PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

New Methods of Identifying Organic Compounds

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New methods of identifying organic compounds

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A 'reversed' geometry mass spectrometer, in which the ion beam passes through the magnetic sector before the electric sector, offers several advantages for the study of large organic molecules. The method used is to select individual ionic species formed in the ion source in turn by using the magnet and to study the fragmentation of these species in the field-free region in front of the electric sector. Either unimolecular or collision-induced fragmentations can be investigated, the masses of the daughter species being determined by scanning the electric sector. By selecting a variety of individual ions, a comprehensive fragmentation 'map' of the molecular species can be constructed. Because it is a voltage that is scanned, the instrument can readily be computer controlled which gives improved reproducibility of scanning, together with other advantages.

The several pathways that often link a particular fragment ion with the molecular ion provide complementary information concerning ion structure. The fragmentation pattern of any ion is often sufficiently characteristic of the ion structure to allow direct identification of structural features present to be made by comparing the pattern from the relevant ion with that of an ion formed from a known reference compound.

By using these methods the molecular structure of large organic molecules can often be deduced. Large isomeric molecules such as steroids, differing only in the structure of a side chain, can be distinguished by selecting only ions containing the side chain for study. The new methods also offer advantages for the detection and identification of individual components in mixtures.

1. Introduction

The advantages of using a mass spectrometer for the analysis of chemical compounds were pointed out by Thomson (1913) but it was not until some 30 years later that a commercial instrument, based on a design used by Dempster (1918), was built and used for the study of petroleum hydrocarbon mixtures (Hoover & Washburn 1940, 1941). The quantitative estimation of the amount of each component in such a mixture depended upon the uniqueness of its 'cracking pattern' when its vapour was bombarded with electrons and the fact that the ion abundances from each component were proportional to their individual partial pressures in the mixture. The use of mass spectrometers in organic chemistry was soon extended to the qualitative identification of compounds from their mass spectra. The method developed depended upon a series of semi-empirical rules that predicted the major fragmentation pathways of molecules containing particular functional groupings (Beynon 1960; McLafferty 1963; Budziciewicz *et al.* 1967). The use of other designs of instrument giving higher mass resolution (Beynon 1959) added to the power of the method by enabling the formulae of molecular and fragment ions to be deduced from the sum of the 'packing fractions' of their constituent atoms (Aston 1927). Most of the published work dealing with the study of organic compounds by mass spectrometry over the past 30 years has been concerned with improvements in technique

and in extending the range of compound types that can be studied. The development of computers led to improvements in the speed and efficiency with which samples could be examined. The combination of gas chromatography with mass spectrometry meant that the components of complex mixtures could be identified and new types of spectrometer, notably the 'quadrupole' (Paul & Steinwedel 1953) were developed for the separation of the ions making up the mass spectrum. However, comparatively little effort was devoted to studying the detailed pathways by which the various ions making up the mass spectrum were formed. It was, of course, known that these arose through a series of competitive and consecutive fragmentations of molecular ions (Rosenstock *et al.* 1952) but lack of detailed knowledge about the resultant 'fragmentation map' was a major weakness in exploiting fully the potentialities of mass spectrometry for the qualitative analysis of organic compounds. The ways in which this difficulty has now largely been overcome are discussed in §§4 and 5.

