International Journal of Mass Spectrometry and Ion Physics, 39 (1981) 339—366
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

FISSION FRAGMENT IONIZATION (^{252}Cf) MASS SPECTROMETRY. POSITIVE AND NEGATIVE SPECTRA AND DECOMPOSITION MECHANISMS FOR SEVEN COMPOUNDS

B.T. CHAIT, WILLIAM C. AGOSTA and F.H. FIELD

The Rockefeller University, New York, NY 10021 (U.S.A.)

(First received 17 November 1980; in final form 18 February 1981)

ABSTRACT

The ^{252}Cf fission fragment ionization mass spectra (both positive and negative ions) have been determined for alanine, arginine, sucrose, guanosine, 5′-adenosine monophosphate, alanylalanylalanine, and lysyltyrosylthreonine. The lower *m/z* limit is 18 for the positive and 26 for the negative spectra tabulated. Mass to ion charge values are given with millimass precision, which permits deduction of atomic compositions for many of the observed ions. Reasonable mechanisms for the production of some of these ions are postulated.

The significant findings of the study are:

(1) The amount of fragmentation occurring is large both in that quasi-molecular ion intensities are low and that many small ions are produced with large intensities. For example, only for alanine is the intensity of the quasi-molecular ion greater than 10% of the total ionization, and for arginine the CN^- ion comprises 56% of the total negative ionization.

(2) The spectra observed have characteristically a quasi-molecular ion, a large gap without ions, and many ions at low mass.

(3) Detectable amounts of quasi-molecular ions are present in all the spectra.

(4) Reactions invoking known chemistry of gaseous positive and negative ions can be written for the formation of many of the fragment ions.

(5) The results indicate that the ionization process occurring is primarily a relatively high-energy one since it produces much fragmentation, but it has a low-energy component which is involved in the formation of the quasi-molecular ions observed here and in previously published work.

An ancillary finding is that the fission fragment ionization method seems to have a high sensitivity for producing anions from certain inorganic trace impurities.

INTRODUCTION

Californium-252 fission fragment ionization mass spectrometry is a technique whereby molecular entities are desorbed and ionized directly from a solid surface by the passage of an energetic fission fragment from californium through a surface coated with a compound of interest. The fragments which are produced by the spontaneous fission of ^{252}Cf have bimodal

distributions of masses and energies peaked around 105 and 141 daltons and 104 and 79 MeV, respectively [1]. Since its discovery in 1974 by Macfarlane and Torgerson [2—4], ^{252}Cf fission fragment ionization mass spectrometry has generated much interest because of the ability of the method to produce significant yields of quasi-molecular ions of thermally labile, highly involatile, and large molecules of biological significance. For example, quasi-molecular ions have been observed for involatile amino acids [2], di- and tri-peptides [5], polypeptides with as many as 31 residues [3,6], antibiotics [6,7], nucleosides [6,7], nucleotides, dinucleotides, and trinucleotides [8—10], protected oligodeoxyribonucleotides extending up to a decanucleotide [11], palytoxin (MW 2681) [12] and chlorophyll-*a* oligomers extending up to a heptamer [13]. A second ^{252}Cf time-of-flight mass spectrometer was subsequently constructed by Wien and his co-workers [14], and their investigations have included spectral measurements of alkaloids, steroids, sugars, glycosides, vitamin B_1, the antibiotic chloramphenicol [15] and three quaternary ammonium salts [16].

Several groups of workers have proposed models and/or mechanisms for ionization and desorption by heavy-ion impact. It has been suggested that the observed phenomena may result from a transient high-temperature thermal pulse [17], from molecular excitation followed by desorption due to a shock wave [18], from a high-frequency perturbation of the electron plasma effected by the passage of the fission fragment [19,20], and from a thermalized ion explosion [21]. None of these theories has as yet received general acceptance. Dück and co-workers [22] are making systematic investigations designed to elucidate mechanisms, but no definitive results have yet been obtained.

We have constructed a ^{252}Cf time-of-flight mass spectrometer and in the present paper report the complete positive- and negative-ion mass spectra obtained from seven biologically interesting compounds of low volatility. These are: alanine, arginine, sucrose, guanosine, 5′-adenosine monophosphate (5′-AMP), the tripeptide alanylalanylalanine (AlaAlaAla), and the tripeptide lysyltyrosylthreonine (LysTyrThr). To date, few complete mass spectra (which include the low mass range) have appeared in the literature, and virtually no information is given about the fragmentation processes involved in the formation of the spectra. The discipline of fission fragment ionization mass spectrometry will obviously remain incomplete until such knowledge is accumulated, and our efforts have been made to contribute to this accumulation.

EXPERIMENTAL

The apparatus is similar to that described by Macfarlane and Torgerson [4]. The spontaneous decay of ^{252}Cf ($t_{1/2}$ = 2.6 years) results in the emission of two highly energetic fission fragments traveling in opposite directions. One fragment passes through a thin nickel foil (0.0005 mm), producing a

shower of electrons which is detected by a channeltron electron multiplier (the start detector) to provide the zero-time mark for a fission event. The complementary fission fragment traveling in the opposite direction passes through another thin nickel foil (thickness 0.001 mm) with the material of interest adhering to its far side, and this effects the volatilization and ionization of the sample. The sample ions thus produced are accelerated by a system of three highly transparent grid electrodes and then pass down a 3-meter flight tube. They are detected by a planar chevron microchannel plate electron multiplier detector. The time of flight of the ions provides a direct measure of their mass-to-charge ratio.

The mass spectrometer is constructed from 4 in. (10.2 cm) outside diameter stainless-steel tubing using standard copper-gasket-sealed flanges (Varian ConFlat). The source electrodes are stacked using a system of sapphire balls to provide accurate spacing, good dimensional stability, and good electrical insulation. The system is evacuated by a liquid-nitrogen trapped diffusion pump (Consolidated Vacuum Corp., PMCS-4B; 690 l s^{-1} capacity) to provide pressures of $\sim 10^{-7}$ torr.

The ^{252}Cf source (Isotope Products Laboratories, Burbank, CA) consists of a 3 mm diameter spot of electro-deposited californium oxide centered on a nickel foil 0.001 mm thick and covered with a layer of sputtered gold (400 μg cm^{-2}). The nominal strength of the source is 15 μCi, which in the source geometry used corresponds to ~200 fission fragments striking the sample foil per second. With sample loads of several micrograms this produces about 10 collected sample ions per second.

Since ^{252}Cf is highly toxic and is known to self-transport [23], we investigated the escape of californium from the foil to adjacent metal surfaces. A vacuum vessel was constructed, and using silicon surface-barrier detectors in this vessel the escape of californium from the gold-covered face of the source was determined to be ~70 pCi h^{-1}. This was deemed unacceptable. The californium foil was then sandwiched between two nickel foils 0.005 mm thick. With this arrangement the escape-rate limit was found to be $< 3 \times 10^{-15}$ Ci h^{-1} during a period of 42 days. This was deemed an acceptable upper limit for escape, and this sandwich technique constitutes a satisfactory method for containing californium.

The sample of interest is dissolved in a suitable solvent and electrosprayed [24] onto the thin (0.001 mm) nickel sample foil, which is stretched flat on a sample foil holder. This holder is mounted at the end of a probe which may be inserted rapidly via a vacuum lock into position between the californium source and the first ion-accelerating grid. The acceleration voltage is supplied by a well-regulated power supply (Spellman RHSR 10PN60 with ripple <100 mV RMS and stability better than $\pm 0.005\%$ h^{-1}). Both positive and negative ions may be studied by reversing the polarity of the potentials applied to the sample foil and grids. Typical potentials employed are ±6.5 kV on the sample foil and 0 to ±5 kV on the first grid. The other two grids are held close to ground potential. The spacing between the sample foil and the

first grid electrode is 5 mm, and that between subsequent pairs of grids is also 5 mm.

An electrostatic particle guide [4,25] is used to increase the transport efficiency of ions in the flight tube. This guide consists of a central wire running the length of the flight tube. A potential of $\sim\pm12$ V with respect to the wall of the flight tube is applied to the wire. This produces a purely radial electric field having the effect of capturing ions with small transverse components of velocity into stable spiral orbits. A transport efficiency enhancement of ~25 results. No significant loss of time-resolution resulting from the use of the particle guide at the potentials normally applied in our experiments has been observed.

