

Advances in
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Editors

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MASS SPECTROMETRY AS A STRUCTURAL TOOL

By R. I. REED, *The University, Glasgow, Scotland*

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I. Historical Introduction and Instrument Design

The positive ray analysis of Thomson (99) by which he separated the different masses in a single element was further developed by Aston (5) who produced the first mass spectrograph, in which the multiplicity of the masses in a given substance was determined by a photographic method. At about the same time, methods of analyzing the relative abundances of the species present were developed by Dempster (23) and other workers, particularly Nier (76), who have greatly increased the accuracy as well as the reliability of the instrument.

The following method is commonly employed (77) for the separation of the isotopic particles and the determination of their relative abundance. The material is introduced into the instrument in the vapor phase and at low gas pressures. The molecules are then bom-

barded by a beam of electrons whose energy can be varied but is usually 50 or 70 e.v.

Negative as well as positive ions are formed although mass spectrometric studies are usually confined to the latter. The positive ion beam is repelled electrostatically through a system of focusing and beam-defining plates and into the field of an electromagnet.

Electrostatic repulsion accelerates the ions in accordance with the equation $\frac{1}{2} Mv^2 = eV$, where M is the mass of the ion, e the charge on an electron, V the voltage of the repeller, and v the velocity of the ion. Charged particles moving in a magnetic field suffer a deflection from their direction of motion according to the relation $Mv^2/r = HeV$ where H is the field strength of the magnet and r the radius of gyration of the ion. The combination of these two effects leads to $M/e = H^2r^2/2V$. The result is that the original beam of particles is resolved into a series of such beams in which all charged particles having the same mass/charge ratio (M/e) follow the same path while different ion beams follow different paths in the instrument. The practice is to align the selected beam upon the collector plate either by varying the magnetic field or by varying the electrostatic potential used to accelerate the ions into the magnetic field. Various designs of instrument differ in the path traveled by the ions, which is 180° of arc in Dempster's design (24) and 60° or 90° (49,50,66) (see Fig. 1) in others. The positively charged ions are received upon the collector plate which is negatively charged and the current so produced is proportional to the number of ions which arrive. Thus the magnitude of the current is a direct measure of the amount of the particular species present. The minute currents obtained are usually amplified before measurement, and machines employing conventional amplifiers usually require a current of the order of 1×10^{-15} amp. Modern developments include the introduction of electron multipliers which are able to measure currents of the order of 1×10^{-18} amp. and lower.

The resolving power of such instruments varies with the length of the trajectory and the field strength of the magnet as well as with the width of the defining slits used to collimate the ion beam. The complete resolution of adjacent beams, while desirable, is not necessary in most organic applications of mass spectrometry. Thus conventional instruments may be used for substances of much higher molecular weight than would otherwise be possible. Equally,

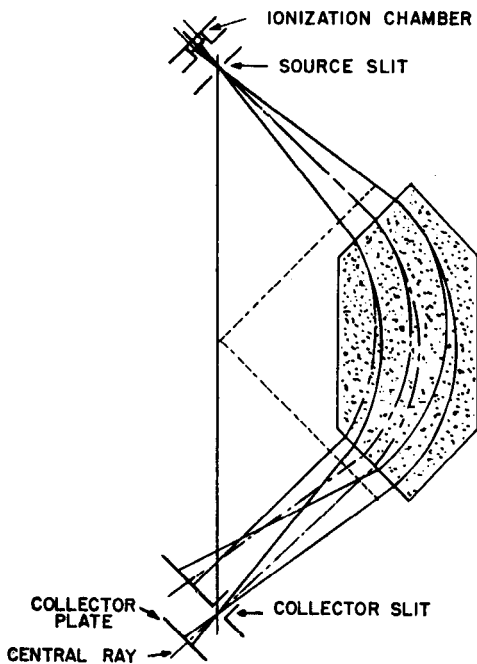


Figure 1.

for many applications the estimation of the relative abundance of different ion beams of widely different intensities need not be more than 0.5% of the least abundant, although specially designed instruments (22) of much higher precision have been constructed for special purposes.