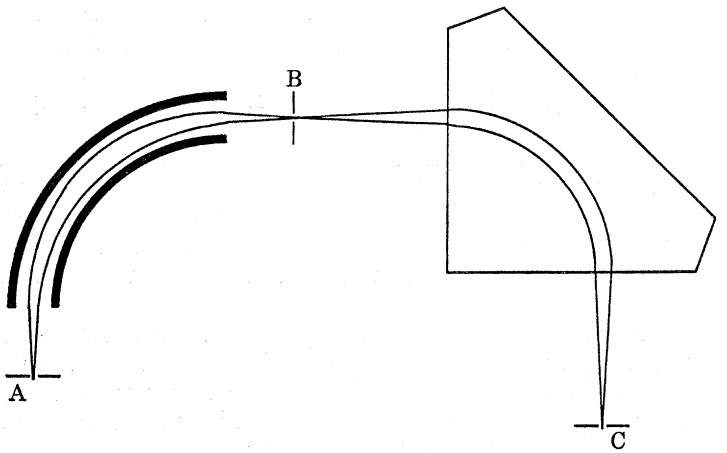


FIGURE 1. If a beam of monoenergetic ions all of the same mass issues from slit A and passes through an electric sector field it is brought to a 'directional' focus at slit B. Diverging from this slit it is refocused at slit C by a magnetic sector field.

2. CONVENTIONAL INSTRUMENTS AND EXPERIMENTAL METHODS

The mode of operation of a typical high performance mass spectrometer of a type typically employed for the identification of organic compounds is illustrated in figures 1–4.

Figure 1 shows a double-focusing mass spectrometer based on the design of Johnson & Nier (1953). A beam of monoenergetic, singly charged ions diverging from slit A is brought to a focus at an intermediate slit B by the electric sector field. The beam diverging from this slit is focused on slit C by the magnetic sector field. Both the electric and magnetic sector fields are said to have independent directional focusing actions. Figure 2 shows the action of the electric sector field in dispersing a collimated ion beam according to the translational energies of the ions in the beam.

Figure 3 shows the action of the magnetic sector field in dispersing such an ion beam according to the range of momenta of the (singly charged) ions contained in it. As was shown by Herzog (1934) and Mattauch & Herzog (1934) the *combination* of an electric sector field and a magnetic sector field can lead to a so-called double-focusing action by which a beam of ions issuing from slit A with a range of energies and of directions can be brought to a focus at slit

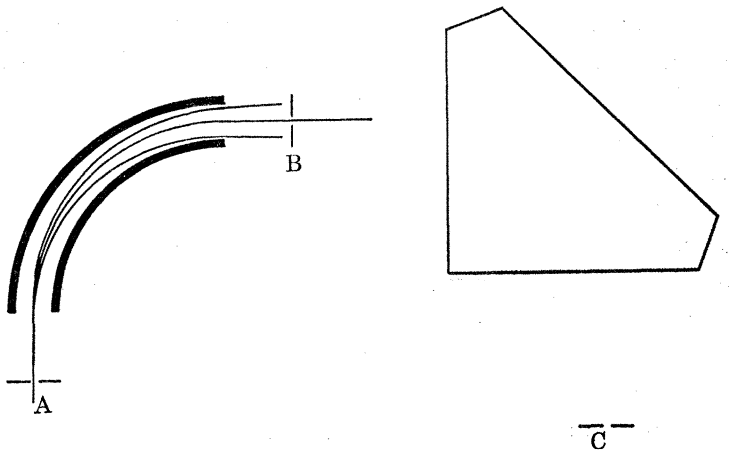


FIGURE 2. If a collimated beam of ions issues from slit A, it is dispersed by the electric sector field according to the range of translational energies of the ions contained in it.

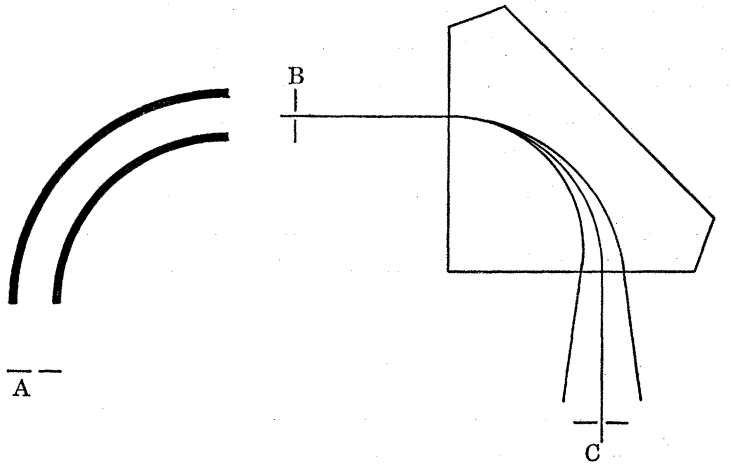


FIGURE 3. If a collimated beam of ions issues from slit B, it is dispersed by the magnetic sector field according to the range of momenta of the ions contained in it.

