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Aspects of Mass Spectrometry in Organic Chemistry

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The term mass spectrometry touches off quite different associations in the minds of members of the various scientific and professional fields connected with natural sciences. Conceptions as diverse as atomic weight, geological age, meteorites, planetary atmosphere, vapour pressure, quantitative mixture analysis, bond energies, fast reactions, atom bomb, semiconductors, structure of molecules, toxicology, metabolism, gas chromatography, high vacuum technique or nuclear reactor processes amongst others are thought to be typical subjects for the method. The reason for this is an extraordinary wide range of potential applicability[1] which is surprising if one considers that in principle one is only dealing with a method for separation and measurement of masses. The history of the technique is a nice example of the way a scientific experiment can continuously promote new applications as their time comes, and start surprising developments in fields often only remotely connected with the original purpose.

The advent of mass spectrometry was initiated by the observation of WIEN and THOMSON at the end of the last century that cathode rays can be deflected by strong magnetic fields. This provided the first experimental proof for the phenomenon of charge transport by ions in the gas phase, which 10 years later resulted in the first great achievements in this field; namely detection of stable isotopes and measurement of their accurate mass and relative abundance. Thereafter, for more than 30 years, mass spectrometry remained a tool exclusively for physicists before its potentialities in chemistry were recognized and brought to bear. Some of the applications in use today are listed in the Table, which can by no means claim anything like completeness. The instrumentation is about as diverse as the scope of applications. It ranges from huge constructions extending over whole buildings for special problems in physics, down to pocket-size instruments which are shot into space in rockets to measure particles of the ionosphere or of planets.

In organic chemistry alone, a whole spectrum of applications has already developed, among which at the present time isotope analysis and qualitative structure analysis are the most important ones. As is shown by the diagram in Figure 1, the principle of the technique is quite simple. The instrument, which operates under high vacuum, consists of an ion source into which the sample is introduced in vapour form. Bombardment of this target gas with electrons produces a large number of positively charged molecule- and fragment-ions. An electrical acceleration field applied to the end of the source then gives these ions a velocity v, which is proportional to both their charge and the field potential, and inversely proportional to their mass according to the equation

$$v = \frac{\sqrt{2eU}}{m}.$$

Typically they leave the source with a velocity of the order of 100 km/sec. These ions are moving charge carriers and are equivalent to a current in an electrical conductor. As such they are subject to a force when in a magnetic field and are therefore deflected. (This is in fact the principle on which the electric motor works.) In addition to their charge and velocity the degree of ion deflection depends on the mass of the individual charge carriers and the strength of the magnetic field. The greater mass and velocity, the greater the forces of inertia and the flatter the ion trajectory. A slow light ion will for example follow a strongly curved path and a fast heavy ion in comparison an almost flat one. Thus a fan of individual ion beams is formed, each one comprising ions of the same mass to charge ratio, resulting in a separation according to mass for equally charged ions. By gradually increasing or decreasing the magnetic field strength successive ion beams comprising the fan may be focussed in turn at a collector slit. They are then detected by a Faraday cup or electron multiplier

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[1] F. A. WHITE, *Mass Spectrometry in Science and Technology* (John Wiley and Sons, New York, London, Sydney 1968).

situated directly behind this slit and registered by means of mirror galvanometers on a photographic chart. The greater the number of ions within a specific beam, the stronger the signal obtained in the spectrum. There is a variety of other methods available for ion production as well as for mass separation and registration but the described arrangement is presently the most important one for general analytical purposes in organic chemistry.

The dominating primary process in the ion source is the removal of a single electron from a molecule. The mass of the ion remains practically the same, since with less than 10^{-27} g the contribution of a single electron to the overall mass is negligible in this context and need not be taken into account. This so-called molecule ion, if it can be registered, immediately provides one with the precise molecular weight of the compound and thereby gives a most important piece of information to the chemist.