II. Analytical Applications

The uses of the mass spectrometer as a structural tool in organic chemistry are many and varied and accordingly the field has been treated in several sections beginning with the historically important analysis of mixtures of aliphatic hydrocarbons.

A. HYDROCARBON MIXTURES (95)

Although it seems likely that it may in the future be combined with other techniques such as gas-liquid chromatography (44,47),

this study represents the first important application of the identification of molecules by their electron-induced fragmentations. The determination of the relative abundance of the species present in isotope analysis is obtained by measurement of the intensity of the respective molecular ion beams. In the analysis of hydrocarbons, however, the bulk of the molecules of each compound present is decomposed by electron bombardment and the estimation of the species present is based on the "cracking patterns" and not on the abundance of the molecular ions.

Analytical studies require that the sensitivity of the instrument should remain the same for any given compound independent of the other compounds present. Moreover, the intensities of all the fragment ions in the cracking pattern of the compound should be in constant relation to each other, as well as being directly proportional to the quantity of this compound present in the sample. This implies that the contributions of various compounds to any given fragment ion are strictly additive and that by an analysis of the intensities of selected peaks, the nature and relative concentrations of the molecular species present can be made. The first of these conditions is generally true, at least over fairly short operating times, and the variations for longer periods of a week or more have been found to be about 5% (75). The second condition is nearly, but often not exactly, obeyed and for accurate analysis it is convenient to calibrate the instrument with a standard, known mixture of about the same composition as that expected in the unknown. The cracking pattern of the unknown mixture is then subject to a suitable mathematical analysis from which the identity and relative abundance of the compounds present may be determined (33).

In view of the advances in the technique of gas-liquid chromatography, however, a more promising analytical technique involves the mixture being first separated by passing through such a column and the quantity of each component determined. The issuing gas is then admitted to the mass spectrometer and identified by its cracking pattern. As this latter instrument requires only a sample of 0.01 mg. or, in special cases, even smaller quantities for analysis (94,103), this combination should provide a powerful means of analyzing minute quantities of mixtures and has been very successfully employed in the analysis of the materials trapped in

bubbles in glass. Samples as small as 0.05 μ l.-atom. of gas may be analyzed with an accuracy of 10% (74).

Since it appears that the gas-liquid chromatographic technique will be capable of further extension to compounds of boiling point higher than those now commonly employed, an important future use of the mass spectrometer, also developed to manipulate high boiling substances (98), may well be the identification of pure substances by their characteristic cracking patterns and the elucidation from such patterns of structural entities in molecules.

B. MOLECULAR WEIGHT DETERMINATION

Frequently a necessary preliminary to the determination of molecular structure, and certainly for the identification of the molecular formula, is the determination of the molecular weight. As will be discussed later (see Sections IV-B, IV-F) the intensity of the molecular ion beam is often weak in the case of large molecules, but it is not commonly absent. The presence of carbon atoms in a compound implies that there will be at least two ions corresponding to parent molecular ions, one from the molecule which contains only carbon-12 atoms and the next higher mass in which one carbon-12 is replaced by carbon-13. The ratio of the intensities of these ions will be calculable if the material examined has the normal isotopic abundance of carbon-13.

Some compounds are known which do not have an ion of mass corresponding to the parent molecular ion. Some of these are relatively simple structures, e.g., dimethyl acetal and diborane. Hydrocarbons such as 3,3-dimethylheptane which possess a quaternary linked carbon atom do not give rise to a parent molecular ion. Such a condition is easily recognized, since the ion of greatest mass in the spectrum is often of the same intensity as that of the ion one mass unit less, and not in the ratio to be expected from the relative abundances of the isotopes present in the compound.

In many compounds having no parent molecular ion, however, the molecular weight can be determined by the ingenious micro-effusion technique of Eden et al. (26). This can presumably be used to determine the molecular weight of any vapor which neither associates nor becomes strongly absorbed on the instrument during the experiment. Thus, with due precautions, molecular weights can be determined this way to an accuracy of better than 1%.