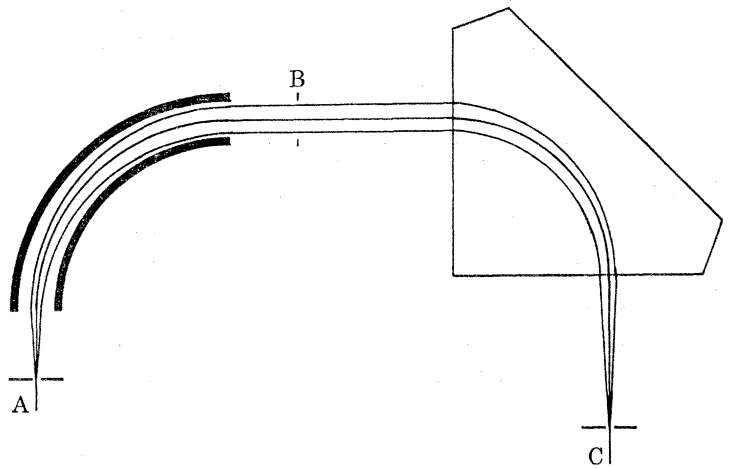


FIGURE 4. If a beam of ions all of the same mass but containing a range of translational energies diverges from slit A and passes through an electric sector field and a magnetic sector field arranged as shown, it will be brought back to a focus at slit C. This focusing of the beam despite its range of energies and directions is called double focusing.

C as shown in figure 4. By narrowing slit B, placing a detector behind it and varying the voltage across the electric sector plates, a spectrum of the translational (kinetic) energies contained in the ion beam is produced (Beynon *et al.* 1969). This ion kinetic energy spectrum gives information about any ion fragmentations that occur in the field-free region between slit A and the electric sector, as when an ion fragments it loses kinetic energy in proportion to the mass lost. Thus, the position of a peak in the ion kinetic energy spectrum can be used to connect a parent ion of mass m_1 with its daughter ion of mass m_2, and thus to gain information about one part of the fragmentation 'map'. Only the ratio of m_1 and m_2 is obtained in this way and to find m_1 and m_2 uniquely a second measurement must be made by moving the detector located at slit B aside, thus allowing the daughter ion to traverse the magnetic sector so that its momentum, and thus its mass, may be found. Metastable ions (Hipple & Condon 1945) that decompose spontaneously in the appropriate field-free region may be studied in this fashion. Alternatively, more intense signals from a greater variety of fragmentation processes can be studied by introducing a collision gas at low pressure (*ca.* 10^{-3} Pa) into the field-free region (Jennings 1968; Haddon & McLafferty 1968). This has the effect of producing electronic excitation in the ions thus inducing fragmentation in a variety of ways. The process has been called collisional activation by McLafferty. Although the use of a double-focusing mass spectrometer in this way enables the complete fragmentation map to be constructed, it has the serious disadvantages that overlapping of peaks in the ion kinetic energy spectrum is common and that determination of the exact mass of m_2 in a scan of the magnetic field is difficult. An alternative method, involving scanning of the ion accelerating voltage (Barber & Elliott 1964; Futrell 1965; Jennings 1965), also has serious disadvantages (Cooks *et al.* 1973).