This is the basis of the simplest procedure for isotope analysis in organic compounds. If in a molecule (for example benzene) one atom of a light isotope (such as in this case ^{1}H) is replaced by a heavy one (that is ^{2}H), the mass difference between the 2 isotopes adds to the normal molecular weight and the mass of the molecular ion is increased by that amount. Not much else is changed by such a replacement and the chemical behaviour remains to a large extent the same. If one looks at the mass spectra of the 2 compounds in the molecular ion region (Figure 2) they appear to be almost identical, at least at reduced ionizing voltages where no loss of hydrogen occurs. The only major difference is a mass shift of the molecular ion signals by one mass unit. The molecular weights are 78 and 79 respectively and both molecular ions have the same satellite signal at the following mass number. This latter signal has its origin in the fact that every ninety-first C-atom in nature is a ^{13}C-isotope. Since the compound contains 6 C-atoms there are 66 molecules among every 1,000, which contain a ^{13}C-isotope and have a higher molecular weight, 79 and 80 respectively in this case. A 1:1 mixture of the 2 compounds gives a spectrum the same as that which would be obtained by addition of the spectra of equal intensity of both of the individual components, i.e. 2 molecular ions whose relative abundance after correction for the natural ^{13}C-isotope contribution corresponds exactly to their relative amounts in the mixture. In the same manner one obtains from partially labelled compounds a separate molecular ion for every stage of isotope incorporation and one can by measurement, quantitatively determine their relative amounts. This yields important additional information as compared with the results of classical combustion methods which only show the overall content of heavy isotopes. The result of an exchange reaction may look similar to Figure 3, where the molecular ion regions of educt and product in a ^{1}H/^{2}H exchange experiment with cyclodecene as determined at reduced ionizing voltage are shown. The product exhibits a whole series of molecular ions, ranging from mass 138 of the unchanged educt up to 154 corresponding to molecules containing 16 ^{2}H-isotopes. A maximum is observed at mass 149 with 11 ^{2}H-atoms incorporated. In comparison, a combustion analysis would merely indicate about 9.5 ^{2}H-atoms per molecule. Such a mass spectrometric determination has, besides giving the additional information about isotopic distribution, the further advantage that it is

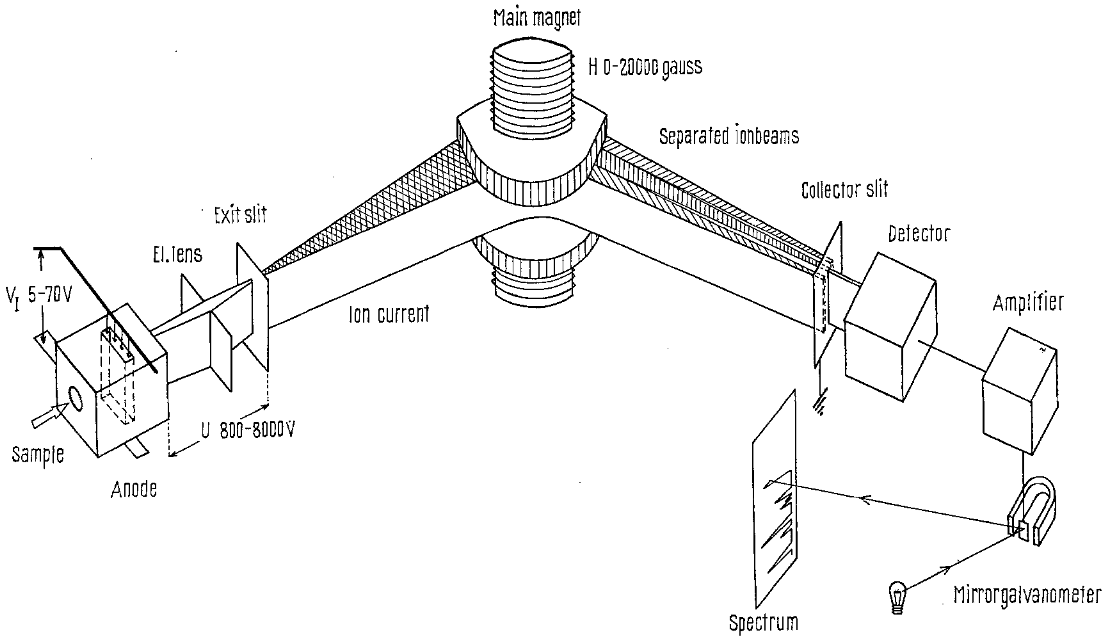

Fig. 1. Scheme of a magnetic sector type single focussing mass spectrometer.

independent of impurities of lower molecular weight (such as residual solvents), that it is applicable to all elements in the same way, and that only microgram amounts of sample are required to carry out the analysis. However, as compared to labelling with radioactive isotopes the method is less sensitive. With standard analytical instruments even under ideal conditions one cannot reliably measure enrichments in stable isotopes of less than 0.2–0.3%. The application of isotope dilution techniques is therefore restricted in this connection and the low sample requirements cannot fully compensate this drawback. On the other hand stable isotopes have the advantage of allowing one to work without regard to the various hazards and safety requirements dictated by work with radioactive isotopes. In addition it is frequently possible to locate the position of the labelled atom in the molecule directly by interpretation of the spectrum without prior chemical degradation.