In normal mass spectra, the peak corresponding to the molecular ion which contains the most abundant isotopic species of each element present is readily identified. The ions of higher mass correspond to molecular ions containing the less abundant isotopes of constituent elements and are consequently less abundant. Thus in Figure 2 the parent molecular ion corresponding to molecular weight 400 is followed by two weaker ones of molecular weights 401 and 402, respectively.

One field in which such determinations are of particular value is that of the steroids and triterpenes. In these series, particularly with the hydrocarbons, it is difficult to decide the molecular com-

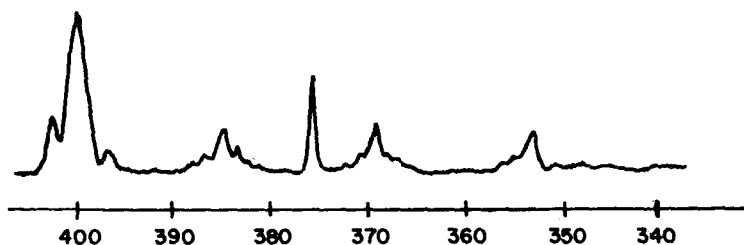


Figure 2.

plexity of the compound by conventional combustion analysis. Even in compounds containing a functional group such as hydroxyl it is often necessary to prepare a series of derivatives in order to decide the probable molecular species. Extensive studies have now been made upon such series both to show the common fragmentations which occur upon electron bombardment (36) and also to use the characteristic breakdown patterns to deduce the length of the side chain in the molecule (32).

Such polycyclic compounds are particularly well suited to the determination of molecular weights by electron impact, since the parent peak is well defined and the determination of the molecular formula facilitated.

Two other series of compounds in which this technique is of great utility are the polycyclic aromatic compounds and the larger molecules of general formula C_nH_{2n} . In the former class there is usually so little hydrogen relative to carbon as to make difficult an estimation of the molecular formula by conventional analysis.

In the latter, the olefins and cycloparaffins, the carbon/hydrogen ratio is constant and conventional molecular weight determinations may not be sufficient accurate.

The method described above is thus a powerful aid to determining molecular structure where the elements present in the compound as well as the category to which it belongs have been determined by other methods.

C. ELEMENT ANALYSIS—ISOTOPE ABUNDANCE

It is also possible by a mass spectrometric examination of a compound to determine the nature of many of the elements present. Two stages of refinement of this technique are possible. The first makes use of the isotopic peaks which, as already mentioned, are also present with the parent molecular ion. These bear a certain fixed relation to the intensity of the parent ion.

As a simple example the molecules carbon monoxide, nitrogen, and ethylene all have parent molecular ions of mass 28. If the intensity of the most abundant parent molecular ion is regarded as unity, then the intensity of the next greater peak may be calculated on the normal isotopic abundance of the elements present in the compound and expressed as a percentage of the commonest species. Thus if mass 28 is $\text{C}^{12}\text{O}^{16}$ mass 29, which is $\text{C}^{13}\text{O}^{16} + \text{C}^{12}\text{O}^{17}$, has an intensity of 1.12%. Similarly, the intensities of $\text{N}^{14}\text{N}^{15}$ and $\text{C}^{12}\text{C}^{13}\text{H}_4$ are 0.38% and 2.24%, respectively.

Comparison with the observed percentages of 1.16, 0.38%, and 2.2% would enable a decision to be made among these species. This principle can be extended to more complicated systems although the possibilities increase with an increasing number of elements and also with an increasing molecular weight. In certain types of compounds, the isotopic peak is larger than predicted because of hydrogen addition to the molecular ion (see Section VIII), but the effect is not often sufficiently large to cause confusion in the identification.

In the case of halogen atoms the identification of systems containing chlorine, in which the natural abundance of $\text{Cl}^{35}:\text{Cl}^{37} = 3:1$, and for bromine, in which it is $\text{Br}^{79}:\text{Br}^{81} = 1:1$, may be readily made. Moreover, as the distribution of the two species is a statistical one, the recognition of the number of halogen atoms present

as well as their nature may be easily determined by an application of the well-known binomial expansion.