3. Instruments of reversed geometry

The double-focusing action of a combination of electric and magnetic fields can be achieved irrespective of the order in which the fields are traversed and the disadvantages discussed in §2 can be overcome simply by reversing the geometrical arrangement. The instrument illustrated in figure 5 is based on a design of Hintenberger & König (1957), and has recently been developed as a commercial instrument (Morgan *et al.* 1978). Some of its capabilities were described by Craig *et al.* in the preceding lecture at this symposium. The production of a complete fragmentation map is now performed in the following fashion. An accelerated ion m_1^+ is selected on the basis of its momentum (and thus of its mass) by the magnetic sector field and transmitted through the intermediate slit E. Collision gas is introduced into the field-free region between slit E and the electric sector (either into the entire region or, preferably, into a special electrically insulated collision-gas cell) (Morgan *et al.* 1978) and thus causes collision-induced fragmentations. The fragmentation products are analysed on the basis of their translational energies by the electric sector field. A single scan of the electric sector voltage gives information concerning all the products of fragmentation of the chosen m_1^+ ion. The most important ion of the mass spectrum, the molecular ion, is selected first and all of the first generation daughters determined. These can then be selected in turn for study of their fragmentation reactions. What one is trying to do is to 'identify' each of the ions making up the fragmentation map and an analogy can be drawn showing that this method, in which mass separation precedes ion kinetic energy analysis, is almost the same as the method used in traditional analytical chemistry to identify each of the components of a mixture. The mixture of ions that

has been prepared in the ion source goes through a 'purification' stage in which a single ion species is selected for study by the magnetic sector field. The chosen ion is made to undergo a number of test reactions by collisional activation and the products are individually 'analysed' by the electric sector field. In contrast, the alternative method, corresponding to the conventional instrument geometry, of causing all of the components in the mixture to undergo their reactions together before attempting any operation or analysis, is not one that would commend itself to an analyst even though, in principle, the same total amount of information should be available. There is another important incidental experimental advantage that results from using the 'reversed' geometrical configuration. The voltage across the electric sector plates is directly proportional to the mass of m_2^+, the transmitted daughter ion. Thus, knowing the electric sector voltage E corresponding to transmission of the parent ion m_1^+, it is possible to scan this voltage, under computer control (Bolton *et al.* 1978) in steps of magnitude E/m_1 and to be sure that the peak current corresponding to transmission of every possible daughter ion will be recorded (Bozorgzadeh *et al.* 1979); no time need be wasted scanning the region between peaks. This makes it possible either to perform rapid scans in times of just a few seconds or, alternatively, to obtain maximum sensitivity for small samples by signal averaging procedures.

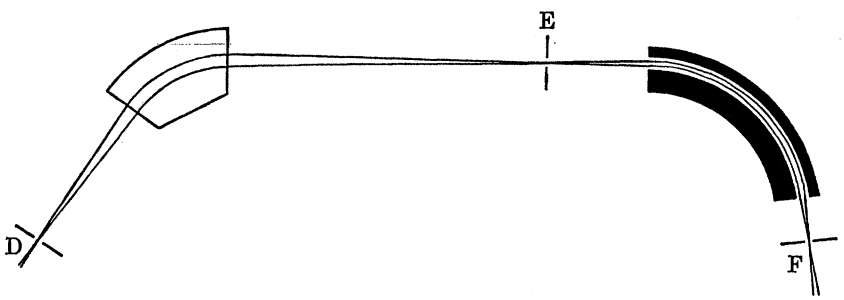


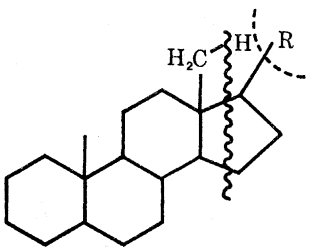
FIGURE 5. The double focusing arrangement of 'reversed' geometry, due to Hintenberger & König, in which the ion beam traverses the magnetic sector field before the electric sector field.