The applicability of mass spectrometry to qualitative structure analysis is basically a by-product of ion production rather than a direct consequence of mass analysis. Upon electron impact the molecules not only loose an electron but at the same time acquire a certain amount of excitational energy which can induce all sorts of chemical reactions. These result in degradation and formation of smaller fragments which are of course also separated and registered according to their mass if they carry a positive charge. The various reaction mechanisms and results of such degradation depend, apart from the amount of excitational energy involved, mainly on the structure of the compound. After all it is chemistry that is taking place and it is a fundamental law of chemistry that structure and reactivity are dependent upon one another.

If a chemist is asked what the compounds 1–3 in Figure 4 might do if each were ionized and forced to react chemically by excitation without offering them a partner for reaction, he will easily predict that compound 1 would break apart symmetrically and then, if forced to react further, loose carbon monoxide. The expected signals would be at mass 190, 95 and 67 respectively. The spectrum ties in with such a prediction beautifully (Figure 5). The molecular ion has mass 190 with an isotope satellite just right for 10 carbon atoms. The product of symmetrical cleavage at mass 95 is very abundant and in fact looses 28 (CO) to yield 67. The only additional signal of appreciable intensity has mass 39 and is formed from the furylion (m/e 67) by further loss of CO.

In compound 2 no particular reaction is immediately obvious and the chemist would not easily make any prediction. Seemingly the compound does not know either and undergoes no specific decomposition. The molecular ion signal is very strong but otherwise loss of hydrogen and double ionization are the only significant reactions (Figure 6).

Compound 3 finally offers so many possibilities for reactions of comparable probability that the chemist would hesitate in predicting a specific one to predominate. The spectrum gives a corresponding result. The compound does not decide to undergo any one

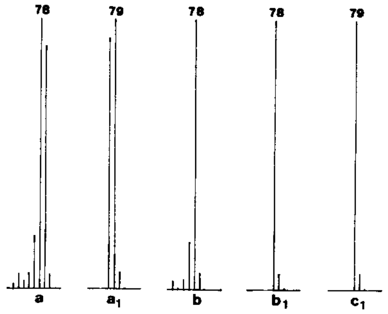

Fig. 2. Molecular ion region of benzene (b, b_1), monodeuterobenzene (c_1) and a 1:1 mixture of both (a, a_1). Suffix '1' indicates reduced electron energy.

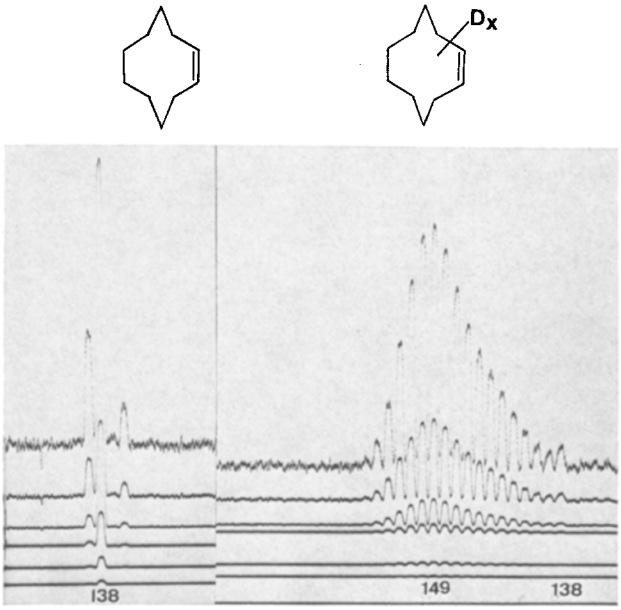

Fig. 3. Molecular ion region of cyclodecene and partially deuterated cyclodecene at reduced electron energy.

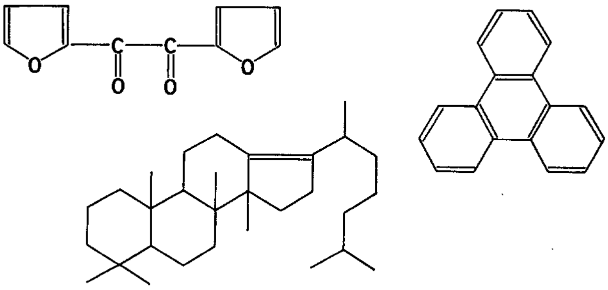

Fig. 4. Structures of 3 types of compounds. 1, furil; 2, 9,10-benzophenanthrene; 3, anti-isotirucallene.

specific reaction but prefers many different ones. Consequently the spectrum consists of a forest of signals of similar intensity, whereby a certain amount of experience is required before any structural conclusions may be drawn (Figure 7).