The ion detector consists of a pair of microchannel electron multiplier plates (Varian, LSE; Palo Alto, CA; non-imaging quality) in tandem, which yields a gain of $\sim10^7$. These plates provide a large collection area (2.5 cm^2) parallel to the sample foil. The short-duration (~1 ns) charge pulse generated when an ion strikes this detector is fed into a fast preamplifier (Ortec 9301; risetime <1.5 ns) and thence into a timing filter amplifier (Ortec 454). The amplified signal is then fed into a 100 MHz leading-edge discriminator (Ortec 436). Similar electronics are employed for the zero-time start detector, except that a constant-fraction timing discriminator (Ortec 463) is used. The timing signals are then fed into a digital flight-time measuring instrument built in this laboratory and described in detail elsewhere [26]. This timing unit is linear with an integral linearity of better than 7 parts in 10^7; it has a resolution of 5/8 ns, with a dynamic range of 0—1300 μs; and it is able to measure the arrival times of as many as 15 secondary ions generated by a single fission fragment. The minimum detection time between two sequential events (the deadtime) is low (<10 ns).

The measured times of flight of ions are transferred from the digital timer to the memory of a PDP 11/34 computer to form an array, each element of which corresponds to a time bin which may be set arbitrarily to values $>5/8$ ns. Intensity data are accumulated by incrementing the number of counts in the relevant time bin upon measurement of the flight time of an event. The intensities given in Tables 2—13 are derived by summing the counts in the five bins encompassing the top of the peak.

At the conclusion of the spectral data acquisition, the event times are manipulated by the computer using standard mass-spectrometric computational procedures to identify the presence of peaks corresponding to the several ions in the spectrum and to calculate their time centroids. The time-to-mass conversions are made using an equation appropriate to a time-of-flight mass spectrometer, namely,

$$t = K_1\sqrt{m} + K_2 \qquad (1)$$

where K_1 is a combined constant including the acceleration voltages and the flight path, and K_2 is an empirical constant which accounts for unknown higher-order effects. The values of the two constants are determined using

the times observed for two known masses in a spectrum. For positive-ion spectra the ever-present ions at m/z 1 (H^+) and 23 (Na^+) are often used, at least for initial calibrations. For negative-ion spectra the ever-present ions at m/z 1 (H^-) and 25 (C_2H^-) serve this function. It is usually possible to use such initial calibrations to determine other ionic masses which provide a more accurate calibration in higher mass ranges of interest. Using this technique we routinely and reliably determine ionic masses up to m/z 1000, and have no reason to doubt that the method will be useful at higher masses. Indeed, the ease and reliability of mass identification is a pleasing and useful feature of fission fragment ionization mass spectrometry.

Figure 1 shows the portion of a typical time-of-flight spectrum between 16.985 and 18.235 μs, corresponding to masses between 55 and 63, for alanine. The data were recorded with time bin widths of 1.250 ns. The peaks are well shaped, the signal-to-noise ratio is high, and the resolution is such that the doublets at m/z 57 and 63 are partially resolved. The width (FWHM) of the m/z 58 peak in Fig. 1 corresponds to an FWHM resolution of ~1000. In other experiments, FWHM resolutions as high as ~1500 in the mass range m/z 100—1000 have been obtained.

The solvent generally used in the sample electrospraying operation has been a methanol—water mixture, which dissolves many substances and the properties of which enable it to be electrosprayed satisfactorily. In the present study we wished to obtain spectra which included the low mass region (<100 daltons), where both the supporting nickel foil and the solvent can produce background ions. Because of this a procedure was developed

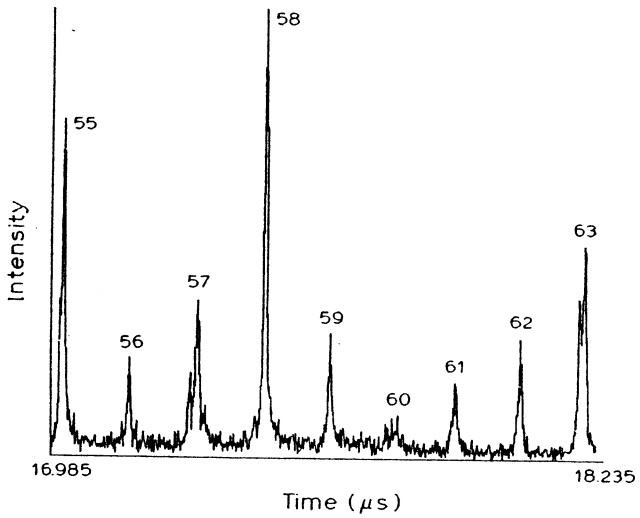


Fig. 1. Partial positive-ion spectrum of alanine. Ion-accelerating voltage = 9500 V, time bin width = 1.25 ns.

whereby backgrounds arising from these sources can be subtracted. The solvent is sprayed onto a sample foil which has been cleaned thoroughly with de-ionized distilled water and ultrapure methanol. A background spectrum produced by this foil is obtained. This foil is then again thoroughly cleaned with water and methanol, after which it is electrosprayed with a solution of the material of interest, and the spectrum determined. The background spectrum is subtracted from the sample spectrum making use of the arbitrary criterion that sample peaks must have at least twice the intensity of the background peak at the same m/z value to be retained in the net sample spectrum. This background subtraction procedure has associated with it considerable uncertainty, especially at the lower masses, since it has not been firmly established whether the background and sample spectra can be subtracted linearly. This is particularly the case for the large background peaks almost always observed at m/z 1 (H^+) and 23 (Na^+) in positive-ion spectra and at m/z (H^-) and 25 (C_2H^-) in negative-ion spectra. We have arbitrarily adopted the convention of not including these ions in the net sample spectra. For all measurements the concentration of the solute and the volume of solution electrosprayed were adjusted so that 5—20 μg of solute was deposited onto the sample foil. The area of the sample foil is ~1 cm^2.

The compounds studied were obtained from commercial sources as follows and were used without further purification: arginine (MCB), sucrose (Fisher), guanosine (Sigma), 5′-adenosine monophosphate (Sigma), AlaAla-Ala (Sigma), alanine (Sigma), and LysTyrThr (Bachem). The solvent used was methanol (Aldrich Gold Label). The water used was laboratory de-ionized and distilled in glass.

RESULTS

Complete positive- and negative-ion mass spectra were obtained for the compounds studied. The spectra were measured from m/z 1 to values ~75 mass units above the molecular weights of the compounds. Duplicate determinations were made on independently prepared samples of all compounds. The reproducibility of the duplicate determinations was acceptable.

Arginine and alanine

The results for these two amino acids are complementary and will be discussed together. Arginine is highly polar and non-volatile, and it constitutes a kind of standard test-case for evaluating new techniques for obtaining mass spectra of labile, refractory materials. Alanine is structurally simpler and more volatile, and its spectrum serves as a guide to understanding that of arginine.

Arginine was the first compound carefully investigated in this work, and it was used as a subject for the determination of the precision and, to a degree, the accuracy of our measurements of the exact masses of the ions in

a spectrum. As pointed out originally by Macfarlane and Torgerson [4], ^{252}Cf time-of-flight mass spectrometry offers the possibility of determining ionic masses with millimass precision in a particularly easy and straightforward way. The basic reason for this is the operation of a highly accurate and reproducible scan function (eqn. (1)), coupled with the availability of a highly stable supply for the ion-accelerating voltage. These exact masses offer information about the atomic compositions of the corresponding ions, but there are limitations on the mass—composition relationship resulting from the fact that the mass spectrometer is not energy-focusing and has limited ability to resolve ion multiplets at a given nominal mass.

