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High-Resolution Mass Spectrometry

Elemental composition and structural parts of a molecule are indicated from a submicrogram sample.

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The mass spectrometer yields a "line spectrum," in contrast to the overlapping band spectra from many other types of spectrometers. This makes the mass spectrum extremely useful for purposes of analysis. For example, molecular ion signals from ethane (C_2H_6), mass 30, and methyl amine (CH_3NH_2), mass 31, are completely separated. This advantage is markedly increased in spectra from high-resolution mass spectrometers, which have recently become commercially available. The high-resolution instrument can even distinguish between molecules such as ethane and formaldehyde (CH_2O), although the latter also has a nominal molecular weight of 30. The molecular ions of C_2H_6 and CH_2O are isobaric—of the same nominal mass, but of different elemental compositions. Monoisotopic atomic weights related to an atomic weight of carbon of 12.00000 are not exact whole numbers (see Table 1); thus, the masses of $C_2H_6^+$ and CH_2O^+ are at 30.0469 and 30.0105, respectively, and the mass of CH_4N^+, an isobaric fragment ion of ethylamine, is 30.0344. A high-resolution mass spectrum of a mixture of these compounds exhibits three separate peaks in the mass-30 region, whose heights (relative abundances) are a measure of the composition of the sample.

Another recent advance in analytical mass spectrometry has greatly increased the value of, and need for, information obtained with high-resolution instruments. Previously, vapor-pressure requirements had limited studies mainly to samples with molecular weights below 500. This limit has been raised to 1000 to 2000 by the development of systems for vaporizing the sample directly in the ion source (at a pressure of about 10^{-7} torr). However, the increase in the size of the molecules that can be studied is accompanied by an increase in the complexity of the spectra. The possible molecular combinations of the elements carbon, hydrogen, nitrogen, and oxygen increase from a few at nominal mass 30 to over 50 at mass 310 (Fig. 1). The inclusion of all possible fragment ions greatly increases the complexity of the problem of identifying an unknown spectral peak which the low-resolution instrument identifies only as mass 310. Many elements in addition to these four are commonly found (see Table 1), so that, for a nominal molecular weight of 1000, literally millions of different possible elemental formulas can be written. Although present high-resolution instruments can differentiate only a small proportion of the exact mass classifications for these formulas, use of these instruments helps substantially to offset the complexity of the mass spectra of samples of this molecular weight.

The advantages of high-resolution mass spectrometry were first recognized and demonstrated by J. H. Beynon (*1, 2*) and his group at Imperial Chemical Industries. Recently many developments and applications have been reported by Klaus Biemann and his co-workers at the Massachusetts Institute of Technology (*3–6*) and from the laboratory of Associated Electrical Industries, a manufacturer of mass spectrometers (*7, 8*). These reports, plus the recent commercial availability of instruments, have led to a great awakening of interest in the last few years. Many laboratories have acquired or are planning to install such equipment.

Principles of Mass Spectrometry

Many excellent reviews on the fundamentals and instrumentation in the field of mass spectrometry are available (*1–3, 7–12*).

The ion source of the mass spectrometer may be visualized as a continuous reactor in which the sample molecules are bombarded by energetic electrons (energies of about 70 volts). Background pressures in the ion source are kept below 10^{-7} torr (10^{-10} atmosphere). By limiting the sample so that the pressure it exerts is less than 10^{-4} torr, secondary reactions of the ion, radical, and neutral products formed are minimized. To obtain the mass spectrum, the mixture of positive ions is electrostatically accelerated from the ion source and separated according to mass, and the amounts of the ions are measured.

A wide variety of methods are employed for separation according to mass (*2, 9–12*); magnetic deflection is the most common. For high resolving power, it is necessary to focus the ions with respect to velocity as well as direction. Figure 2 shows an instrument which utilizes the Mattauch-Herzog type geometry, one of the two common geometries for achieving double focusing. In this geometry the ions are first sorted by the electrostatic analyzer into monoenergetic paths. The deflection of these paths by means of the magnetic separator compensates for the differences in energy of the sorted ions, so that the ions are refocused along a plane according to mass (actually, according to

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the mass-to-charge ratio m/e). The spectrum can be scanned by the conventional sweeping of the magnetic field or ion accelerating potential, the ions being recorded as they come in focus on a fixed exit slit. Additionally, this geometry has the advantage that all the ions can be simultaneously recorded on a photographic plate placed in the focal plane of the instrument. Such an integrating recorder is especially valuable for obtaining the complete high-resolution spectra of microsamples introduced into the instrument at rates which change rapidly, as in the continuous monitoring of effluents from a gas chromatography column (*5*).

Modern electron multipliers are able to detect single ions, so that sensitivities of a few parts per million can be achieved despite the low ion currents produced. The low requirement for sample pressure in the small ion chamber can be met by direct vaporization of 1 microgram or less of sample, although careful control of volatilization and fast recording methods are necessary. In a conventional inlet system the vaporized sample is stored in a large reservoir at a pressure higher than that of the ion chamber and allowed to leak continuously into the ion source through a pinhole opening. This system makes possible a nearly constant flow rate and highly reproducible spectra, but it requires milligram-size samples and much higher vapor pressure than is necessary when the sample is introduced directly into the ion source.

The interaction of a bombarding electron with a molecule of sample can result in the ejection of another electron from the molecule. The resulting positive ion usually has excess energy. According to the "quasi-equilibrium theory" (*13*), this energy is distributed among the available energy states of the ion. Many of these states are unstable, each such state leading to the decomposition, at a particular rate, of the molecular ion to a product ion plus a neutral fragment or fragments. The mass spectrum observed is thus composed of the remaining molecular ions plus the product ions, the relative amounts of molecular and product ions being determined by the rates of formation and of subsequent decomposition. For most instruments, the effective reaction times are microseconds or less.

Table 1. Nuclides frequently found in high-resolution mass spectrometry.

Nuclide	Exact mass
H^{1}	1.0078252
C^{12}	12.0000000
C^{13}	13.003354
N^{14}	14.003074
O^{16}	15.99491415
F^{19}	18.998405
Si^{28}	27.976927
P^{31}	30.973763
S^{32}	31.972074
Cl^{35}	34.968855
Br^{79}	78.918348
I^{127}	126.904352

Relation of Spectra to Structure

For many molecules, the molecular ion is stable enough so that at least a measurable concentration of these ions reaches the collector. Thus the molecular ion will appear as the peak of highest mass in the spectrum. Measurement of this mass with sufficient accuracy can give absolute identification of the elemental composition (the absolute number of the atoms of each chemical element which composes the particular molecule) or can at least reduce the possibilities to a small number from which one can choose the correct formula by using other information concerning the sample. Because of the mass spectrometer's submicrogram sample requirements, speed, and unlimited range of elements, it is rapidly proving to be a valuable complement to classical combustion analysis for the determination of the elemental composition of organic compounds. Knowledge of the elemental formula of the molecule also permits one to compute the number of rings and double bonds in the molecule from a formula derived from the valences of the elements (*14*).