Evidently mass spectrometry is in the qualitative analytical respect more than anything else a reaction spectroscopy. To interpret a spectrum by putting together the pieces as in a puzzle requires one to be familiar with chemical reactivity. The better the chemist, the higher the chances for a successful structural interpretation. It must however be stated that practical experience is also important in this field, because priorities within alternative ways of degradation are not always to be judged on the basis of chemical arguments only. One should remember that chemistry in the laboratory deals with intermolecular reactions while in the ion source of a mass spectrometer it is intramolecular processes that are almost exclusively taking place. The reactions are carried out at pressures around 10^{-6} mm Hg as a result of which the individual molecules are well separated from one another. In cases where the results of electron impact induced reactions, and reactions in the laboratory are the same, one may in general safely assume, that even in solution the reaction was of intramolecular nature.

This chemical basis of structure analysis by mass spectrometry is to be acknowledged as well in the training of students, who are expected to become familiar with these modern analytical techniques. There is little point in teaching such applications before a fund of chemical knowledge is attained.

If the method in fact is based on chemical reactions, then the mass spectrum is a collection of products of degradation and synthesis and the mass spectrometer a kind of chemical factory, a quite proficient one at that, producing hundreds of compounds at the same time within micro-seconds. This poses the question as to whether one could not make use of this for synthetic purposes on a preparative scale by collecting the products. The possibilities would be unique. Collection of molecular ion m/e 78 of benzene for example would yield a compound which does not yet exist, namely a completely uniform benzene consisting only of ^{12}C and ^{1}H. If this was carried out on a large scale on any compounds considered desirable one could really begin to find out what significance in nature stable isotopes have or what their function is. The ^{13}C isotope peak of CO_2 would yield carbon dioxide containing exclusively $^{13}C^{16}O_2$. Many a spectrocopist would be more than happy to have that compound if it was available. In the spectrum of pyridine (Figure 8) the most abundant fragmentation occurs at mass 52 corresponding to the elemental composition C_4H_4. It could (although need not) be cyclobutadiene, a small molecule but nevertheless a tricky compound that has in fact resisted synthetic effort up to now, although it may be produced in hundreds of mass spectrometers every day.

Such preparative intentions are hampered by the very small transmission of substance in a mass spectrometer. A rough estimation gives the following picture:

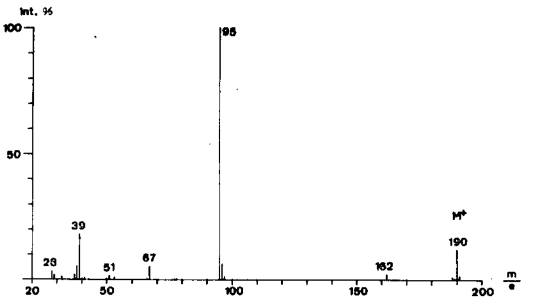

Fig. 5. Mass spectrum of furil.

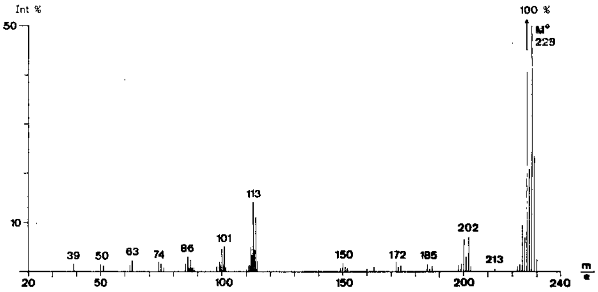

Fig. 6. Mass spectrum of 9,10-benzophenanthrene.

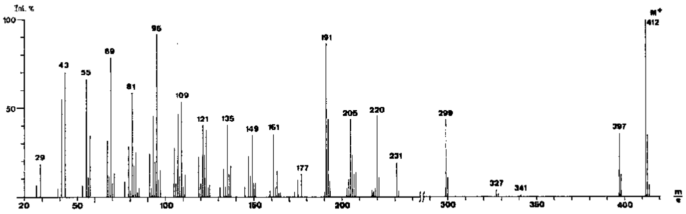

Fig. 7. Mass spectrum of anti-isotirucallene.