To provide information about the behavior of the apparatus for precise mass determination, six measurements were made of the positive-ion spectrum of arginine during a period of three weeks. The results of precise mass determinations for the major ions are given in Table 1. The constants in eqn. (1) were obtained using the Na^+ impurity peak at $m/z = 22.9898$ and the

TABLE 1

Precise mass determinations [a] from six replicate positive-ion spectra of arginine, $C_6H_{14}N_4O_2$ (MW = 174)

m/z	Average precise mass	Std. dev.	Possible ion	Calc. mass	Δmass
18	18.036	0.001	NH_4^+	18.035	+0.001
28	28.015	0.0004	$HC{=}NH^+$	28.019	−0.004
30	30.033	0.0004	$CH_2{=}NH_2^+$	30.034	−0.001
41	41.024	0.002	$C_2H_3N^{+\cdot}$	41.027	−0.003
			$C_3H_5^+$	41.039	−0.015
43	43.020	0.003	$C_2H_3O^+$	43.018	+0.002
			$CH_3N_2^+$	43.030	−0.010
44	44.038	0.002	$CH_4N_2^{+\cdot}$	44.037	+0.001
59	59.047	0.002	$CH_5N_3^{+\cdot}$	59.048	−0.001
60	60.060	0.003	$C_2H_6NO^+$	60.045	+0.015
			$CH_6N_3^+$	60.056	+0.004
70	70.082	0.004	$C_5H_{10}^+$	70.078	+0.004
			$C_4H_8N^+$	70.066	+0.016
87	87.093	0.003	$C_4H_{11}N_2^+$	87.092	+0.001
			$C_5H_{11}O^+$	87.081	+0.012
			$C_5H_{13}N^+$	87.105	−0.012
			$C_3H_9N_3^+$	87.080	+0.013
100	100.076	0.011	$C_4H_{10}N_3^+$	100.087	−0.011
112	112.144	0.007	$C_6H_{12}N_2^+$	112.100	+0.044
130	130.107	0.010	$C_5H_{14}N_4^{+\cdot}$	130.122	−0.015
175	175.120		$(M + 1)^+$	*calibration mass*	
176	176.119	0.008	$^{13}C(M + 1)^+$	176.123	−0.004
197	197.100	0.011	$C_6H_{14}N_4O_2Na^+$	197.102	+0.002

[a] Mass scale calibrated at m/z 23 and 175 $[(M + 1)^+]$.

peak at nominal m/z 175, which was reasonably assumed to be the arginine $(M + 1)^+$ ion (m/z = 175.1195). The low values of the standard deviations of the precise experimental masses, tabulated in column 3 of Table 1, indicate a high degree of reproducibility in the measurements. The experimental precise masses in column 1 (average of the six replicates) may be compared with the calculated masses (column 5) for several possible ion compositions (column 4). We have no independent information about the compositions of the ions produced from arginine by ^{252}Cf ionization, so the conclusions to be drawn are not unequivocal. However, we think that the compositions given in the table at m/z = 18, 28, 30, and 197 are likely (the identity of the $(M + 23)^+$ ion at m/z = 197 is almost a certainty), and many of the other compositions indicated by the several precise masses are reasonable. The generally small values of Δmass shown in column 6 give promise that useful information about atomic compositions may be obtained even for fragment ions. The policy in this paper is to use precise mass information to postulate the atomic composition of ions, but the postulated compositions must be as chemically reasonable as we are able to adduce.

The spectra and ionic reactions of alanine are discussed first because of its relatively simple structure. The positive- and negative-ion spectra are given in Tables 2 and 3, respectively. These two spectra were obtained using the same

TABLE 2

Positive-ion spectrum [a] of alanine, $C_3H_7NO_2$ (MW = 89)

m/z	Intensity (counts)	Percent of total ionization	Meas. mass	Ion	Calc. mass	Δmass
1	915			H^+		
18	220	4.1	18.032	NH_4^+	18.034	−0.002
23	2919		*calibration*	$^{23}Na^+$		
28	119	2.2	28.018	$HCNH^+$	28.019	−0.001
30	108	2.0	30.030	$CH_2{=}NH_2^+$	30.034	−0.004
42	189	3.5	42.030	$C_2H_4N^+$	42.034	−0.004
43	313	5.9	43.034	$C_2H_5N^{+\cdot}$	42.042	−0.008
44	2370	44.4	44.046	$C_2H_6N^+$	44.050	−0.004
45	164	3.1	45.037	$^{13}C^{12}CH_6N^+$	45.053	−0.016
46	129	2.2	46.063			
58	319	6.0	58.073			
90	703	13.2	*calibration*	$(M + 1)^+$		
91	49	0.9	91.053	$^{13}C(M + 1)^+$	91.058	−0.006
112	138	2.6	112.045	$(M + Na)^+$	112.037	+0.008
134	108	2.0	134.021	$(M - 1 + 2\,Na)^+$	134.019	+0.002
179	115	2.2	179.117	$(2\,M + 1)^+$	179.102	+0.015

[a] Total experimental ionization = 5341 counts, total tabulated ionization = 5044. Relative intensities >1% of total ionization tabulated. Calibration masses: Na^+ (22.9898); $(M + 1)^+$ (90.0555). Run time = 1184 s.

TABLE 3

Negative-ion spectrum [a] of alanine, $C_3H_7NO_2$ (MW = 89)

m/z	Intensity (counts)	Percent of total ionization	Meas. mass	Ion	Calc. mass	Δmass
1	1295			H^-		
25	1427		*calibration*	C_2H^-		
26	2323	26.5	26.006	CN^-	26.003	+0.003
40	130	1.5	40.014	$C_2H_2N^-$	40.019	−0.005
41	165		41.006	C_2HO^-	41.003	+0.003
42	366	4.2	42.002	CNO^-	42.998	+0.004
43	246	2.8	43.008	$HCNO^{-\cdot}$	43.006	+0.002
45	303	3.5	45.002	$HCOO^-$	45.998	+0.004
50	263	3.0	50.001	C_3N^-	50.003	−0.002
59	525		59.019	$C_2H_3O_2^-$	59.013	+0.006
65	184	2.1	65.010			
66	165	1.9	66.003			
71	194	2.2	71.014	$C_3H_3O_2^-$	71.013	+0.001
86	395	4.5	86.018	$(M-3)^-$	86.024	−0.006
88	3270	37.3	*calibration*	$(M-1)^-$		
89	241	2.7	89.028	$^{13}C(M-1)^-$	89.043	−0.015
177	273	3.1	177.042	$(2M-1)^-$	177.086	−0.044

[a] Total experimental ionization = 8766 counts, total tabulated ionization = 8353. Relative intensities >1% of total ionization tabulated. Calibration masses: C_2H^- (25.0078); $(M-1)^-$ (88.0399). Run time = 1298 s.

sample; that is, the alanine sample on the sample foil was inserted into the mass spectrometer and the spectrum of one polarity was obtained. The potentials of the spectrometer were then reversed and the spectrum of the other polarity was obtained. This procedure was utilized for all of the spectra reported in this paper. Only in these two spectra for alanine do we include the H^+ and Na^+ intensities (positive) and the H^- and C_2H^- intensities (negative), as an example of the intensities found for these ions. Since the intensities of these ions in the general background spectra are often comparable to the intensities in the sample spectra, we decided not to include them in the net spectra of samples.

An examination of the spectra given in Tables 2 and 3 reveals that much fragmentation (by soft ionization standards) occurs in alanine. Thus in the positive-ion spectrum only 21% of the total ionization comprises quasi-molecular ions (*m/z* 90, 91, 112, 134, and 179), and the corresponding figure in the negative spectrum is 43%. We have not found a published field desorption (FD) spectrum of alanine, but Winkler and Beckey [27] reported a spectrum of valine which contains only two ions [$(M+1)^+$ = 77% of total ionization and $(M+1-46)^+$ = 23%], and we expect that the FD spectrum of alanine will not be very different. The CH_4 chemical ionization (CI) spectrum

[28] of alanine contains 32% of the total ionization at $(M + 1)^+$, but the i-C_4H_{10} CI spectrum may be expected to show much less fragmentation. Again we found no published spectrum of alanine, but the i-C_4H_{10} CI spectrum of valine has as much as 99% of the total ionization in quasi-molecular ions [29]. On the other hand, the amount of fragmentation in fission fragment ionization (FFI) is less than that in electron ionization (EI), for the intensity of the $M^{+\cdot}$ ion in EI is only 0.1% of the total ionization [30]. Comparisons of the negative FFI spectrum with those produced by other methods are limited because of lack of other data. The only possible comparison is with the OH^- CI spectrum of leucine [31], for which 79% of the total ionization appears at $(M - 1)^-$. We make these comparisons here because a recurrent theme throughout the remainder of this paper will be that fission fragment ionization is basically a high-energy process producing large amounts of fragmentation. Although less fragmentation occurs for alanine than for any other compound we have studied, the above comparisons show that even in this case the amount of fragmentation is relatively large.