Only in recent years have chemists recognized the value of the mass-spectral fragment ions for providing information about molecular structure. In simplest terms, obtaining structural information from a mass spectrum may be likened to assembling a three-dimensional jigsaw puzzle. The molecular ion has been decomposed into a wide variety of fragments, and high-resolution mass spectrometry reveals their elemental compositions and relative abundances. To interpret the spectrum, hypothetical molecules containing these fragments are constructed, and the types of ion decomposition predicted for these models are checked against the ion abundances found. Unimolecular ion decomposition reactions—an interesting new field of chemistry—are often surprisingly similar to other chemical reactions. Intensive studies of such mechanisms have been made recently, and our understanding of them is growing rapidly. Such knowledge, plus study of similar reference spectra of known compounds, has produced a growing literature on elucidation of the structure of complex molecules.

The low-resolution spectrum of

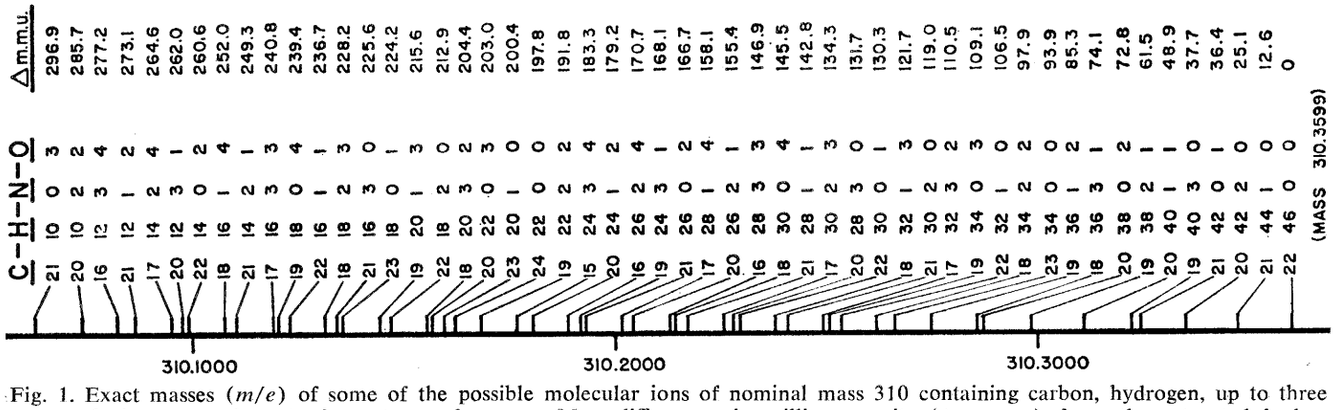

Fig. 1. Exact masses (m/e) of some of the possible molecular ions of nominal mass 310 containing carbon, hydrogen, up to three atoms of nitrogen, and up to four atoms of oxygen. Mass differences in millimass units (Δ m.m.u.) from the saturated hydrocarbon are shown.

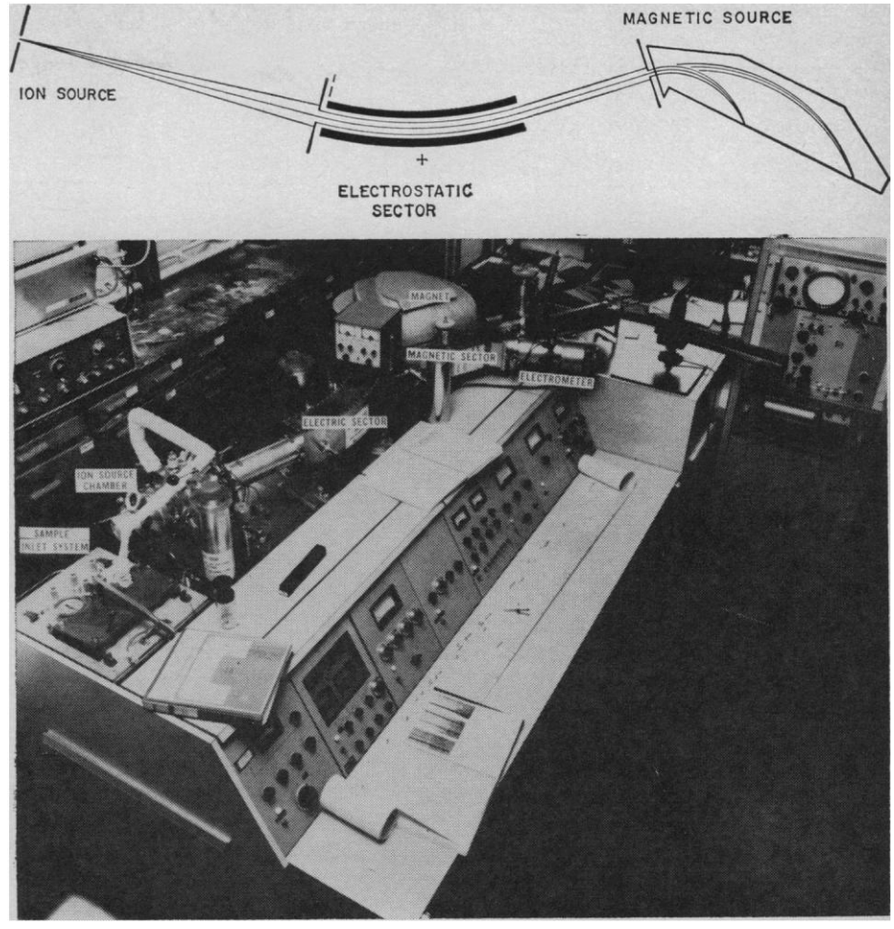

Fig. 2 (top right). Schematic diagram and top view of the high-resolution mass spectrometer of Mattauch-Herzog geometry (Consolidated Electrodynamics Corporation Model 21-110) in the chemistry laboratory at Purdue University. Double focusing of the ion beam by the electrostatic and magnetic analyzers makes possible mass measurements of an accuracy of a few parts per million. Spectra can be recorded electrically or on a photographic plate placed in the focal plane of the magnetic analyzer.