As an example of the application of this theorem, consider the spectrum of trichloropropane. For simplicity of demonstration, the occurrence of molecules containing carbon-13 will be neglected. Since two chlorine isotopes are present and the molecule contains three chlorine atoms, there are four molecular species $\text{C}_3\text{H}_5\text{Cl}_3^{37}$, $\text{C}_3\text{H}_5\text{Cl}_2^{37}\text{Cl}^{35}$, $\text{C}_3\text{H}_5\text{Cl}^{37}\text{Cl}_2^{35}$, and $\text{C}_3\text{H}_5\text{Cl}_3^{35}$ having molecular weights of 152, 150, 148, and 146 respectively. The relative abundances of these species will be given by the coefficients of the expansion $(\text{Cl}^{35} + \text{Cl}^{37})^n$. In this instance $n = 3$, as there are three chlorine atoms, and since the expansion is only concerned with the relative abundance of the various species, the expression may be rewritten as $[(\text{Cl}^{35}/\text{Cl}^{37}) + 1]^3 = (Y + 1)^3 = Y^3 + 3Y^2 + 3Y + 1$. Now, for a natural abundance of chlorine, $Y \sim 3$ as already stated, and therefore the relative abundance of each species is

$$\text{C}_3\text{H}_5\text{Cl}_3^{35}:\text{C}_3\text{H}_5\text{Cl}_2^{37}\text{Cl}^{35}:\text{C}_3\text{H}_5\text{Cl}^{37}\text{Cl}_2^{35}:\text{C}_3\text{H}_5\text{Cl}_3^{37} = 27:27:9:1$$

D. EXACT MASS DETERMINATION

The second method for deducing the elementary constitution of a compound depends upon the determination of the mass of the molecular ion to a very high degree of accuracy, and its introduction for use with double-focusing machines represents a striking advance in analytical technique (7).

In one method of molecular weight determination with single-focusing instruments the magnetic field of the mass spectrometer is held constant and the appropriate ion beams focused by altering the potential of the repeller plate. Ideally, the relationship between the ion mass focused on the collector and the ion accelerating potential is an inverse one $(M_n/e) [\alpha(1/V_n)]$ where M_n represents the mass of the ion of unit electronic charge and V_n the ion accelerating potential. Accordingly $M_1/M_2 = V_1/V_2$. If V_1 and V_2 can be measured precisely and the value of M_1 is known exactly, M_2 can be accurately determined. In practice, however, in many conventional single-focus mass spectrometers the problem is rarely as simple as this, principally because of the existence of other potentials in the ion source which help to focus the ion beam and to draw the ions out into the tube. In general, these potentials do

not change uniformly with variations in the main potential, and the above simple determination becomes insufficiently precise. This difficulty may be overcome by removing the small potential gradients from within the source, as is done in exact studies upon appearance potentials (102); however, this leads to a concomitant loss of sensitivity in the instrument such that the required beam is often only detectable with difficulty. Moreover, for many compounds of high molecular weight the ion accelerating voltages are necessarily small. In some case there is also a falling off in the sensitivity of sector instruments at low accelerating potentials and this, with the effect referred to above, often precludes the use of that technique.

A second approach is to examine a series of compounds of known molecular weight in the neighborhood of the unknown. A graph is constructed by plotting the mass of the known molecular ions against the appropriate ion accelerating voltages necessary to focus them on the collector. The mass of the unknown may then be obtained by interpolation. This method is more arduous but has the advantage that the machine may be run at its maximum sensitivity.

Some further gain in sensitivity, at the expense of resolving power, can also be achieved by increasing the width of the defining and collecting slits. This leads to a peak with a flat top trapezoidal in shape if properly focused. If such a peak is plotted out with the ion accelerating voltage as abscissa and ion beam intensity as ordinate the true value of the accelerating voltage is given by the value of the abscissa of the centroid of the peak.