A further consideration of the data in Tables 2 and 3 shows that quasi-molecular ions $(M \pm 1)^{\pm}$ are formed rather than molecular ions ($M^{+\cdot}$ or $M^{-\cdot}$), and the fragment ions produced seem (with one minor exception) to have an even number of electrons. As emphasized above, no generally accepted detailed mechanism for volatilization and ionization by the FFI method exists, but Macfarlane and Torgerson [2—4] suggested that the ionization in FFI might result from a disproportionation reaction wherein the high-energy condition resulting from the passage of a fission fragment caused the transfer of a proton to an adjacent molecule, i.e.

$$M + M \rightarrow (M + H)^+ + (M - H)^- \qquad (2)$$

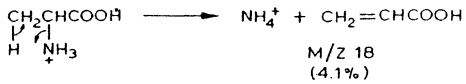
Further reactions of the quasi-molecular ions can occur to produce the observed spectra. On the whole our results fit well with this mechanism and it will be used in postulating reactions for the production of the ions observed in our spectra.

Considering now the positive-ion spectrum given in Table 2, the ion at m/z 112 is a cationized alanine, where the Na^+ added to the molecule is present in the sample as an impurity. Ions such as that at m/z 134 [$(M - 1 + 2\ Na)^+$] have been observed previously by Macfarlane and Torgerson [2—4], and the ion is formed by a combination of Na^+ addition and Na—H exchange. This ion is of much chemical interest, for no information exists about either its structure or the reaction by which it is formed.

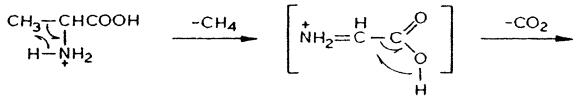
The most intense fragment ion is that at m/z 44, and its precise mass corresponds to the composition $C_2H_6N^+$. This is the $(M + H - H_2CO_2)^+$ ion, which is the most intense fragment ion in the EI and CI spectra of alanine and in the FI spectrum of valine. The reaction producing the ion is probably:

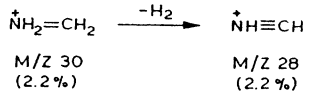
$$M \xrightarrow{H^+} CH_3\underset{\overset{|}{\overset{+}{N}H_3}}{CH}COOH \longrightarrow \underset{\text{M/Z 44}\ (RI = 44\%)}{CH_3CH{=}\overset{+}{N}H_2} + HCOOH \qquad (3)$$

Plausible reactions can be written similarly for the production of the *m/z* 18, 28, and 30 ions, namely:



(4)

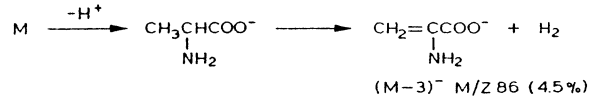




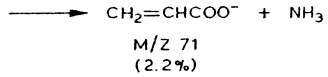
(5)

Ions at *m/z* 43 and 42 are found with low intensities in the EI spectrum of alanine, and it is interesting that they also appear in our FFI spectrum. They could be formed by the loss of H and H_2, respectively, from the *m/z* 44 ions. The important conclusion to be drawn from this spectrum is that much of it may be understood in terms of well-established concepts of gaseous-ion chemistry.

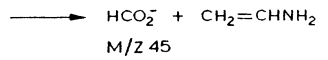
The negative-ion spectrum given in Table 3 also indicates extensive fragmentation, although slightly less than that found for the positive-ion spectrum. The most intense fragment ion is CN^- at *m/z* 26. This ion is formed copiously from all the nitrogen-containing compounds which we have investigated. There is no known precedent for this observation. A characteristic of gaseous negative ions which was emphasized in our study of OH^- CI mass spectrometry [31] is an enhanced tendency for precursors to decompose by undergoing four-center reactions. A number of the fragment ions found in the alanine negative-ion spectrum (including CN^-) may be formed in this way. Thus:



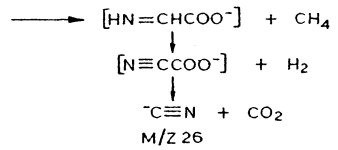
(6a)



(6b)



(6c)



(6d)

The order of CH_4 and H_2 loss given in reaction (6d) is arbitrary and may be reversed.

Ions with low intensities are observed at *m/z* 40, 42, and 43. The compositions deduced from the masses are given in Table 3, even though reactions for their formation cannot be postulated. At these low masses the numbers of possible compositions are small, and the experimental exact mass becomes definitive. Furthermore, with regard to the ion at *m/z* 42, which is indicated by its mass to be CNO^-, this ion has been found in the spectra of all the nitrogeneous compounds we have studied. It is also formed in mixtures of CH_4 and N_2O [31], and this apparent ubiquity is compatible with its presence in the alanine spectrum.

A large proportion of the total ionization in the negative-ion spectrum of alanine may be postulated in terms of established reactions of gaseous anions, which parallels the situation found for the positive spectrum.

Two other ions included in Table 3 require comment, namely *m/z* 59 and 41. The composition indicated for *m/z* 59 is $C_2H_3O_2^-$, the formation of which from alanine can be rationalized only with difficulty. As a matter of experimental interest, the FFI method seems to be very sensitive to the formation of certain anions, and it is quite possible that the ion observed at *m/z* 59 is the acetate anion formed from a small amount of acetate impurity in the alanine. The *m/z* 41 ion can be formed by loss of H_2O from the *m/z* 59 ion. We have determined experimentally that the major ions in the negative spectrum of sodium acetate are at *m/z* 59 and 41, which is consistent with this argument.

The complete positive- and negative-ion spectra of arginine are given in Tables 4 and 5. The amount of fragmentation occurring is great in both spectra, for the quasi-molecular ions comprise only 4.5 and 3.3% of the total ionization in the positive- and negative-ion spectra, respectively. Considering first the positive-ion spectrum, a marked difference between the spectra of arginine and alanine is that for alanine the $(M + 1 - HCOOH)^+$ ion has the greatest intensity (44% of total intensity), but the corresponding ion from arginine (*m/z* 129) is only 0.2% of total intensity. Similarly, the *m/z* 129 ions produced from arginine by FD [27], CI with FD wires [32], and rapid-heating CI [33], exhibit zero or low intensity. A reasonable explanation may be advanced for this difference in behavior; namely, alanine protonates on the α-amino group, and the reaction represented in eqn. (3) may occur. In arginine, protonation occurs on the more basic guanidino group, and reaction (3) is absent or inhibited.

However, this argument cannot be applied consistently, for it was postulated above that the NH_4^+, $HCNH^+$, and $CH_2{=}NH_2^+$ (*m/z* 18, 28, and 30) ions are produced in alanine by reactions (4) and (5) starting from the $(M + 1)^+$ ions protonated on the amino group. Since these ions are also formed with relatively high intensities from arginine, the reactions producing them must be different and/or additional proton-transfer reactions occur in the case of arginine.

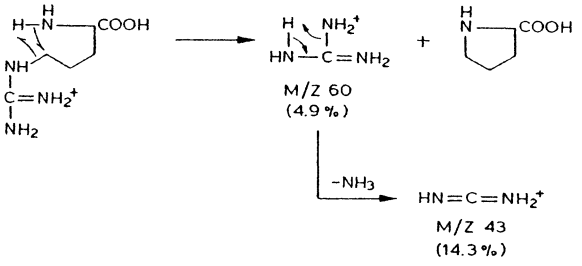
TABLE 4

Positive-ion spectrum [a] of arginine, $C_6H_{14}N_4O_2$ (MW = 174)

