XV seems to be the most likely from other spectroscopic evidence.

Experimental

The spectra of most of the compounds discussed in this chapter were obtained at a time when the mass spectrometer was not equipped

with a vacuum lock and an adjustable probe. Introducing the samples by means of the heated inlet system resulted in considerable pyrolysis. The probe system available[112] proved to be unsuitable as the compounds were too volatile at the source equilibration temperature, thus making it difficult to obtain reproducible spectra. The rapid depletion of the sample also meant that it was often impossible to perform more than a very few mass measurements before the tedious reintroduction procedure had to be repeated. Excellent results could be obtained using the other available direct evaporation system[112] but this had the drawback that it was difficult to remove excess sample after completion of the measurements. To circumvent these difficulties the direct evaporation system illustrated in Fig. XVIII was devised. The glass bulbs served to hold the "probe" in position in the ion source and, if the sample was introduced in solution and the solvent removed by a side-pump, served to provide a large surface area of sample which favoured a steady rate of evaporation. By using these glass bulbs the sample was placed further from the hot source block than could be obtained by the commercially-available probe; thus for the coumarins discussed previously, a suitable vapour pressure could be obtained at the source equilibration temperature

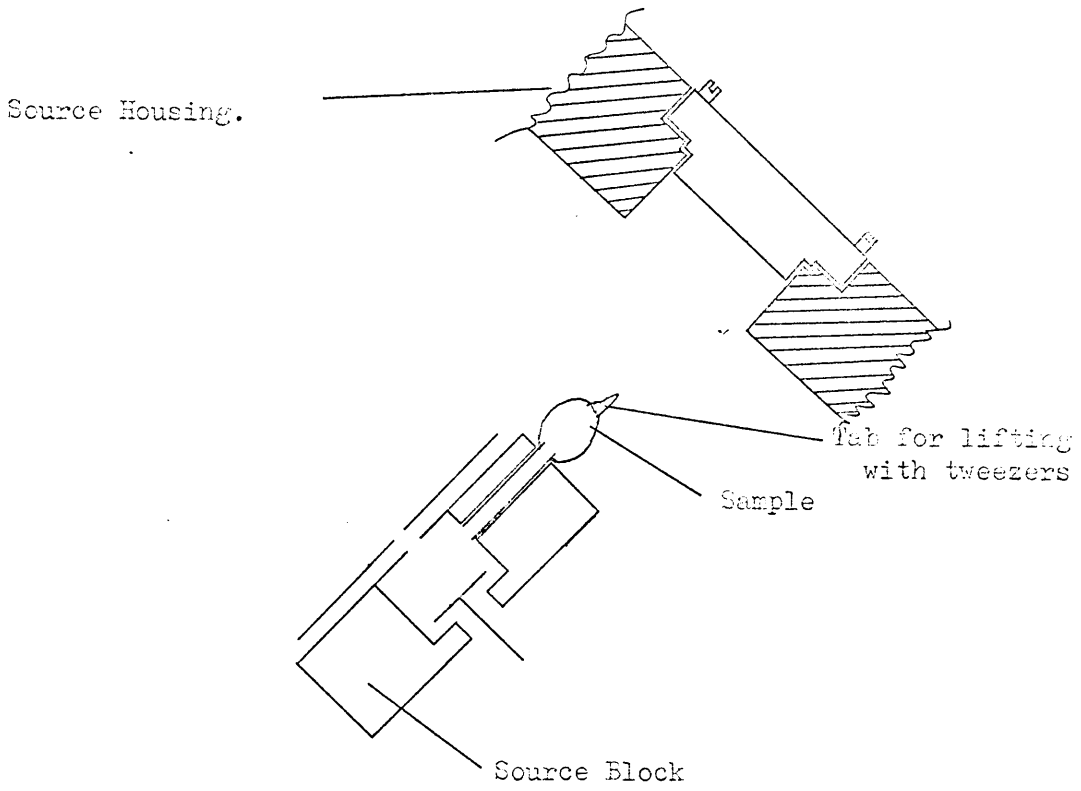

Source Housing.
Tab for lifting with tweezers
Sample
Source Block

Fig. XVIII.

or slightly above; when it was possible to control the rate of evaporation by the source-block heaters. Cleaning of the bulbs presented no problem as a new one was used for each compound.

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