4. EXAMPLES OF ANALYTICAL APPLICATIONS

Even before the advent of the combination of ion kinetic energy analysis and collisional activation, it had been shown that it was possible, without prior separation, to identify an unknown component in a mixture by using metastable ions to link together pairs of parent and daughter ions in the mixture mass spectrum (Beynon 1968). The extra advantages of the new methods for mixture analysis were immediately apparent and were stressed several years ago (Cooks *et al.* 1973). It is obvious that in some cases ions of the same mass will arise from more than one component and that the probability of this occurring is reduced if the number of peaks in the mass spectrum is reduced by using a 'soft' method of ionization. The problem to be solved is, of course, an easier one if it is merely required to confirm the presence of a particular substance. Detection of individual alkaloids in whole plant material (Kondrat *et al.* 1978) provides a recent example of the use of the technique in this simple way at high sensitivity. The method of sample introduction used is extremely important in this as in all analyses. In the example quoted the sample was introduced by a direct insertion probe and this limited the attainable information to a qualitative demonstration of the presence of a particular constituent. The sensitivity with this method of sample introduction depends to a very large

extent on the relative vapour pressure of the component to be detected compared with that of other components present.

Two further examples will serve to demonstrate the power of the new methods in the analysis of mixtures. The first concerns mixtures of isomeric compounds of very similar structure. Consider the mass spectrum of a large molecule such as a steroid **1** having a side-chain R.



1

Most of the ions in the spectrum will reflect the structure of the molecules and few the structure of R˙. If there are two such isomeric steroids in a mixture it will thus be very difficult to analyse for each component. However, there are two fragmentation processes, one of which is indicated by the wavy line and which leads to an ion $[R+C_3H_6]^+$ and a less probable one leading to the ion R^+. By isolating each of these ions in turn by means of the magnetic sector in an instrument of 'reversed' geometry, their collisional activation spectra can be studied. Thus, the structural investigation of just *part* of the molecular structures is possible, and none of the ions arising from the part of the molecule that is identical in each component is considered in the analysis. Indeed, the ability to isolate particular ions suspected to correspond to functional groups such as, say, pyridyl or acetyl is very important even in the case of a pure substance. Comparison of the collisional activation spectra of such ions with those from a small library of the spectra of known groups will often enable unambiguous identification of the groups present in the studied compound to be made. This has not hitherto been possible in mass spectrometry but now adds considerably to the power of mass spectrometry as a technique for the identification of unknown compounds.

The second example is one in which the mixture of compounds to be studied is produced by the pyrolysis of a DNA sample directly into the ion source of the spectrometer from a direct insertion probe. The controlled pyrolysis of normal DNA produces a mixture of the bases adenine, cytosine, guanine and thymine, all of which give relatively simple mass spectra containing abundant molecular ions. It is thought that the presence of modifications along the DNA chain has significant influence on the regulation and control functions of DNA. Only a very small fraction of the units of one particular base in the DNA chain, of the order 0.1%, need be modified by the presence of a substituent group such as methyl, ethyl, hydroxyl or thiohydroxyl for these effects to occur. The search for such modified bases may be made with very high sensitivity by the use of an instrument of reversed geometry (J. L. Wiebers 1978, personal communication). The magnet is first set to transmit ions corresponding to the molecular mass of the modified base suspected to be present, collision gas is introduced into the field-free region in front of the electric sector and the pyrolysis started. As soon as any ion current is detected corresponding to the transmitted mass, repetitive scanning of the electric sector voltage under computer control is begun and a series of spectra collected. The ion current

transmitted by the magnet shows when the base is being produced; the spectra confirm the identity of the base. The entire pyrolysis–analysis sequence takes only about a minute.

Alternative systems of producing mass selected ion kinetic energy spectra involve simultaneous scanning of two of the fields in the mass spectrometer, typically the magnetic sector field, B, and the electric sector field, E, keeping the ratio of these two fields constant (Boyd & Beynon 1977; Millington & Smith 1977; Bruins *et al.* 1978). This has the effect of narrowing the individual ion kinetic energy peaks, thus improving the resolution between adjacent peaks and preventing overlapping. This advantage is countered by the disadvantage that detailed peak shapes in the conventional ion kinetic energy spectra do form part of the 'fingerprint' by which a compound is recognized and these are lost when linked scans are performed. The linked scans have not yet made any appreciable impact in the analytical field partly because the accuracy with which the ratio B/E can be held constant throughout a scan has only reached a satisfactory level when a fairly large computer is used to control the scanning (Haddon & Young 1978).