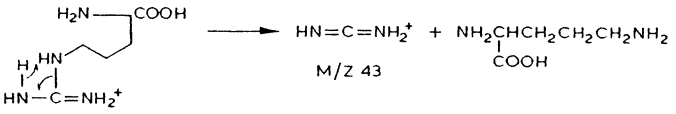
m/z	Intensity (counts)	Percent of total ionization	Meas. mass	Ion	Calc. mass	Δmass
18	737	7.5	18.036	NH_4^+	18.035	+0.001
27	287	2.9	27.017			
28	966	9.8	28.015	$HCNH^+$	28.019	−0.004
30	794	8.1	30.033	$CH_2{=}NH_2^+$	30.034	−0.001
41	302	3.1	41.024	$C_2H_3N^{+\cdot}$	41.027	−0.003
42	280	2.8	42.023	$CH_2N_2^{+\cdot}$	42.022	+0.001
43	1405	14.3	43.020	$CH_3N_2^+$	43.030	−0.010
44	483	4.9	44.038	$CH_4N_2^{+\cdot}$	44.037	+0.001
59	332	3.4	59.047	$CH_5N_3^{+\cdot}$	59.048	−0.001
60	483	4.9	60.060	$CH_6N_3^+$	60.056	−0.004
70	1141	11.6	70.082	$C_4H_8N^+$	70.066	+0.016
87	153	1.6	87.093			
100	57	0.6	100.076	$C_4H_{10}N_3^+$	100.087	−0.011
112	73	0.7	112.144			
129	24	0.2	129.154			
130	66	0.7	130.107	$C_5H_{14}N_4^{+\cdot}$	130.122	−0.015
131	30	0.3	131.087			
175	367	3.7	*calibration*	$(M + 1)^+$		
176	56	0.6	176.119	$^{13}C(M + 1)^+$	176.123	−0.004
197	23	0.2	197.100	$(M + Na)^+$	197.102	−0.002

[a] Total experimental ionization = 9839 counts, total tabulated ionization = 8059. Relative intensities >2% of total ionization for $m < 100$ and >0.2% of total ionization for $m \geq 100$ tabulated. Calibration masses: Na^+ (22.9898); $(M + 1)^+$ (175.1195). Run time = 1152 s.

The two most intense ions from arginine are those with *m/z* 43 ($CH_3N_2^+$) and *m/z* 70 ($C_4H_8N^+$). The *m/z* 43 ion, because of its two N-atoms, is almost certainly formed from the guanidino portion of the molecule. Two possible reactions may be written, but we cannot distinguish between them. Thus:



(7a)



(7b)

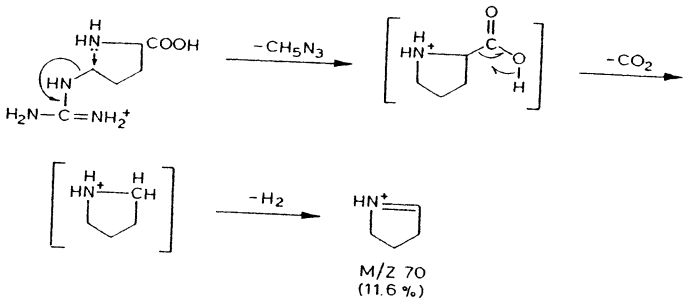
TABLE 5

Negative-ion spectrum [a] of arginine, $C_6H_{14}N_4O_2$ (MW = 174)

m/z	Intensity (counts)	Percent of total ionization	Meas. mass	Ion	Calc. mass	Δmass
26	4495	55.9	26.006	CN^-	26.003	+0.003
40	282	3.5	40.013	$C_2H_2N^-$	40.019	−0.006
41	842	10.5	41.018	CHN_2^-	41.014	+0.004
42	501	6.2	42.003	CNO^-	41.998	+0.005
45	143	1.8	45.004	$COOH^-$	44.998	+0.006
50	338	4.2	49.995	C_3N^-	50.003	−0.008
64	223		63.976	SO_2^-	63.962	+0.014
65	211	2.6	65.015	$C_3HN_2^-$	65.014	+0.001
66	326	4.1	66.008			
80	794		79.964	SO_3^-	79.957	+0.007
81	566		80.987	HSO_3^-	80.965	+0.022
97	3151		96.965	$H^{32}SO_4^-$	96.960	+0.005
99	220		97.954	$H^{34}SO_4^-$	97.955	−0.001
131	120	1.5	131.127	$C_5H_{11}N_2O_2^-$	131.082	+0.045
173	255	3.2	*calibration*	$(M-1)^-$		
195	60		195.005	$(H_2SO_4 \cdot HSO_4)^-$	194.928	+0.077
217	96		216.895	$(H_2SO_4 \cdot NaSO_4)^-$	216.910	−0.015

[a] Total experimental ionization = 8047 counts, total tabulated ionization = 7513. Relative intensities >2% of total ionization for $m < 100$ and >1% of total ionization for $m > 100$ tabulated. Calibration masses: C_2H^- (25.0078); $(M-1)^-$ (173.1038). Run time = 1200 s.

Reaction (7a) also accounts for the ion observed at m/z 60 ($CH_6N_3^+$). The m/z 70 ion ($C_4H_8N^+$) can be produced as follows:



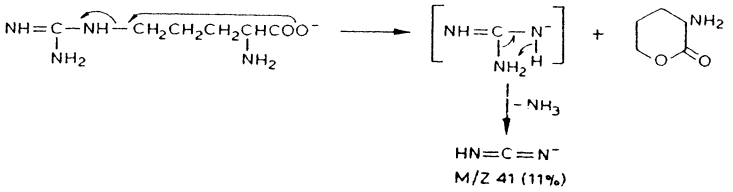
(8)

Our spectrum is qualitatively similar to that given by Macfarlane and co-workers [2] (lower limit of spectrum given in ref. 2 is m/z 120). The spectra obtained by FD [27], CI with FD wires [32], and rapid-heating CI [33] show considerable differences among themselves and from our FFI spectrum. Perhaps the most important difference is that all three other methods show a large intensity at $(M + 1 - NH_3)^+$ (m/z 158), whereas we do not observe this ion at all. This is the first example of behavior observed frequently in the compounds investigated here; namely, that the tendency for

fragmentation to occur by the loss of small molecules (H_2O, NH_3, H_2, etc.) from $(M + 1)^+$ is much less in FFI than in the soft ionization processes CI and FI. In FFI, the fragmentation to produce low-mass ions appears to be much more extensive. The laser desorption spectrum of arginine [34] also shows either a small or zero intensity at m/z 158, and this tends to agree with the FFI results. Chemical ionization with FD wires [32] produces a strong signal at m/z 70. Most other literature measurements were not made or reported to low enough masses to permit comparisons with our low-m/z results.

The negative-ion spectrum of arginine exhibits a low-intensity $(M - 1)^-$ ion and a high-intensity m/z 26 ion (CN^-). The amount of fragmentation is thus much greater in arginine than in alanine. We suggest that CN^- is produced from arginine by a reaction analogous to eqn. (6d), with the neutral entity initially lost being $C_3H_7NH{-}\underset{\underset{NH}{\|}}{C}{-}NH_2$ instead of CH_4. We have no explanation for the greater rate of occurrence of the reaction in arginine. Remarks made above in connection with the negative-ion alanine spectrum concerning the ions at m/z 40, 42, and 45 also apply to the negative-ion arginine spectrum.

A set of ions not found in alanine appear in arginine at m/z 131, 65, 50, and 41, and the sum of their intensities comprises ~20% of the total ionization. Reactions for the formation of these ions can be written by postulating that bond fissions can occur at different places along the arginine side-chain and by making use of the tendency of anions to eliminate small molecules by four-center reactions. One example of such a reaction is:



(9)

Included in Table 5 is the set of ions with m/z values of 64, 80, 81, 97, 99, 195, and 217, which we believe constitutes another example of the high sensitivity of negative FFI to certain impurities. All of the ions in this set show very marked mass deficiencies which are incompatable with their being ions from arginine. An experiment showed that sulfuric acid sprayed onto a sample foil produced ions at the same nominal m/z values and with roughly the same pattern of intensities. The agreement between the measured masses and the masses calculated assuming the ions to be derived from H_2SO_4 or some sulfate salt is very good. The arginine stock which provided the sample on which the spectral measurements recorded in Table 5 were made is of good quality, and although it was not specifically purified, there is no reason to expect that it is contaminated.