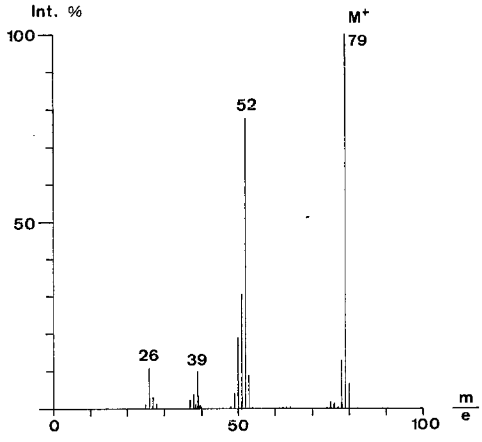

Fig. 8. Mass spectrum of pyridine.

Using a Faraday cup as collector the limit of ion detectability is in the range of 10^{-14} amp. Since a single charge carrier, independent of its nature, has a charge of about 1.6×10^{-19} Coulomb (and $1A = 1C/sec$), 10^{-14}A corresponds to about 100,000 ions/sec for a signal intensity close to that of the noise level. The usual dynamic range, i.e. the intensity ratio between strongest and weakest usable signal in the spectrum, is in general 10,000:1 whereby a very strong signal comes to represent around 10^9 ions/sec. If one adds a factor of 1,000 which may be possible to achieve by adjusting all parameters for maximal ion yield and by working at as high as possible a sample pressure, one obtains about 10^{12} ions/sec. In other words, to get 1 mg of a compound of a molecular weight 100, one would need to collect the ions of a very strong peak continuously for 12 days. Clearly from weak signals months or years would be necessary to isolate such an amount of sample. This does not then seem such an interesting proposition even if one is planning to collect several products simultaneously.

The material collected within less than 1 h from a very abundant ion peak would on the other hand be sufficient to be reintroduced for a check on the correctness of the expected structure by mass spectrometry. Here lies the great strength of this technique with respect to other methods of analysis, for in spite of the low yield of ionization mass spectrometry is extremely sensitive. The amount of sample represented by a normal mass spectrum is of the order of 10^{-14} *g*. Practically one can do analytical work with 1 μg or even less of a suitable sample although often for ease of handling rather more is used.

As an analytical technique used for isotope determination and structure elucidation mass spectrometry plays a predominantly auxiliary role in organic chemistry, providing a means for the chemist to speed up his work and check and confirm his results. A series of problems arises in this connection, the solution of which is allotted as a task to scientific research.

One of the problems concerns the instrumental equipment, which should be adjusted to suit the most important applications in an optimal fashion. Presently the development should be directed in two directions: instruments with very high performance with respect to mass separation and accuracy of mass determination on the one hand, and low cost instruments sufficiently powerful and rugged to be used as self-service instruments for general analytical purposes in the laboratory or as combination instruments directly connected to gas chromatographs. Today an analytical mass spectrometer of medium resolving power still costs about $50,000. Although such an investment pays off reasonably fast even in a research or production group of moderate size, there is an enormous need for smaller simpler instruments. To a large extent though this aspect should be the task of industrial research.

A second problem originates from the vast amount of data coming out of a mass spectrometer, especially when high resolution measurements of whole mass spectra are wanted or direct combination with gas chromatography is carried out. In the former case one obtains within minutes from a compound of moderate molecular weight several hundred signals, the individual masses of which must be measured with very high accuracy (to about 0.001 mass unit) and converted into elemental compositions. Both measurement and calculation on such a scale are beyond the range of manual handling. In the latter case one may produce several hundred spectra within hours from a complex mixture. Careful mass assignments to all peaks as well as many intensity measurements are necessary to make them interpretable or ready for comparison with reference data. This can be done manually in isolated cases but for routine operation it is impossible to take full advantage of the capability of such an instrument and manual data handling is no longer feasible. If one thinks of high resolution work in direct combination with gas chromatography manual data acquisition makes no sense at all. In this connection information problems are involved, which even for modern computer techniques are serious and call for a major mathematical effort. Automatic data acquisition and reduction systems are necessary here which can cope with the requirements of speed and accuracy. The