octan-4-one given in Fig. 3 shows major peaks at masses 43, 57, 71, and 85, which can arise through initial cleavages of the molecular ion at the bonds adjacent to the carbonyl group. For example, an expected reaction for such compounds is cleavage of the 4,5-carbon-carbon bond to form the acylium ion $CH_3CH_2CH_2CO^+$ (mass 71) and the butyl radical $CH_3CH_2CH_2CH_2\cdot$. Further decomposition of this ion through loss of the stable carbon monoxide molecule yields the propyl ion $CH_3CH_2CH_2^+$ (mass 43). The reaction would be as follows:

$$CH_3CH_2CH_2COCH_2CH_2CH_2CH_3 \rightarrow CH_3CH_2CH_2CO^+ + \cdot CH_2CH_2CH_2CH_3$$

$$CH_3CH_2CH_2CO^+ \rightarrow CH_3CH_2CH_2^+ + CO$$

The propyl ion can also be formed directly by cleaving the molecular ion. Peaks at masses 58, 86, and 100, however, cannot be explained readily in terms of combinations of simple bond cleavages. Studies with isotopically labeled molecules show that the ions which produce these peaks arise through specific rearrangement processes. Cleavage of the 5,6-carbon-carbon bond, accompanied by rearrangement of a hydrogen atom from carbon-7 to the carbonyl oxygen atom, yields a resonance-stabilized radical ion of mass 86 and accompanying ejection of the stable propylene molecule. The involvement of a sterically favorable six-membered-ring transition state and the stability of the products formed are thought to be strong driving forces for this reaction. Note that this rearrangement (Fig. 4)

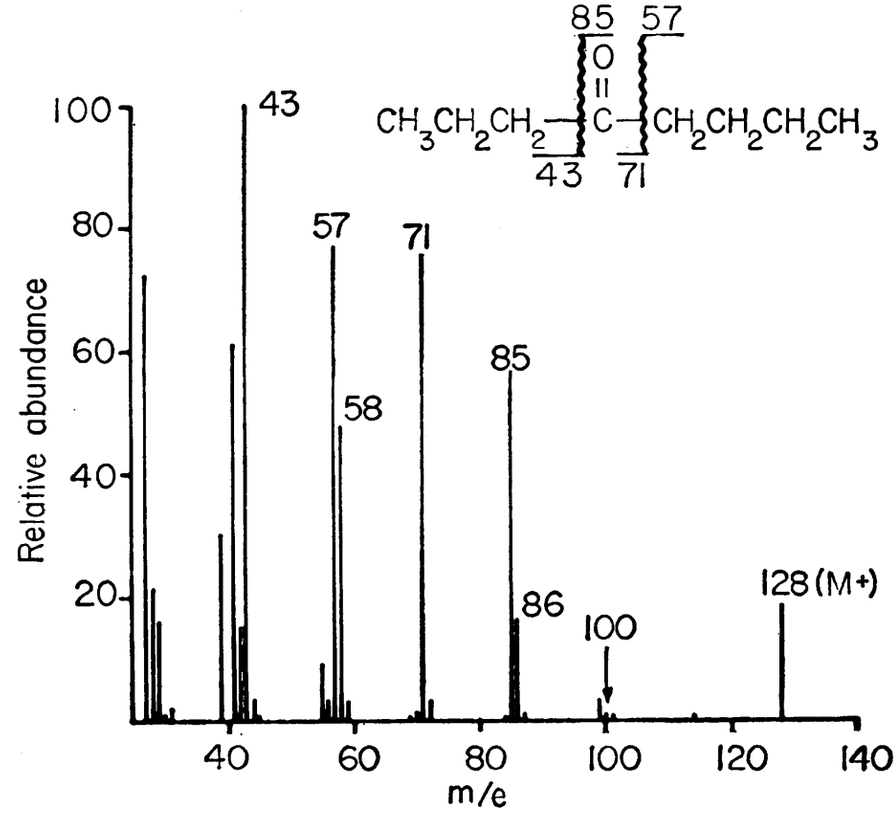

Fig. 3 (bottom right). Mass spectrum of octan-4-one (see *11*). Four of the largest fragment ions can be related to the cleavage of the labile bonds adjacent to the carbonyl group. Peaks at *m/e* 58, 86, and 100 are due to specific rearrangement reactions involving cleavage of the α-β carbon-carbon bond with rearrangement of the hydrogen atom on the γ carbon (see Fig. 4).

Table 2. Possible formulas considered for the alkaloid vobtusine (*12*).

Empirical formula	Molecular weight	Carbon content (%)	Hydrogen content (%)	Nitrogen content (%)
$C_{45}H_{54}N_4O_8$	778.3941	69.46	6.95	7.20
$C_{42}H_{48}N_4O_6$	704.3574	71.57	6.86	7.95
$C_{42}H_{50}N_4O_7$	722.3679	69.78	6.97	7.75
$C_{43}H_{50}N_4O_6$	718.3730	71.84	7.01	7.79
$C_{42}H_{46}N_4O_7$	718.3366	70.17	6.45	7.79

allows formation of a new bond for each one that is broken (*9*).

Careful study of a number of such rearrangement processes has given us sufficient understanding of them to make them quite useful for the elucidation of structure. For example, the peak at mass 58 ($C_3H_6O^+$), formed by a sequence of two such rearrangement reactions (the reaction involving cleavage of a bond between α- and β-carbon atoms with rearrangement of the γ-hydrogen) gives evidence that neither of the carbon atoms adjacent to the carbonyl group is substituted.

Viewing this spectrum as an unknown poses some new problems which illustrate the value of high-resolution mass spectrometry. From the explanation given above of the spectrum of Fig. 3 one might conclude that abundant ions of masses 71 and 85 would indicate the presence of propyl and butyl ketones, respectively. Extrapolating from this explanation to lower homologs, one might expect to find that masses 43 and 57 are typical of methyl and ethyl ketones, respectively. Although $C_2H_3O^+$ and $C_3H_5O^+$ can indicate such compounds (*15*), for the spectrum of Fig. 3 major components of the peaks at masses 43 and 57 are the isobaric ions $C_3H_7^+$ and $C_4H_9^+$, indicative of the alkyl substituents on the carbonyl group. Obviously, information on elemental composition such as is provided by high-resolution mass spectrometry would simplify the interpretation of an unknown spectrum of this kind.