Sucrose

The positive- and negative-ion spectra of sucrose are given in Tables 6 and 7. Fragmentation is extensive. No $(M + 1)^+$ ion is found in the positive-ion spectrum, but a quasi-molecular ion accounting for 2% of the total ionization is formed at *m/z* 365 $[(M + Na)^+]$ by the addition of impurity sodium

TABLE 6

Positive-ion spectrum [a] of sucrose, $C_{12}H_{22}O_{11}$ (MW = 342)

m/z	Intensity (counts)	Percent of total ionization	Meas. mass	Ion	Calc. mass	Δmass
15	316	3.0	15.025	CH_3^+	15.023	+0.002
19	633	5.9	19.016	H_3O^+	19.018	−0.002
27	712	6.7	27.013	$C_2H_3^+$	27.023	−0.010
29	548	5.1	29.027	$C_2H_5^+$	29.039	−0.012
31	823	7.7	31.021	CH_3O^+	31.018	+0.003
38	230	2.1	38.005	$C_3H_2^{+\cdot}$	38.016	−0.011
39	926	8.7	39.010	$C_3H_3^+$	39.023	−0.013
41	541	5.1	41.036	$C_3H_5^+$	41.039	−0.003
43	585	5.5	43.031	$C_2H_3O^+$	43.019	+0.012
45	362	3.4	45.032	$C_2H_5O^+$	45.034	−0.002
50	216	2.0	49.995	$C_4H_2^{+\cdot}$	50.016	−0.021
51	240	2.2	51.005	$C_4H_3^+$	51.023	−0.018
55	278	2.6	55.037	$C_3H_3O^+$	55.018	+0.019
57	622	5.8	57.053	$C_2HO_2^+$	57.049	+0.004
61	351	3.3	61.021	$C_2H_5O_2^+$	61.029	−0.008
69	353	3.3	69.052	$C_4H_5O^+$	69.034	+0.018
73	312	2.9	73.036	$C_3H_5O_2^+$	73.029	+0.007
85	176	1.6	85.039	$C_4H_5O_2^+$	85.029	+0.010
87	85	0.8	87.029	$C_4H_7O_2^+$	87.045	−0.016
97	115	1.1	97.037	$C_5H_5O_2^+$	97.029	+0.008
101	73	0.7	101.043			
115	29	0.3	115.029			
127	53	0.5	127.027	$C_6H_7O_3^+$	127.039	−0.012
128	29	0.3	128.012			
145	54	0.5	145.114	$C_6H_9O_4^+$	145.050	+0.064
163	222	2.1	163.108	$C_6H_{11}O_5^+$	163.065	+0.043
185	20	0.2	185.007	$C_6H_{10}O_5Na^+$	185.047	−0.040
203	43	0.4	203.056	$C_6H_{12}O_6Na^+$	203.101	−0.045
365	200	1.9	*calibration*	$(M + Na)^+$		
366	61	0.6	366.111	$^{13}C(M + Na)^+$	366.109	+0.002

[a] Total experimental ionization = 10701 counts, total tabulated ionization = 9208. Relative intensities >2% of total ionization for $m < 80$, >0.8% of total ionization for $80 < m < 100$, and >0.2% of total ionization for $m > 100$ tabulated. Calibration masses: Na^+ (22.9898); $(M + Na)^+$ (365.106). Run time = 1182 s.

TABLE 7

Negative-ion spectrum [a] of sucrose, $C_{12}H_{22}O_{11}$ (MW = 342)

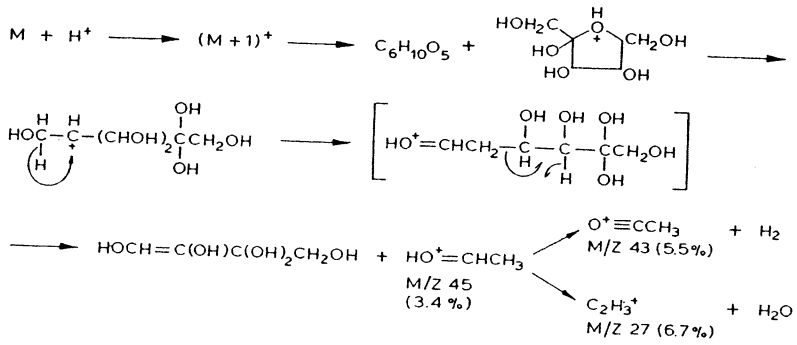
m/z	Intensity (counts)	Percent of total ionization	Meas. mass	Ion	Calc. mass	Δmass
41	663	6.3	40.997	HC_2O^-	41.003	−0.006
43	319	3.0	43.024	$C_2H_3O^-$	43.018	+0.006
45	753	7.2	45.000	HCO_2^-	44.998	+0.002
55	326	3.1	55.028	$C_3H_3O^-$	55.018	+0.010
57	212	2.0	56.998	$C_2HO_2^-$	56.998	0.000
58	417	4.0	58.024	$C_3H_6O^{-\cdot}$	58.042	−0.018
59	1512	14.4	59.030	$C_2H_3O_2^-$	59.013	+0.017
69	403	3.8	69.005	$C_3HO_2^-$	68.998	+0.007
71	1731	16.5	71.020	$C_3H_3O_2^-$	71.013	+0.007
73	248	2.4	73.004			
86	308	2.9	85.998	$C_3H_2O_3^{-\cdot}$	86.000	−0.002
87	619	5.9	87.013	$C_3H_3O_3^-$	87.008	+0.005
88	363	3.5	88.016	$C_3H_4O_3^{-\cdot}$	88.016	0.000
89	487	4.6	89.040	$C_3H_5O_3^-$	89.024	−0.016
99	270	2.6	99.010	$C_4H_3O_3^-$	99.008	+0.002
101	143	1.4	101.026	$C_4H_5O_3^-$	101.024	+0.002
113	81	1.1	113.048			
115	79	0.8	115.006	$C_4H_3O_4^-$	115.003	+0.003
117	70	0.7	117.002			
119	134	1.3	119.048			
129	91	0.9	129.030	$C_5H_5O_4^-$	129.019	+0.011
159	65	0.6	159.033	$C_6H_7O_5^-$	159.029	+0.004
179	67	0.6	179.060	$C_6H_{11}O_6^-$	179.056	+0.004
203	42	0.4	203.004			
341	162	1.5	*calibration*	$(M-1)^-$		
342	53	0.5		$^{13}C(M-1)^-$		
377	21	0.2	377.062			

[a] Total experimental ionization = 10482 counts, total tabulated ionization = 9639. Relative intensities >2% of total ionization for $m < 100$, >0.6% of total ionization for $100 < m < 200$, and >0.4% of total ionization for $m > 200$ tabulated. Calibration masses: C_2H^- (25.00782); $(M-1)^-$ (341.1084). Run time = 1443 s.

ions to sucrose molecules. In the negative-ion spectrum an $(M-1)^-$ quasi-molecular ion appears at *m/z* 341 with an intensity accounting for 1.5% of the total ionization. A striking feature of both spectra is that virtually no fragment ions with *m/z* values above 180 are observed; that is, the minimum amount of fragmentation that occurs entails rupture of the glycosidic bond between the glucose and fructose rings in the molecule. Loss of small molecules such as H_2O from the intact molecule to form $(M+1-18)^+$ ions, etc., does not seem to occur. In the positive-ion case, 75% of the total ionization appears at *m/z* values of 73 or less, and for the negative-ion spectrum the corresponding value is 65% of the total ionization. The most intense peak in

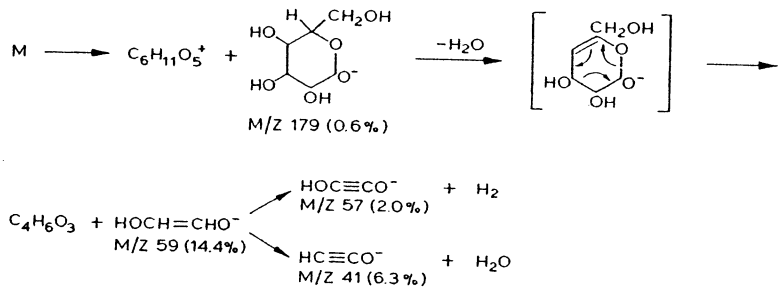
the positive-ion spectrum (m/z 39) accounts for only 9% of the total ionization, and in the negative-ion spectrum the most intense peak accounts for 17% of the total ionization. These results indicate that the dominant ionization process occurring is of high energy.