This exact molecular weight which corresponds to the combined atomic weights of the most abundant isotopic species of each element may then be used to determine the elements present. As an example, the above system of CO, N₂, and C₂H₄ can be again considered in which the molecular weights of 27.990, 28.00, and 28.30, respectively, are obtained.* As it is practicable to determine the molecular weight to within 0.02 of a mass unit, this result will decide whether the compound under examination is ethylene or either nitrogen or carbon monoxide. The distinction between nitrogen and carbon monoxide is best made by consideration of the intensities of the isotopic peaks.

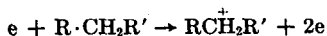
* Using C¹² = 12.00000.

This process may be extended to more complex examples. The molecular weight of the most abundant molecular species in diphenylmethane is 168.09 while that of dibenzofuran is 168.05. These may thus be distinguished from one another by an accurate molecular weight determination. The utility of the method depends upon the possession of a list of accurate molecular weights calculated from the atomic weights of the most abundant species ($H^1 = 1.0075$, $C^{12} = 12.0000$, etc.) and such lists are being compiled (9). In general it would appear that a combination of these two methods—accurate molecular weight determination and the measurement of the intensity of the ions of the rarer isotopic constituents—will provide a solution to many structural analytical problems. Even if a unique solution may not be obtained by these means, investigations of this kind will greatly restrict the number of possibilities.

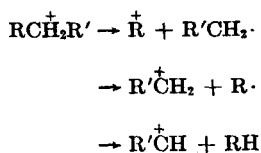
The extension of this method of the accurate determination of molecular weights is not applicable to fragment ions, at least with conventional mass spectrometers, owing to the possibility of these derived ions being formed with kinetic energy (7). This problem is overcome by the use of double-focusing spectrometers which have not yet, however, come into general use. Such a deficiency in technique is not serious in the elucidation of molecular structure for, as will be detailed later, characteristic molecular units have a characteristic cracking pattern.

III. Cracking Patterns

These patterns are usually obtained by the bombardment of molecules with electrons of 50–70 e.v. energy. This gives rise to molecular ions

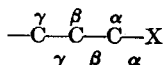


The molecular ions then break down to give fragment ions, radicals, and sometimes molecules, the last being formed by hydrogen transfer or abstraction reactions



Of these fragments only those which are positively charged are recorded by the mass spectrometer and the deductions as to the structural nature of the original molecule have to be made on the basis of these. Even with this restriction substantial progress has recently been made in an attempt to correlate the cracking patterns of compounds with particular structural features in the molecule. In the past six years a very large field of compounds has been surveyed, principally by McLafferty and Friedel with their respective collaborators. The problem is a most complex one but certain general conclusions can be drawn. McLafferty (60) has examined and classified the effect of the functional groups upon the position of cleavage in the molecule. Broad correlations have been found between the point of fission in the molecule and the electron attracting or releasing properties of the groups present.

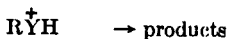
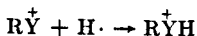
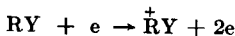
In the discussion of these fissions, the following nomenclature is used. The carbon atoms attached in a chain to the functional group X are designated α , β , γ , etc., and the corresponding bonds are referred to as the α -bond, β -bond, etc., as in the following diagram



Three main categories of fragment ions are present.

(1) Fragment ions are produced which contain the functional group X in a molecule RX. Electronegative substituents have been shown to favor fission of the α -bond (i.e., R—X in the molecule RX) or β -bond fission accompanied by a hydrogen rearrangement.

These are the commonest forms of electron-induced dissociations of organic molecules. Even compounds possessing an electro-positive group, particularly if one atom of the group possesses an unshared pair of electrons, may well behave in this way by reason of a sequence involving a hydrogen abstraction process followed by the decomposition of the "onium" ion.



The influence of functional groups is sometimes modified by their effect upon each other (e.g., the alkoxy alcohols (80)) and less frequently by the nature of the hydrocarbon residue.

(2) These are ions which arise by decomposition of the hydrocarbon residue. Ions of this type provide most of the spectrum in the case of the long-chain alcohols, esters, and fatty acids.