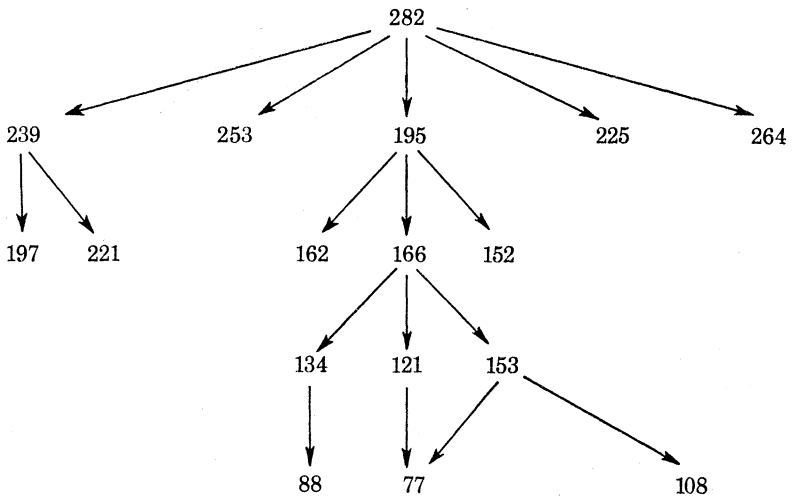


FIGURE 6. The fragmentation 'map' of a compound of molecular mass 282. The fragmentations shown were induced by introducing a gas into the special collision cell located near the intermediate slit of an instrument of 'reversed' geometry. Only the main fragmentation routes are shown. The vertical arrows indicate the main fragmentation process at each stage.

5. THE IDENTIFICATION OF UNKNOWN COMPOUNDS

The power of the new methods for identifying unknown compounds and the logic used to deduce structural formulae have already been described (Bozorgzadeh *et al.* 1978) and can best be illustrated by considering a detailed example. The major processes making up the fragmentation map of an unknown compound are shown in figure 6 and from this pattern it seems clear that the molecular mass of the unknown is 282. Examination of the conventional mass spectra shows an isotope peak at m/z 283 of abundance about 16.5% of the peak at m/z 282 and an isotope peak at m/z 284 of about 8.5% of the abundance of the m/z 282 peak. This suggests that the compound probably contains about 15 carbon atoms and two atoms of sulphur. The molecular ions fragment with loss of neutral particles of masses 18, 29, 43, 57 and 87. This is indicated in figure 7. The first of these losses (H_2O) indicates the

presence of oxygen. Thus the formula is, perhaps, $(C_{15}S_2+38)$ i.e. $C_{15}S_2OH_{22}$ or $C_{15}S_2O_2H_6$. The losses of 29, 43 and 57 suggest $C_2H_5^{\cdot}$, $C_3H_7^{\cdot}$ and $C_4H_9^{\cdot}$.

To understand the loss of the fragment of mass 87 (the major fragmentation process undergone by the molecular ion) the magnetic sector is tuned to transmit ions of m/z 283. These ions all contain one heavy isotope and this label is randomly distributed through the molecule. The natural abundance of ^{13}C is greater than that of ^{2}H, ^{17}O or ^{33}S and so, to a good approximation,