We have conceived of possible ionic reactions for the production of 10 ions in the positive spectrum accounting for 32% of the total ionization, and of 12 ions in the negative spectrum accounting for 66% of the total ionization. The possible reaction pathways for the positive ions involve known, straightforward gaseous-ion reactions, and while those for the negative ions are perhaps less well known, they are analogous to the reactions for the positive ions. Consequently, to save space, only examples of the kinds of reaction which may occur are given here. Thus, for positive ions:



(10)

and for negative ions:



(11)

Retro-Diels–Alder decompositions such as that shown in reaction (11) are useful in rationalizing both the positive- and negative-ion spectra of sucrose.

Daves [35] has summarized the mass spectra of sucrose obtained by different ionization techniques. Exact comparisons with our results are often difficult because many published spectra do not contain the degree of detail given in Tables 6 and 7 here, but it can be seen that the amount of fragmentation in FFI is significantly greater than in FD and laser desorption. Much fragmentation is observed in so-called in-beam CI and EI, and in EI flash-desorption, but exact comparisons with our results cannot be made. The

laser desorption results exhibit intense cationized quasi-molecular ions, and the intensities of these will in all likelihood depend in some way upon the number of cations present in the sample and on the desorbing surface. Since this will vary from experiment to experiment and apparatus to apparatus, exact comparisons between our results and the laser desorption results should not be made until this variable is controlled.

Guanosine

Positive- and negative-ion spectra of guanosine are given in Tables 8 and 9. Fragmentation is again extensive. The $(M + 1)^+$ ion is not observed, but alkali-ion cationized quasi-molecular ion species with low but significant intensities are observed. The most intense ion in the positive-ion spectrum is at *m/z* 72, and this ion has an appreciable mass defect. The precise mass corresponds to that of Na^+-cationized sodium cyanide, and the possibility of salt

TABLE 8

Positive-ion spectrum [a] of guanosine, $C_{10}H_{13}N_5O_5$ (MW = 283)

m/z	Intensity (counts)	Percent of total ionization	Meas. mass	Ion	Calc. mass	Δmass
28	2616	4.0	*calibration*	$HC{\equiv}NH^+$		
43	2752	4.2	43.031	$H_2N^+{=}C{-}NH$	43.030	+0.001
50	2503	3.8	50.000	$(NaCN)H^+$	50.000	0.000
72	9504	14.6	71.977	$(NaCN)Na^+$	71.983	−0.006
135	1675	2.6	135.021	$(B + 2 - NH_3)^+$	135.031	−0.010
136	364	0.6	136.034	$^{13}C(B + 2 - NH_3)^+$	136.034	0.000
151	553	0.8	151.027	$C_5H_5N_5O^{+\cdot}$	151.049	−0.022
152	9603	14.7	152.048	$(B + 2)^+$	152.057	−0.009
153	1609	1.6	153.040	$^{13}C(B + 2)^+$	153.060	−0.020
154	301	0.5	154.051			
174	6900	10.6	174.042	$(B + 1 + Na)^+$	174.039	+0.003
175	1038	1.6	175.019	$^{13}C(B + 1 + Na)^+$	175.042	−0.023
196	2606	4.0	196.030	$(B + 2\,Na)^+$	196.021	+0.009
197	554	0.8				
214	345	1.9	214.019			
216	671	1.0	216.000			
218	392	0.6	218.043			
306	2336	3.5	*calibration*	$(M + Na)^+$		
307	677	1.0	307.054	$^{13}C(M + Na)^+$	307.084	−0.030
328	1078	1.7	328.039	$(M - 1 + 2\,Na)^+$	328.063	−0.024
329	309	0.5		$^{13}C(M - 1 + 2\,Na)^+$		

[a] Total experimental ionization = 65248 counts, total tabulated ionization = 47846. Relative intensities >1.5% of total ionization for $m < 120$ and >0.5% of total ionization for $m > 120$ tabulated. Calibration masses: $HCNH^+$ (28.019); $(M + Na)^+$ (306.081). Run time = 7831 s.

TABLE 9

Negative-ion spectrum [a] of guanosine, $C_{10}H_{13}N_5O_5$ (MW = 283)

m/z	Intensity (counts)	Percent of total ionization	Meas. mass	Ion	Calc. mass	Δmass
26	16500	29.9	26.008	CN^-	26.003	+0.005
40	808	1.5	40.007	$CN_2^{-\cdot}$	40.006	+0.001
41	2216	4.0	41.019	CHN_2^-	41.014	+0.005
42	2017	3.7	42.000	CNO^-	41.998	+0.002
50	1574	2.9	50.001	C_3N^-	50.003	−0.002
65	1993	3.6	65.014	$C_3N_2H^-$	65.014	0.000
66	5965	10.8	66.011	$C_2N_3^-$	66.009	+0.002
75	1405	2.5	75.000	$C_2H_3O_3^-$	75.008	−0.008
90	1945	3.5	90.011			
106	882	1.6	106.024			
108	924	1.7	108.028	$C_4H_2N_3O^-$	108.020	+0.008
132	576	1.0	132.035	$(\text{sugar} - 1)^-$	132.042	−0.007
133	1395	2.5	133.021	$(B - NH_3)^-$	133.015	+0.006
134	346	0.6	134.033	$^{13}C(B - NH_3)^-$	134.026	+0.007
149	1429	2.6	149.026	$(B - 1)^-$	149.034	−0.008
150	3282	7.0	*calibration*	B^-		
151	337	0.6	151.043	$^{13}C(B^-)$	151.050	+0.007
172	280	0.5	171.988			
178	201	0.4	178.034			
282	224	0.4	282.082	$(M - 1)^-$	282.084	−0.002

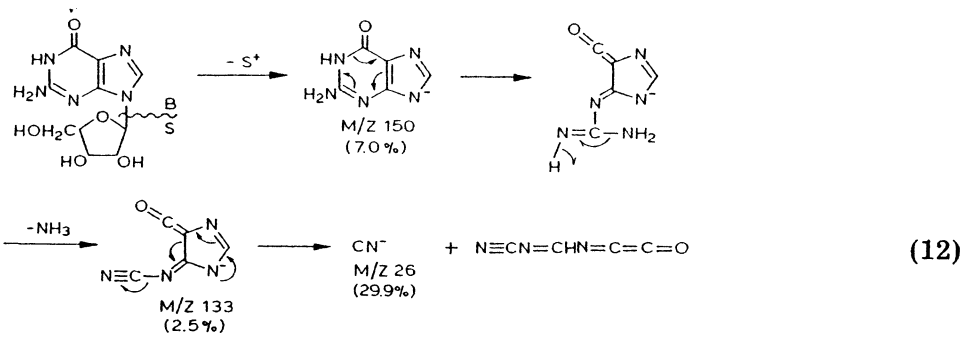
[a] Total experimental ionization = 55184 counts, total tabulated ionization = 44299. Relative intensities >1.5% of total ionization for $m < 120$, >1.0% of total ionization for $120 < m \leq 150$, and >0.35% of total ionization for $m > 150$ tabulated. Calibration masses: C_2H^- (25.008); (B^-) (150.047). Run time = 2121 s.

impurities producing ions of significant intensity has been pointed out above. An ion of moderate intensity is observed at *m/z* 50, and its precise mass corresponds to that of protonated sodium cyanide. Of the ions that can be attributed unequivocally to guanosine, the most intense ion in the positive spectrum is the $(\text{base} + 2)^+$ ion at *m/z* 152. Our FFI results in this regard are in agreement with results obtained by CI [32] and FD [36]. Intense ions corresponding to the cationized base moiety are also observed at *m/z* 174, 175, and 196. We suggest that the reactions producing these ions may be the same or analogous to those observed in CI, namely, protonation (or cationation) of the base followed by decomposition with hydrogen transfer from the sugar to the base.

The negative-ion spectrum is dominated by the CN^- ion, which constitutes 30% of the total ionization. Some of this ionization may result from the presence of cyanide impurities in the guanosine, but we have no real information about this.

We have conceived of reaction pathways for the production of 10 of the ob-

served fragment ions, but again only an illustrative example is given. Thus:



(12)

Other routes to CN^- may exist.