(3) There are often prominent ions which are not obviously derivable from known molecular structures but are obtained by rearrangements of either the molecular ion or the first formed fragment ion. The use of isotopically enriched molecules, as for instance *o*-deutero-2-phenylethanol (42), has shown that in many cases extensive rearrangements may occur and that the fragment peaks resulting from these rearrangements are often very intense. These, in general, do not mislead in the identification of a given material but may suggest the presence of impurities which do not exist. Occasionally, they are useful in the identification of structural units. In this article their discussion will be limited to the cases in which identification is aided or hindered by their presence. A study of rearrangements which hinder the determination of structure has been made by McLafferty (60) and reference should be made to this for further details.

Finally, before beginning a detailed discussion of selected molecules, it should be stressed that the fragmentation is not of a neutral molecule but of the molecular ion. This is often the reason for the breakdown of any particular compound not following the route expected from a study of its normal chemical reactions.

A. THE CRACKING PATTERN OF HYDROCARBONS

As previously mentioned, much of the cracking pattern observed in the fragmentation of molecules is derived from the breakdown of the carbon skeleton. Accordingly, much of the interpretation of a spectrum will depend upon the recognition of the fragments derived from it. This, the most studied and best documented of the mass spectra of molecules, is often the most difficult of interpretation. The carbon skeleton is conveniently discussed under the following categories, namely, alkanes (including alkenes and alkynes), the alicyclic hydrocarbons and aromatic compounds.

1. Alkanes

The first of these groups has been extensively studied (78,79) with the general conclusion that the distribution of fragment ions from the normal alkanes varies slightly with chain length. As the series is ascended the most intense fragment corresponds to a C_3 or C_4 residue and the intensity of the molecular ion diminishes (18). A further characteristic of such decompositions is that ions of odd mass

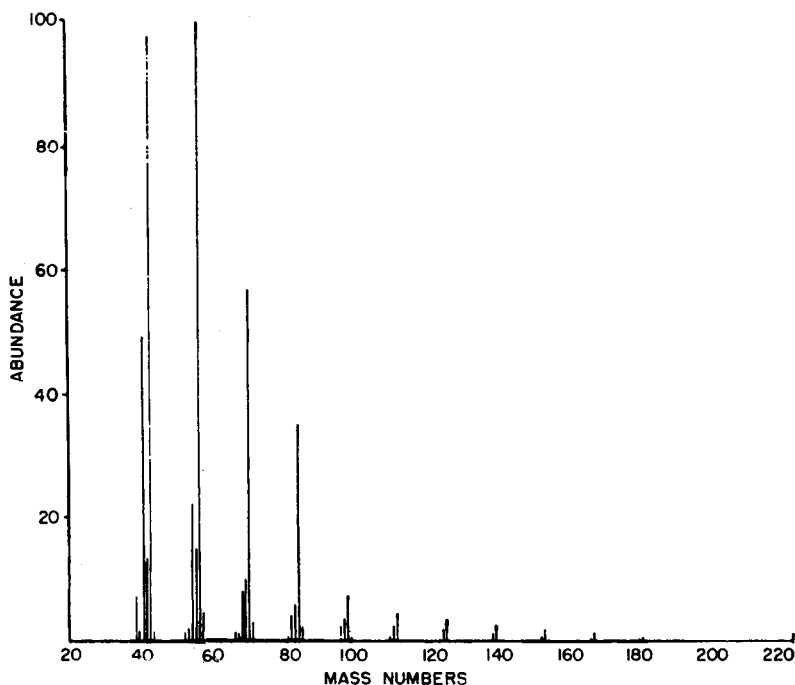


Fig. 3. *n*-Hexadecane.

are more abundant than those of even mass (64,65,101) and the authors in the above-cited references have provided a theoretical explanation for this in terms of the break down of the molecular ion. An interesting feature of such studies is the good agreement observed between the abundance of the various ions obtained by the electron impact-induced dissociation of *n*-octane and the calculated prob-