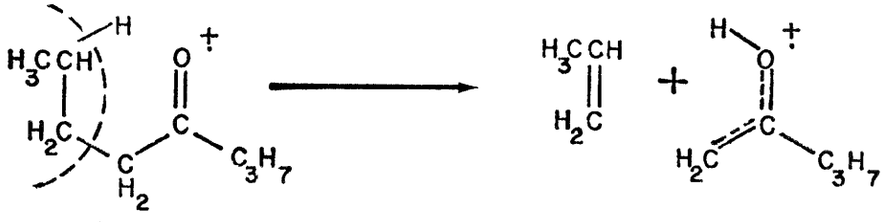

Fig. 4. Mechanism for the formation of the *m/e* 86 ion in the spectrum of octan-4-one (Fig. 3) through the specific rearrangement of a γ-hydrogen atom.

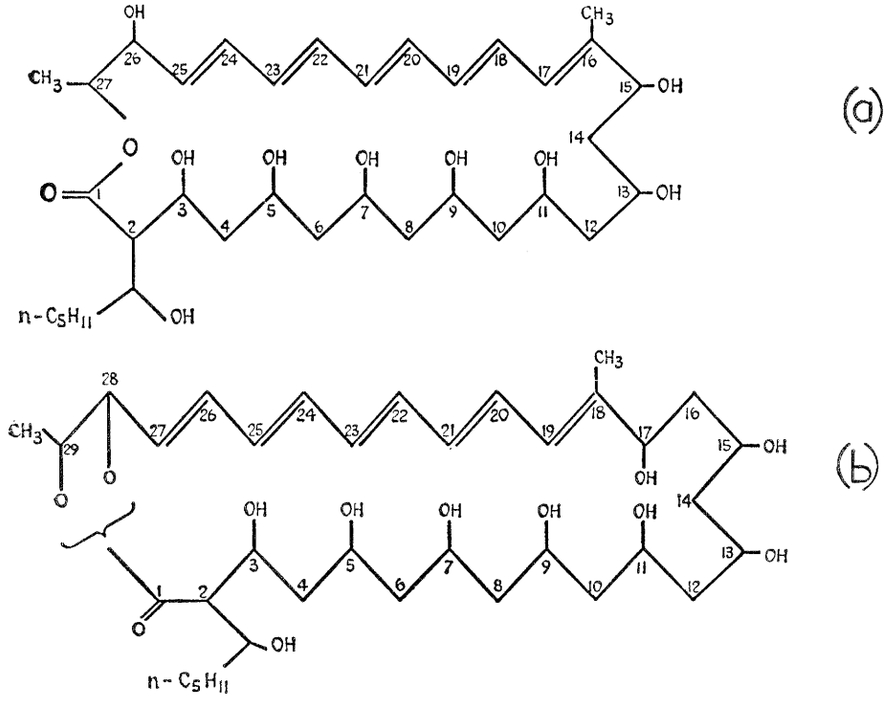

Fig. 5. Alternative structures proposed for filipin, on the basis of spectrochemical evidence. The elemental composition is $C_{35}H_{58}O_{11}$, as determined from high-resolution mass spectrometry of the pertrimethylsilyl ether derivative; this composition corresponds exactly with structure *a*.

Determinations of Molecular Structure

Djerassi and his co-workers have collected a number of interesting examples (*12*) of natural products whose assigned elemental compositions were shown, by mass spectrometry, to be incorrect. Classical elemental carbon, hydrogen, and nitrogen analysis often cannot differentiate unequivocally between two possible components, such as large molecules differing only by a CH_2 group. The data given in Table 2 for the alkaloid vobtusine illustrate this point. From microanalyses and other information it was concluded that the empirical formula was one of the following: $C_{45}H_{54}N_4O_8$, $C_{42}H_{48}N_4O_6$, or $C_{42}H_{50}N_4O_7$. A single-focusing mass spectrometer showed the molecular weight to be 718, a value incompatible with any of these three formulas. High-resolution spectrometry showed $m/e =$ 718.3743, clearly indicating that the formula $C_{43}H_{50}N_4O_6$, molecular weight 718.3730, is, unexpectedly, the correct one.

Barber and his co-workers recently investigated (*8*) the structure of filipin, a polyene macrolide antibiotic. Chemical and spectroscopic (infrared, ultraviolet, and nuclear-magnetic-resonance) evidence had led to proposals for two different molecular formulas (Fig. 5). Although these structures are quite similar chemically, they differ in elemental composition by C_2H_4O. A direct answer to this question by mass spectrometry proved difficult, however, illustrating a problem often found in the analysis of complex polar molecules, such as this polyhydroxy compound. Even with direct heating of such large polar molecules in the ion source, often a sufficiently high vapor pressure cannot be obtained without some thermal decomposition or rearrangement. Use of chemical derivatives provides a con-

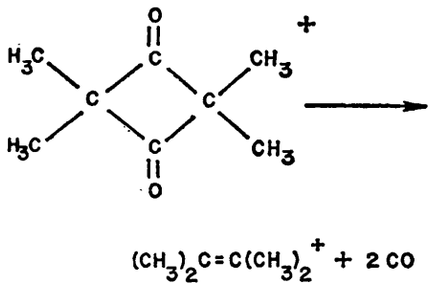

Fig. 6 (left). Rearrangement mechanism for the formation of the *m/e* 84 ion in the spectrum of tetramethylcyclobutan-1,3-dione (see Fig. 7) through the expulsion of two molecules of carbon monoxide.

venient means of increasing the volatility and thermal stability. The hydroxyl groups of filipin and its fully hydrogenated derivative were converted to the corresponding trimethylsilyl ethers, $-OSi(CH_3)_3$. At a source temperature of approximately 170°C, these derivatives give vapor pressures such that mass spectra can be obtained. These spectra conclusively support the validity of structure *a* of Fig. 5. The mass of the molecular ion of perhydrofilipin pertrimethylsilyl ether, measured relative to the $C_{28}F_{51}^+$ (mass 1305) from perfluorolube residues, is 1312.8341, a value within 2 parts per million of the theoretical value of 1312.8319, or $C_{35}H_{59}O_{11}[(CH_3)_3Si]_9$. The spectra of the two compounds are also closely similar to those of lagosin and perhydrolagosin, respectively. Lagosin is known to have the structure of Fig. 5*a* plus a 14-OH group.

When peaks in a spectrum (such as the spectrum of Fig. 3) obtained with a single-focusing spectrometer are compared with the peaks in the high-resolution spectrum of the same sample, it often becomes apparent that the ion decomposition reactions are not as simple as they had appeared to be. In the low-resolution spectrum of tetramethylcyclobutan-1,3-dione, Nicholas Turro (*16*) observed a large peak due to the loss of 56 mass units. He proposed that this peak is due to a facile loss of two molecules of carbon monoxide (Fig. 6), in striking analogy to the photolytic behavior of this compound.