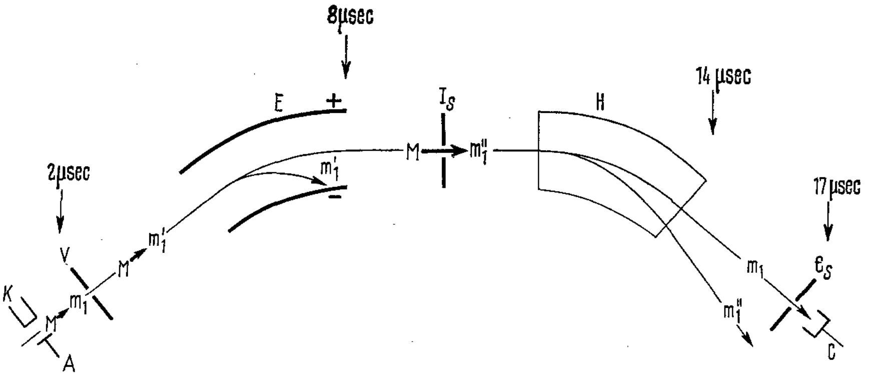

Fig. 9. General scheme of a double focussing mass spectrometer of Nier-Johnson-geometry. A, anode; K, cathode; V, acceleration potential; E, electrostatic sectorfield; I_s, intermediate slit; H, magnetic sectorfield; C_s, collector slit; C, collector.

Fields of mass spectrometry application

Physics	Anorganic chemistry, Geology	Cosmology, Medicine	Physical chemistry	Organic chemistry
Atomic weights	Vapour pressure of salts	Planetary atmosphere	Ionization potentials	Quantitative mixture
Mass and abundance of isotopes	Metal-analysis	Meteorites	Dissociation potentials	Qualitative mixture
Isotope separation	Soil-analysis	Respiration analysis	Kinetic investigations	Trace-analysis
Physics of ion formation	Rock-analysis	Toxicology	Isotope effects	Isotope-analysis
Residual gas analysis	Semiconductor-trapped gases	Metabolism	Radical-analysis	Elementary-analysis
Surface phenomena	Geological age determination		Fast reactions	Structure-analysis
Interactions between ions and matter				Pyrolysis
Nuclear reactions				Combination with separation techniques
				Reaction mechanisms
				Structure of ions
				Life time of ions

development of efficient and practicable systems is very important for full utilization of the tremendous power of the method.

Last but not least, innumerable questions of a chemical nature are posed in connection with structural interpretation of mass spectra of organic compounds. Although practised for nearly 20 years this method of qualitative structure analysis has still a strongly empirical character. The knowledge about stability and reactivity of the ions formed upon electron impact, kinetic factors of monomolecular degradation, energetical premisses and intercorrelations of different structure parameters is not sufficient to make safe a priori predictions. The situation is improving gradually as more and more real chemists are dealing with the subject but much work is still necessary before all the information available in mass spectra can be taken advantage of.

Related to this problem are possibilities inherent to mass spectrometry as a tool of independent basic chemical research. Again, considering the fact that basically one is dealing with a reaction spectroscopy, one can ask the question whether it is not possible to follow these reactions in situ with the mass spectrometer itself. This would mean an effort to determine the real structures of the ions formed, to measure their reaction constants, lifetimes, internal energies and related fundamental characteristics. There are in fact such possibilities, one of which[2] is briefly depicted in the sequel using Figure 9 which shows schematically a double focussing mass spectrometer. Everything corresponds to Figure 1, that is ion source, exit slit/acceleration electrode, magnetic field and collector, except that inserted between source and magnet is an electric sector field with an intermediate slit. The function of this field is to focus the moving ions with respect to their kinetic energy in order to achieve improved resolving power at the collector. The mass spectrometer is an optical system and can be evaluated accordingly. Mass separation efficiency or resolving power (corresponding to the minimal mass difference necessary between 2 ions to be seen by the detector as individual entities) is the better, the sharper an image of the ion source exit slit can be produced by a single ion beam at the collector slit. The magnetic field constitutes a lens, focussing ions of differing direction but not of differing velocities. The velocity of ions of identical mass leaving the ion source is not uniform for several reasons. This causes image distortions and concomitant loss of resolving power, which can be corrected for by the electric sector field.

The mass spectrometer is not only a highly sensitive but also a relatively fast device. A mean time schedule for a singly charged ion of mass 100 (M^+) at 2,000 V acceleration voltage and distances of 20 cm each within and between the different fields looks approximately like indicated in Figure 9: after formation the ions reside for about 1 μsec in the ion source, after about 2 μsec they have passed the acceleration field, 6 μsec later they leave the electric sector field and again 6 μsec later the magnetic field. Thus the overall process of ion formation and mass separation takes about 14 μsec. All signals of normal appearance in a spectrum are produced by ions with lifetimes sufficient to reach the collector. They all are formed within less than 1 μsec and must be relatively stable to reach the collector unchanged. The unstable precursors with lifetimes of less than 1 μsec are not observed. However there are ions with intermediate lifetimes, that is more than 1 μsec and less than 14 μsec, which decompose underway and may therefore be termed metastable. With this somewhat arbitrary but expedient classification the following picture results: The primary products formed in the ion source are the molecular ions M^+. Depending on the amount of excitational energy taken up they are stable, unstable or metastable. Stable ones reach the collector after about 17 μsec and show the molecular weight. Unstable ones decompose in the ion source, loose a neutral particle and form daughter