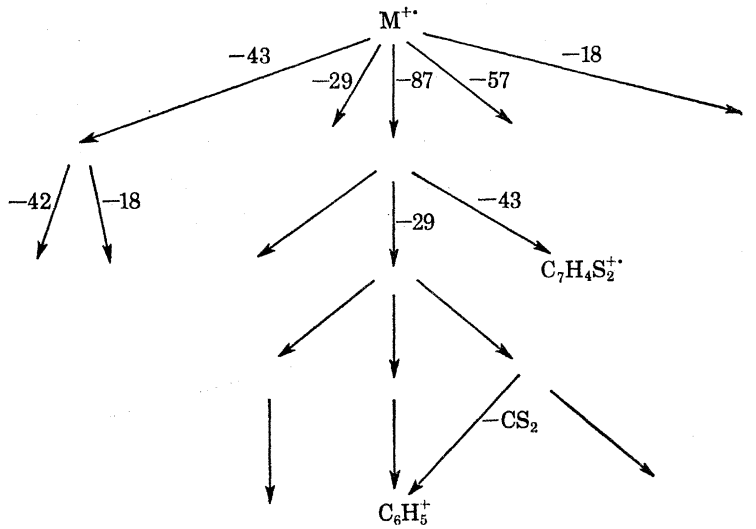


FIGURE 7. The fragmentation pathways used to identify the unknown compound, the more detailed fragmentation map for which is shown in figure 6.

one is dealing with a compound labelled with a single atom of ^{13}C. The m/z 183 ions are found to lose neutral moieties of masses 87 and 88 in the ratio 2.0:1. Thus the number of carbon atoms being lost is exactly one-third of the number present; and the number present must be exactly divisible by 3. Thus, the formula of the mass 87 fragment must be (C_5+27), i.e. $C_5OH_{11}^{\cdot}$ and the molecule can be seen to contain at least 11 atoms of hydrogen, so that its formula must be $C_{15}H_{22}S_2O$. The $C_5OH_{11}^{\cdot}$ group is saturated and so the compound must be either an alcohol or an ether. Loss of mass 18 indicates that it is an alcohol. It can be seen from figures 6 and 7 that the loss of 43 from the molecular ion is followed by loss of 42 (C_3H_6) and 18. This latter loss confirms that the original mass 43 unit lost does not contain the oxygen atom. The C_3H_6 shows the presence of a *second* propyl group in the molecule, but since this loss is not accompanied by loss of 29, it suggests an isopropyl unit.

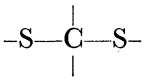
Loss of mass 87, on the other hand, is followed by loss of both ethyl and propyl fragments. It thus appears that it is this group of mass 87 that contains the butyl and isopropyl groups as well as the hydroxyl group and its formula must correspond to **2**.

$$-CH(OH)CH_2CH(CH_3)_2.$$

2

The ions of mass 77 can be shown from their collision-induced fragmentation pattern to be phenyl ions but since they are only formed after a four-step fragmentation process it does not seem that a phenyl group can be present in the complete molecule but that this group is formed by rearrangement. Loss of the two groups $C_5H_{11}O^{\cdot}$ and $C_3H_7^{\cdot}$ leaves a rather stable

ion $C_7H_4S_2^{+\cdot}$ containing fewer than five hydrogens and suggests this as the formula of part of the structure. No sulphur is lost until after two fragmentation steps; the loss of CS_2 suggests that both sulphur atoms are attached to a single carbon so that a group corresponding to **3** must be present.



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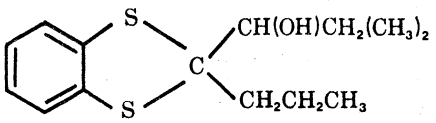
All of the above arguments, taken together, suggest that the structure of the unknown is given by **4**.



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It should be noted that this structure was deduced even without making use of any accurate mass measurements and that it rests on rather firm ground because of the various alternative fragmentation paths that can be investigated, each of which confirms the information deduced from other paths.

Use of the reversed geometry does not appear to have any disadvantages when compared with conventional arrangements; the performance of the instrument when used in the conventional, high resolution mode is good and mass resolution (10% valley definition) of 80000 has been achieved. There appear to be advantages of the new geometry for general applications in chemistry and biology that give promise of its wider use in the future.

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