Our high-resolution spectrum (Fig. 7) confirms this interpretation and illustrates the advantage of photographic-plate recording, which makes all the data available for examination. Measurements from the $C^{13}HCl_2^+$ and $CH_2Cl_2^+$ reference lines conclusively identify the peak at nominal *m/e* 84 as $C_6H_{12}^+$. It seems logical to assume that the peak at *m/e* 83 in the low-resolution spectrum is the $C_6H_{11}^+$ ion. In Fig. 7, such an ion line is found at the pre-

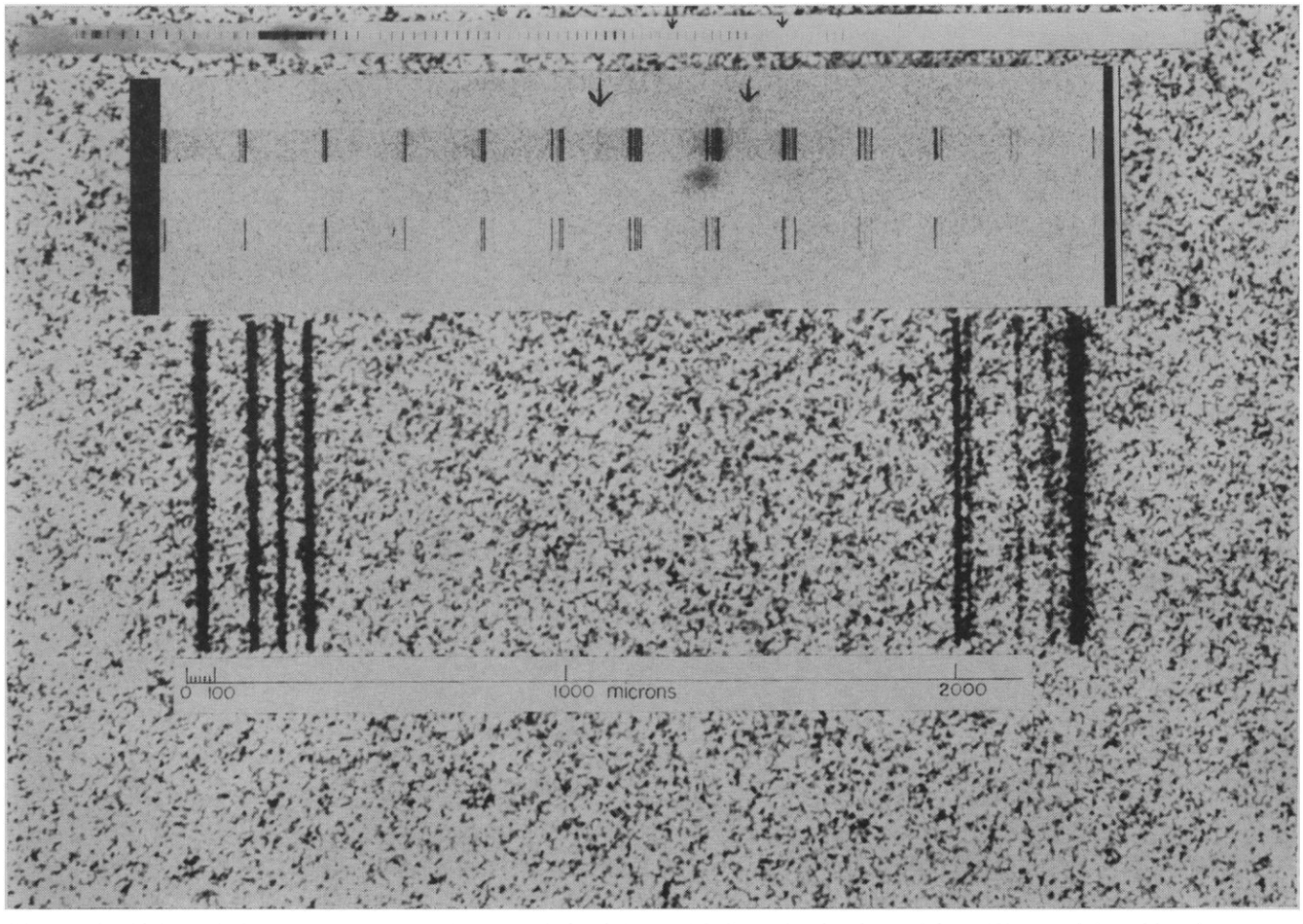

Fig. 7. The high-resolution mass spectrum of tetramethylcyclobutan-1,3-dione (molecular weight, 140) containing chloroform as an internal mass standard. (Top) Magnification of the photographic-plate spectrum for the region from *m/e* 24 to *m/e* 141. The two middle spectra are for the region *m/e* 77 to *m/e* 89 (region between the arrows in the top spectrum), recorded at twice the photographic-plate dispersion; they differ in total ion current by a factor of 10. (Bottom) Magnification of the region between the arrows (from *m/e* 83 to *m/e* 84) of the third spectrum from top. Exact mass measurement of these multiplets identifies the ion lines (left to right) as: *m/e* 83, $CHCl_2$ (reference line), $C_4H_3O_2^+$, $C_5H_7O^+$, and $C_6H_{11}^+$; and *m/e* 84, $CCl^{35}Cl^{37+}$ (reference line), $C^{13}HCl_2^{35+}$, $C_3C^{13}H_3O_2^+$, $C_4C^{13}H_7O^+$, and $C_6H_{12}^+$ (the $C_5C^{13}H_{11}^+$ line is hidden at this exposure). The presence of oxygenated ions had not been suspected.

dicted distance from the $CHCl_2^+$ reference line. However, between these lines lie lines for two additional isobaric ions. Careful measurement shows that these are $C_5H_7O^+$ and $C_4H_3O_2^+$; the structures shown in Fig. 8 may be contributing ion structures.