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[2] E. TAJIMA and J. SEIBL, J. Mass Spectrometry Ion Physics *2* (1969), in press.

ions m_1, which again may be stable, unstable or metastable. Stable ones reach the collector, in somewhat shorter time because of their higher velocity and appear in the spectrum at their nominal mass m_1 as normal signals. Metastable molecular ions with a lifetime around 4 µsec decompose after acceleration into daughter ions m'_1, which have the same mass as m_1 but are not identical in as much as they are slower. They have attained only the velocity of the heavier precursor ion M^+. Since their kinetic energy is smaller than that of normal ions of the same mass, they are caught by the negative sector electrode and do not appear at the collector. Metastable molecular ions with lifetimes of about 9 µsec will pass the sector field just as stable ones but decompose before entering the magnetic field into daughter ions m''_1. These have the same mass and velocity as the previously mentioned ions m'_1 but they do appear in the spectrum. Since their kinetic energy is smaller they are deflected more in the magnetic field than normal daughter ions m_1 and registered at a mass lower than their nominal mass value. Where they appear in the spectrum is determined by their velocity and therefore by the mass of the precursor ion M^+ and can easily be calculated to be m_1^2/M. One finds these products as diffuse peaks of low intensity at the calculated mass. In the literature they are referred to as 'metastable peaks' but they will be called transition signals in this context to avoid confusion with metastable ions. In fact such transition signals are the only experimental evidence in mass spectra, that fragments are formed in sequential processes and not only by simple scission of molecular ions.

Normally acceleration voltage and sector voltage are tuned in a way that all normal ions travel along the axis of the sector field and are registered. If one adjusts the magnetic field for registration of daughter ion m''_1 and then gradually diminishes the sector field voltage, the trajectories of the ions in the field are stretched proportionally and soon the main beam is filtered out by the intermediate slit. At some predictable value of the sector voltage (determined by the mass ratio m_1/M), the trajectory of m'_1 will be suitably stretched and m'_1 will appear at the collector to give a sharp signal. Under these operating conditions only ions m'_1 arising from degradation of a specific type of metastable ions are registered and by comparison with ions of the type m_1 and m''_1 one has a means for measuring reaction rates and other characteristics. One can expand the range of measurement by varying acceleration voltage and residence time of the ions in the source.

If one scans the whole mass range magnetically under these selective conditions, one can sometimes observe besides the sharp peaks due to ions m'_1 again diffuse transition signals, which must arise from further degradation of m'_1 before they enter the magnetic field for mass separation. Thereby two- and even three-step reaction sequences have become directly observable for the first time.

A practical example is illustrated by Figure 10. The upper figure shows the normal mass spectrum of the drawn perinaphtholactone with typical transition signals (indicated by arrows) for 2 decarbonylations and subsequent loss of acetylene, all occurring in the region between magnetic and electric sector fields.

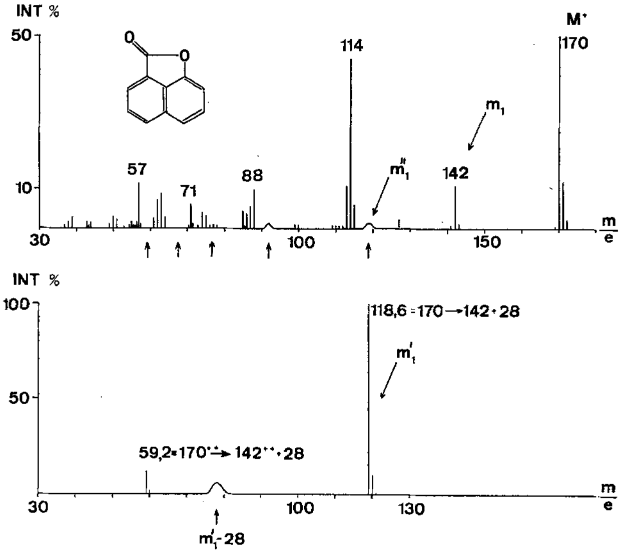

Fig. 10. Normal mass spectrum of 1,8-naphtholactone and selector spectrum from molecular ion decarbonylation ($E/E_0 = 0.835$).