A dramatic example of research of the kind that will be possible with high-resolution mass spectrometry is the research on amino acid sequences in polypeptides, by Lederer and his co-workers, cooperating with Barber and his associates (*17*). From previous studies made with classical and spectrochemical techniques the peptidolipid fortuitine, isolated from *Mycobacterium fortuitum*, was assigned the preliminary structure

$CH_3(CH_2)_nCO$-Val-Val-Val-(Thr-Thr)(-$OCOCH_3$)-Ala-Pro-OCH_3

($n = 18, 20$). Ambiguities in further work led to determination of the mass spectrum, shown in Fig. 9. That fortuitine is a mixture having either C_{20} or C_{22} fatty acid moieties is shown by the groups of peaks, throughout the spectrum, separated by 28 mass units. The molecular weights of the two components are clearly shown to be 1331 and 1359. Peaks indicating another pair of ion groups occur at 1211 and 1239; this pair corresponds to the loss of two acetic acid molecules. Thus fortuitine is a diacetate, not a monoacetate as had previously been supposed. The next pair of ion groups, located at the further loss of 128 mass units, corresponds to the amino acid proline as the terminal methyl ester. In the same way losses of 72 and 83 mass units can be correlated, respectively, with cleavage of an alanine plus hydrogen, and an anhydrothreonine as the next amino acid units of the chain. This assignment is confirmed by a measurement of mass, made at high resolution, for the peak at *m/e* 928 (1331 − 120 − 128 − 72 − 83) as 928.7327, while the formula $C_{53}H_{96}N_6O_7$ for this ion gives a theoretical mass of 928.7340. Further sequential losses of (i) anhydrothreonine minus hydrogen, (ii) methylleucine, (iii) valine, (iv) valine, and (v) methylleucine are discernible in the mass spectrum; the elemental compositions of some of the pertinent ions have been checked by high-resolution techniques. This work gives the whole structure of fortuitine as

$CH_3(CH_2)_{18-20}CO$-Val-MeLeu-Val-Val-MeLeu-Thr(-$OCOCH_3$)-Thr(-$OCOCH_3$)-Ala-Pro-OCH_3

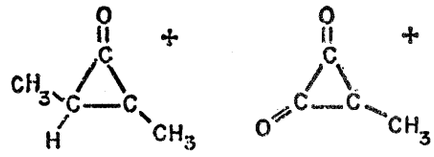

Fig. 8. Possible structures for the unexpected ions C_5H_7O and $C_4H_3O_2$ found at nominal mass 83 in the spectrum of tetramethylcyclobutan-1,3-dione (see Fig. 7).

Further work by Lederer and Barber and their associates and work in our laboratory give promise that amino acid sequence in a number of types of peptides may be determinable. Because only submicrogram amounts of sample are required, and because of the potential speed and ease of the determinations, this is a promising breakthrough.

Element Mapping

In the examples given above, an initial study of the low-resolution spectrum indicated key peaks for which data obtained with high-resolution techniques would be valuable, and these peaks were then measured by means of a double-focusing instrument. For the fortuitine analysis, the exact masses of five peaks were reported. No more than five were measured because considerable structural information was available from other studies, and because the geometry (Nier-Johnson) of the double-focusing instrument (*2*) used requires that individual measurements of the mass of each peak be made while the sample is flowing through the instrument.

Biemann and his co-workers have recently reported a new and revolutionary approach to high-resolution spectrometry which they call "element mapping" (*3, 4*). In this approach, exact measurements of mass are obtained for most of the ions produced, and the possible elemental compositions are calculated. These ions include many which, when studied at low resolution, have been considered of little value for the determination of structure, and thus, with the element-mapping technique, sometimes hundreds of initial exact determinations of mass are made from each spectrum. The spectrum is interpreted, then, not in light of the *mass* of the ions produced but directly according to the elemental formulas of the molecular fragments.

The element map (Fig. 10) is produced by an initial sorting, by computer, of the elemental formulas into columns according to content of hetero atoms; the columns are arranged in ascending order of numbers of carbon and hydrogen atoms. Figure 10 displays the elemental composition and relative abundances of most of the ions of $m/e > 93$ in the mass spectrum of deoxydihydro-N_b-methylajmaline (*3*). Column 1 shows the nominal mass-to-charge ratio of the ions. Column 2 lists the ions that contain no oxygen or nitrogen—$C_7H_{11}^+$, $C_8H_7^+$, $C_9H_7^+$, and $C_{10}H_7^+$. Column 3 indicates that there is no appreciable quantity of ions that contain only one oxygen and no nitrogen atoms. Column 4 shows that there are many ions of the general formula C_xH_yN. Because molecular size increases as one reads across (from left to right) and down the rows of the map, the molecular ion, if present, will be found in the lower right-hand corner (column 7). Thus the elemental composition of this compound is indicated to be $C_{21}H_{30}N_2O$.

The advantage of the element map for interpreting the structural significance of fragment ions is immediately apparent when one examines the other entry in column 7 of Fig. 10—$C_{17}H_{21}N_2O$. Ions of this formula can only be formed by the loss of a C_4H_9 moiety from the molecular ion, and this entry thus directly indicates the saturated C_4 side chain of the molecule. Formation of the next-smaller fragment ion (column 6) is already accompanied by the loss of the oxygen atom and of seven, eight, or nine carbon atoms. The abundant $C_{13}H_{13}N_2$ can be formulated as a resonance-stabilized β-carboline quaternary-type ion. Column 5 contains ions with a high hydrogen-to-carbon ratio, which indicates that the oxygen atom is located in the alicyclic area of the molecule. [The hydrogen-to-carbon ratios for cyclohexane (C_6H_{12}) and

Fig. 10 (right). Element map of deoxydihydro-N_b-methylajmaline (*3*). Column 1 shows the nominal mass-to-charge ratio (*m/e*) of the observed ions of the spectrum. Columns 2–7 give the elemental formulas determined from the exact masses; in each column ions of a particular number of hetero atoms are listed. For example, the first two numbers (6/8) of the first listing of column 4 (headed *CHN*) signify ions of the formula $C_6H_8N^+$. The third number (0) signifies the error of mass measurement, in millimass units. The asterisks indicate the relative ion abundance on a logarithmic scale.