The lower figure shows the spectrum obtained under conditions, where the electric sector voltage is properly set for registration of the first decarbonylation product (M^+-28) as formed in a metastable ion transition in the region between acceleration and electric sector field. The sharp signal at m/e 118 comes from the daughter ion. Another sharp signal at m/e 59 arises from the same reaction but started from the doubly charged molecular ion (M^{++}-28). The additional diffuse signal at m/e 76.5 finally is the product of further loss of CO from (M^+-28) and proves a two-step degradation sequence.

If successful appearance potential measurements can be made of such primary and secondary products of metastable transitions, valuable thermodynamic data would be available which are of general interest to chemistry.

There are other possibilities for similar basic research by mass spectrometry[3] which just barely are starting to be investigated and look like promising fields for scientific activities.

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[3] J. H. Beynon, *Revue in Advances in Mass Spectrometry* (Inst. of Petroleum London and Elsevier, Amsterdam and New York 1968), vol. 4, p. 123.

Some of the aspects of mass spectrometric structure analysis in organic chemistry may be summarized visually as shown by Figure 11, where the unknown mass spectroscopist is trying with apparent concentration and determination to reveal with the aid of his instrument some of the secrets of the shape and coherence of organic molecules.

Fig. 11. The unknown mass spectrocopist.

Zusammenfassung. Massenspektrometrie ist die instrumentalanalytische Methode mit der grössten Anwendungsvielfalt im naturwissenschaftlichen Bereich. Auf dem Gebiet der organischen Chemie sind derzeit qualitative Strukturanalyse und die Analyse stabiler Isotopen die wichtigsten in einer ganzen Reihe von Applikationsmöglichkeiten. In strukturanalytischer Hinsicht handelt es sich im wesentlichen um eine chemische Reaktionsspektroskopie, die als Nebenprodukt der Ionenerzeugung anfällt. Man könnte diese Tatsache für synthetische oder präparative Zwecke ausnützen, wenn der Mengendurchsatz in einem Massenspektrometer nicht so klein wäre.

Der wissenschaftlichen Forschung sind in diesem Zusammenhang Aufgaben gestellt, welche die Entwicklung optimaler Geräte zum Ziel haben, eine wirkungsvolle Bearbeitung und Auswertung der riesigen Datenmengen, die besonders in Zusammenhang mit hochauflösender Massenspektrometrie und in direkter Kombination mit Gaschromatographie anfallen, sowie ein besseres Verständnis für die Vorgänge, die der massenspektrometrischen Strukturanalyse zugrunde liegen, um sichere Voraussagen zu ermöglichen.

In jüngster Zeit zeichnet sich eine Entwicklung ab, die sich das Massenspektrometer als vielversprechendes Instrument der chemischen Grundlagenforschung zunutze macht. Die Untersuchung des Zerfalls metastabiler Ionen scheint eine wirkungsvolle Methode zu werden, um Informationen über die Kinetik und Thermodynamik monomolekularer Abbaureaktionen zu erarbeiten.

SPECIALIA

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Comparative Acetoacetylation of Proteins

Among the acylation procedures for proteins, the acylation method with acetic anhydride[1], the trifluoroacetylation with ethylthioltrifluoroacetate[2] and the succinylation with succinic anhydride[3] have been described. Furthermore, other methods of amino group modification have been proposed, with reagents such as carbon disulphide[4], methylacetamidate or benzimidate[5,6].

The acetoacetyl group, formerly proposed as a protective agent in peptide synthesis[7], seemed to combine the most advantageous characteristics of a reversible blocking and was employed in our laboratory for modification of proteins[8,9].

The acetoacetylation was carried out by using diketene. Hydroxylamine was chosen to remove the acetoacetyl group. Both the acetoacetylation and the deacetoacetylation step after hydroxylamine treatment are represented in the following general scheme:

This general reaction is dependent on the molar ratio of reagent x to protein $R\text{–}(NH_2)_n$, the number of the groups introduced may vary from a small number all the way up to $_n$, that is to complete coverage of amino groups of the protein. The reagent shows a strong preference for amino groups but reacts also with hydroxyl groups under suitable circumstances. An easy removal of the acetoacetyl function from residues other than amino groups has been shown to be possible after a carbonate-bicarbonate buffer treatment[8].

50 mg of lysozyme (Fluka) or ribonuclease (Fluka) were dissolved in 4 ml of distilled water and diketene (T. Schuchard GmbH & Co.) was added in a suitable