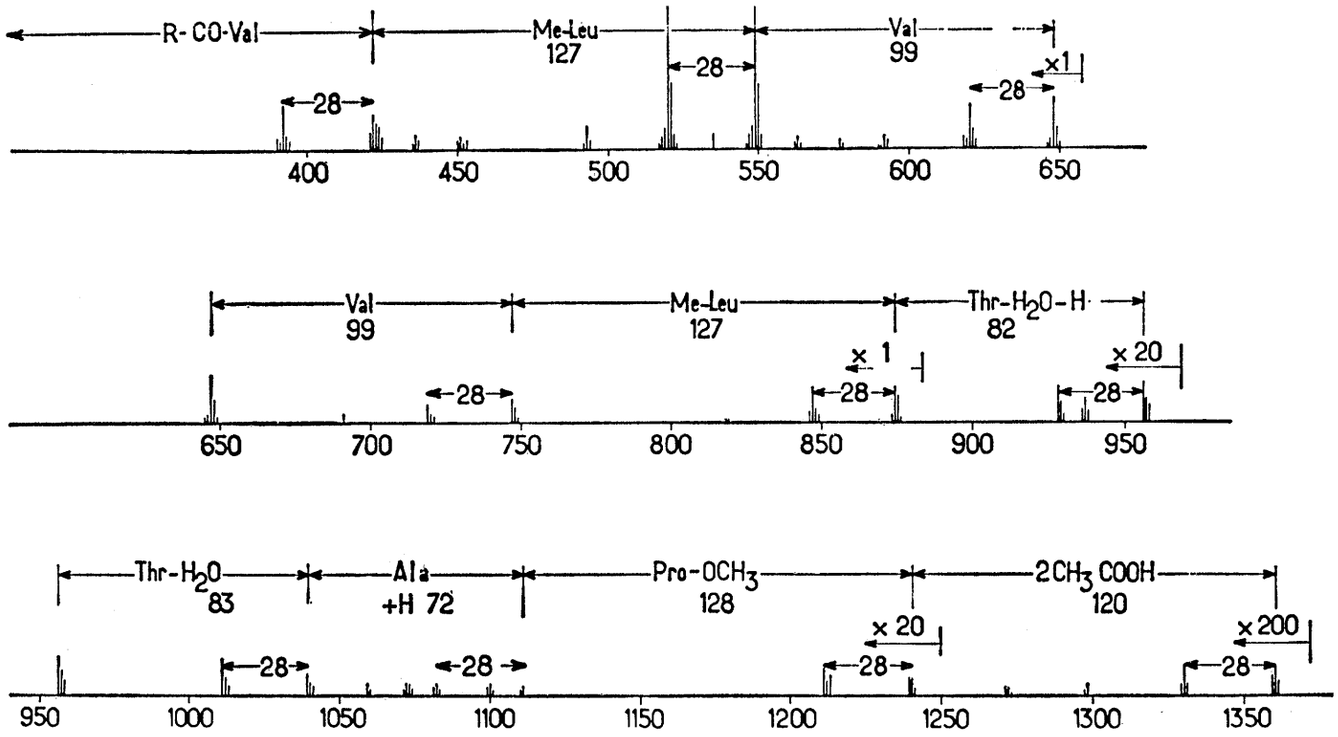

Fig. 9. Mass spectrum of the peptidolipid fortuitine, showing the sequential loss of the amino acid units. The sample is a 1 : 1 mixture of compounds in which the terminal fatty acid moiety *R* is either $C_{19}H_{39}$ or $C_{21}H_{43}$. This mixture gives rise to sets of peaks separated by 28 mass units in the spectrum.

DEOXYDIHYDRO-N_b-METHYLAJMALINE 52-11-2

	CH	CHO	CHN	CHNO	CHN2	CHN2O
94			6/ 8 0****			
95	7/11 0**		6/ 9-0***			
98				5/ 8 1******		
103	8/ 7-0***					
106			7/ 8 0**			
107			7/ 9-0*			
108			7/10-0***			
110			7/12-0****			
115	9/ 7-0***					
117			8/ 7 0**			
118			8/ 8 0*			
120			8/10 1**			
122			8/12 2*			
123			8/13 2*			
124				7/10 0***		
126				7/12-0**		
127	10/ 7-0**					
129			9/ 7 1*			
130			9/ 8 0***			
131			9/ 9-0****			
132			9/10-1***			
142			10/ 8-0*			
143			10/ 9-0**			
144			10/10-0****			
145			10/11-0***			
152			10/18-0****			
154			10/20-0**			
156			11/10-0*			
157			11/11-0***			
158			11/12 0***	10/ 8-0**		
159				10/ 9-0*		
160				10/10-0***		
167			12/ 9-0*			
168				10/18 0**		
170			12/12 0**			
173				11/11 0**		
181			13/11-0***			
182			13/12-0****	11/20 0******		
183					12/11 2**	
197					13/13 0****	
213					14/17-0***	
269						17/21 1*
326						21/30-0*

benzene (C_6H_6) are 2.0 and 1.0, respectively.]

If the oxygen atom were attached to the aromatic ring, the ratios would be much lower. Biemann explains the abundance of $C_{11}H_{20}NO^+$ as implying that the alicyclic part of the molecule can easily be split off; the splitting would remove 11 carbon atoms, leaving 10 with the aromatic part, which contains the other nitrogen atom. This ion species is actually part of a doublet at mass 182; the other component ($C_{13}H_{12}N$) contains only one nitrogen atom and has a low hydrogen-to-carbon ratio, findings which point to the conclusion that the aromatic system can retain up to 13 carbon atoms without involving the second nitrogen atom. A number of other conclusions can also be drawn from this element map, giving a surprisingly comprehensive picture of the molecular structure (*3*).

Combination with Separation Technique

Gas chromatography has done as much as any other new analytical technique in the last decade to revolutionize research in chemistry and allied areas. A great deal of effort has been expended on finding methods for identifying the separated pure components that emerge from a gas chromatograph. The main problem is that the separated pure components often occur in amounts that are much too small for identification by most techniques. However, the sensitivities of mass spectrometers are in general comparable to those of the most sensitive gas-chromatography detectors (*18*). Also, lower vapor pressures are required in mass spectrometry than in gas chromatography. Complete transfer to the mass spectrometer of even the smallest of the separated components from a gas chromatograph can be conveniently effected by collection of the components on the column packing used for the gas chromatographic separation (*19*). Identification of amounts as small as 10^{-11} mole is possible with this technique.

A number of methods are available for recording complete low-resolution mass spectra very rapidly (in from 10 seconds to 10^{-5} second). This rapid recording makes possible direct monitoring of the effluent from the gas chromatograph by a mass spectrometer. The disadvantages resulting from dilution of the sample by carrier gas can be reduced by means of separators which pump off the carrier gas preferentially (*5, 20*). With high-resolution techniques, photographic-plate recordings of spectra of 1-microgram samples with m/e ranging from 28 to 800 can be made satisfactorily even if the sample is in the ion source for only 1 second. From another sample arriving at the ion source only a few seconds later a useful spectrum can likewise be produced and recorded. Great strides have also been made recently in speeding up the recording time necessary for conventional scanning, such as is required with the Nier-Johnson double-focusing geometry. A recording time of 10 seconds has been achieved (*21*) for spectra covering a range in mass of a factor of 10 at a resolution of 10,000 (peaks of ions differing in mass by one part in 10,000 are resolved to a 10-percent valley). This method does suffer the disadvantage that the concentration of the component emerging from the gas chromatograph is changing during the spectral scan. The photographic plate, however, records all the ions from the component continuously, thus recording an integrated spectrum.

The Data-Handling Problem

The dramatically increasing capability of analytical mass spectrometry during the last decade has brought ever-increasing demands for more rapid recording and interpretation of data. Samples of molecular weight from two to five times that of samples studied at the beginning of the decade can now be analyzed, and determinations of mass are made with an accuracy that has increased by a factor of 1000. The time required previously for recording a spectrum was 5 to 20 minutes; now spectra are recorded in a few seconds or less in the monitoring of gas chromatography. Thus, mass-spectral data can now be generated roughly a million times as fast as they could be a decade ago. Various digitization and computation techniques for coping with this problem have been developed. For example, to generate an element map from a photographic plate of a high-resolution spectrum, the Massachusetts Institute of Technology group (*3–6*) utilizes a semiautomatic comparator from which line positions can be digitized directly onto punched cards, the measurements requiring one to several hours per spectrum. By means of computer calculation line positions are converted to exact masses by comparison with reference lines of a standard spectrum recorded simultaneously. Possible elemental compositions are calculated for each mass, sorted, and printed out in the form of the element map of Fig. 10.

The potentialities of the method have led to further efforts to find a way to break this data-handling bottleneck. Recent approaches include the Associated Electrical Industries high-speed scanning (*21*) and use of an automatic photographic-plate comparator coupled with magnetic-tape recording, which has been developed by Burlingame's group at the University of California, Berkeley (*22*).

In our laboratory at Purdue we are now measuring high-resolution spectra, recorded on photographic plates, with a special Grant comparator-microdensitometer (*23*) which has the increased accuracy required for making the line-position and optical-density measurements which are needed if one is to make use of the accurate spectral registration on the plate. The time required for recording the data from the plate has been cut to 14 minutes per spectrum through the use of a Datex high-speed digitization and magnetic-tape assembly (*24*). Measurement of the 30 spectra on one plate is fully automatic, so complete reduction of the data to an element map or similar form is now possible for 100 high-resolution spectra per day.

Future

The mass spectrometer can supply other valuable information on molecular structure—information of a kind which has been little utilized in the past because of the data-handling effort required to obtain it. The purity of a sample can be checked by comparing spectra recorded during the initial and final stages of evaporation of the sample into the ion source. For a given sample the availability of additional spectra recorded at different exposures will increase the accuracy of measurements both of relative abundance and of exact mass. Spectra taken at reduced voltage of the bombarding electrons can provide measurements of the energies necessary for the formation of particular ions in the spectrum, a further indication of molecular environment (*25*). Metastable ions—those ions that decompose after acceleration out of the ion source but before mass analysis—are extremely useful in pinpointing particular ion decomposition reactions. An ad-

ditional spectrum, recorded to show the reactions of metastable ions with maximum sensitivity, could also be digitized and interpreted by the computer. Charge-exchange, negative-ion-spectra, and field-ionization techniques yield additional data of potential usefulness.

The combining of such a variety of data to obtain maximum information on molecular structure obviously requires computer systems. Reference high-resolution spectra of known compounds are being accumulated, and files of these can be rapidly searched by the computer for matching spectra. It should be possible to check for homologous compounds, or for compounds which vary by a particular discrete functional group. Calculation of the molecular composition from a spectrum containing no molecular ion is possible (*6*).

The effects of substituents on spectra can be predicted quantitatively for particular systems, and encouraging parallels have been found with effects (such as Hammett σ constants) in condensed-phase chemical reactions (*26*). It also appears possible that at least part of the interpretation of spectra can be accomplished by programming the computer to recognize known ion-decomposition reactions. Close parallels can often be drawn between these and condensed-phase chemical reactions, so that the chemical behavior of new compounds can be partially predicted from a study of their mass spectra.

It appears feasible to automate most of the mass-spectrometer adjustments necessary to obtain such additional data and still require only microgram samples. The proportion of compounds for which this procedure will provide significant or complete information on molecular structure is growing rapidly as studies are extended to more and more types of molecules. For samples sufficiently volatile to be separable by gas chromatography, these advances bring the dream of the "universal analyzer" much closer to reality. Considerable research is also in progress which may make it possible to obtain spectra of polymers and biologically important macromolecules.

There obviously are large problems to be solved before we can achieve such goals. Considerations of the skills, facilities, personnel, and financial support necessary appear to dictate that these studies be carried out in large research centers. The large number of samples suitable for study with the mass spectrometer would appear to justify the establishment of such centers and permit them to cooperate on research problems from a large number of diverse research groups.

Finally, there is a concomitant development which is, I feel, as important as recognition of the need for these large research centers. This is the growing awareness on the part of scientists in other fields of the power of mass spectrometry for the solution of their research problems. In the last few years the mass spectrometer has become a required research tool in most laboratories exploring low-molecular-weight chemical systems. There has been a great increase in the sale of low-resolution mass spectrometers for chemical work, and many laboratories are now using high-resolution techniques. As has been amply demonstrated in the history of such techniques as ultraviolet, infrared, and nuclear-magnetic-resonance spectroscopy, interest and utilization by the nonspecialist can inspire a proliferation of advances and applications undreamed of by the experts.

Summary

High-resolution mass spectra can be obtained in seconds from submicrogram quantities of compounds of molecular weights as high as 1000 to 2000, including steroids, alkaloids, polysaccharides, and peptides. The monoisotopic molecular weight of the molecule can be measured to accuracies of a few parts per million, and from these measurements the elemental composition of the compound can be calculated directly. In addition to the molecular ion, the spectrum also displays the mass and relative abundance of fragment ions produced by unimolecular decompositions of the sample molecule. The elemental compositions of the fragment ions can also be determined and used to elucidate the molecular structure. An understanding of the mechanisms of such decompositions enables the researcher to reassemble the fragment ions into logical structures. The small sample requirements and the speed with which high-resolution spectra can be obtained make such spectra very valuable for identifying pure components separated by gas chromatography. Recent techniques make possible rapid, fully automatic reduction of data obtained at high resolution, and calculation of elemental compositions and suitable display of spectra by a computer. Abundant additional information is obtainable under other ionizing conditions, and the availability of such information, plus the increasing use of the computer for spectral interpretation and search, promises continuing progress in this field.

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