TABLE 10

Positive-ion spectrum [a] of 5′-adenosine monophosphate, $C_{10}H_{14}N_5O_7P$ (MW = 347)

m/z	Intensity (counts)	Percent of total ionization	Meas. mass	Ion	Calc. mass	Δmass
18	220	3.5	18.046	NH_4^+	18.034	+0.12
28	521	8.2	*calibration*	CH_2N^+		
30	171	2.7	30.035	CH_4N^+	30.034	+0.001
47	337	5.3	46.967			
50	189	3.0	49.997	C_3N^+	50.003	−0.006
55	175	2.8	55.031	$C_2H_3N_2^+$	55.030	+0.001
99	159	2.5	98.975	$H_4PO_4^+$	98.985	−0.010
108	72	1.1	108.057			
109	117	1.9	109.072			
119	264	4.2	119.045	$(B + 2 - NH_3)^+$	119.036	+0.009
121	142	2.2	121.003			
125	121	1.9	124.991			
135	89	1.4	135.079			
136	1603	25.4	*calibration*	$(B + 2)^+$		
137	179	2.8	137.057	$^{13}C(B + 2)^+$	137.065	−0.008
138	79	1.3	138.088			
148	75	1.2	148.047			
158	96	1.5	158.098	$(B + 1 + Na)^+$	158.044	+0.054
178	73	1.2	178.063			
348	31	0.5	348.095	$(M + 1)^+$	348.071	+0.024
370	23	0.4	370.182	$(M + Na)^+$	370.053	+0.132

[a] Total experimental ionization = 6320 counts, total tabulated ionization = 4736. Relative intensities >2% of total ionization for $m < 100$, >1% of total ionization for $100 < m < 200$, and >0.5% of total ionization for $m > 200$ tabulated. Calibration masses: CH_2N^+ (28.019); $C_5H_6N_5^+$ (136.062). Run time = 1159 s.

5'-Adenosine monophosphate

Tables 10 and 11 give the positive- and negative-ion spectra of this nucleotide. The major aspects of its positive-ion spectrum are very similar to those for the nucleoside, guanosine. The quasi-molecular ion intensities are low, and the protonated and/or cationized base intensities are relatively high. Not unexpectedly, adenosine monophosphate produces an ion at m/z 99 which, on the basis of its precise mass and chemical expectations, may be attributed to protonated phosphoric acid. The negative-ion spectrum of adenosine monophosphate is also similar to that of guanosine. Thus, the quasi-molecular $(M-1)^-$ ion intensities are low, CN^- intensities are high, and base ion intensities are moderate. As in the positive-ion spectrum, the negative-ion spectrum of adenosine monophosphate strongly indicates the presence of the phosphate group in the molecule, for the PO_3^- ion at m/z 79 constitutes 41% of the total ionization, and moderate intensities of PO_2^- (m/z 63) and $H_2PO_4^-$ (m/z 97) are also observed. The CN^-, PO_2^-, PO_3^-, and $H_2PO_4^-$ ions together

TABLE 11

Negative-ion spectrum [a] of 5'-adenosine monophosphate, $C_{10}H_{14}N_5O_7P$ (MW = 347)

m/z	Intensity (counts)	Percent of total ionization	Meas. mass	Ion	Calc. mass	Δmass
26	3414	21.1	26.008	CN^-	26.003	+0.005
50	332	2.1	50.002			
63	1284	7.9	62.963	PO_2^-	62.964	−0.001
66	408	2.5	66.010			
79	6684	41.3	78.959	PO_3^-	78.959	0.000
90	490	3.0	90.017			
97	937	5.8	96.979	$H_2PO_4^-$	96.969	+0.010
106	146	0.9	105.997			
117	130	0.8	117.020			
132	98	0.6	132.019			
134	551	3.4	*calibration*	$C_5H_4N_5^-(B^-)$		
159	309	1.9	158.921	$(HPO_3)PO_3^-$	158.926	−0.005
177	86	0.5	176.939	$(H_3PO_4)PO_3^-$	176.936	+0.003
181	83	0.5	180.898			
215	32	0.2	214.842			
221	36	0.2				
223	32	0.2				
346	65	0.4	346.060	$(M-1)^-$	346.055	+0.005
383	32	0.2				

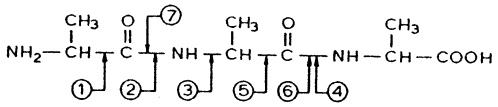
[a] Total experimental ionization = 16183 counts, total tabulated ionization = 15148. Relative intensities >2% of total ionization for $m < 100$, >0.5% of total ionization for $100 < m < 200$, and >0.2% of total ionization for $m > 200$ tabulated. Calibration masses: C_2H^- (25.0078); $C_5H_4N_5^-$ (134.047). Run time = 1200 s.

comprise 76% of the total ionization and indicate very extensive fragmentation.

It is of interest to compare the amounts of fragmentation occurring in the positive-ion spectra obtained by laser desorption [34], field desorption [36], and our FFI experiments. If the base ions are assigned a relative intensity of

TABLE 12

Positive-ion spectrum [a] of alanylalanylalanine, $C_9H_{17}N_3O_4$ (MW = 231)



m/z	Intensity (counts)	Percent of total ionization	Ion structure	Bond cleaved	Type of ion
15	170	1.1			
18	461	3.1			
28	556	3.7			
30	198	1.3			
42	693	4.6			
44	7579	50.8	$^{+}NH_2{=}CHCH_3$	①	*N*-imine
45	323	2.2			
56	215	1.4			
70	151	1.0			
72	74	0.5	$NH_2CHCH_3C{\equiv}O^{+}$	②	*N*-acylium
89	54	0.4	$NH_2CHCH_3CONH_3^{+}$	③	*N*-amide
90	81	0.5	$^{+}NH_3CHCH_3COOH$	④	*C*-ammonium
99	199	1.3			
106	98	0.7			
108	64	0.4			
115	267	1.8	$NH_2CHCH_3CON^{+}H{=}CHCH_3$	⑤	*N*-imine
143	183	1.2	$NH_2CHCH_3CONHCHCH_3C{\equiv}O^{+}$	⑥	*N*-acylium
161	302	2.0	$^{+}NH_3CHCH_3CONHCHCH_3COOH$	⑦	*C*-ammonium
177	56	0.4			
187	68	0.5			
232	357	2.4	$(M + 1)^{+}$		
254	876	5.9	$(M + Na)^{+}$		
255	110	0.7			
276	229	1.5	$(M - 1 + 2\,Na)^{+}$		
277	40	0.3			
294	155	1.0			
296	86	0.6			
316	125	0.8			
318	64	0.4			

[a] Total experimental ionization = 14932 counts, total tabulated ionization = 13834. Relative intensities >1% of total ionization for $m < 80$ and >0.4% of total ionization for $m >$ 80 tabulated. Run time = 1224 s.

1, the quasi-molecular ion intensities observed in laser desorption, field desorption, and FFI are 1, 0.6, and 0.02, respectively. However, we once again emphasize that the laser desorption results in particular show extensive cationation of the molecule and ours do not. This could result from different amounts of cations present in the two experiments, perhaps making the results not exactly comparable.

Alanylalanylalanine

The positive- and negative-ion spectra are given in Tables 12 and 13. In the positive-ion spectrum the most intense ion appears at *m/z* 44, and this is probably the imine ion which is also the most intense in the positive-ion spectrum of alanine. Quasi-molecular ions of moderate intensity are observed at *m/z* 232, 254, and 276. The fragmentation produces the types of ions observed by Field and co-workers [37] in their study of peptide sequencing by *i*-butane chemical ionization. The structures of ions of this type, the bonds

TABLE 13

Negative-ion spectrum [a] of alanylalanylalanine, $C_9H_{17}N_3O_4$ (MW = 231)

m/z	Intensity (counts)	Percent of total ionization	Meas. mass	Ion	Calc. mass	Δmass
26	6536	31.2	26.009	CN^-	26.003	+0.006
40	465	2.2	40.029	$C_2H_2N^{-\cdot}$	40.019	+0.010
42	2588	12.3	42.003	CNO^-	42.998	+0.005
44	208	1.0	44.050	$C_2H_6N^-$	44.050	0.000
50	580	2.8	49.996	C_3N^-	50.003	—0.007
65	249	1.2	65.010			
87	385	1.8	87.070	$C_3H_5NO_2^{-\cdot}$	87.032	+0.038
88	589	2.8	88.061	$C_3H_6NO_2^-$	88.040	+0.021
97	440	2.1	96.976	HSO_4^-	96.960	+0.017
115	1033	4.9	114.927			
117	496	2.4	116.913			
131	484	2.3	130.922			
133	203	1.0	132.892			
206	118	0.6	205.871			
230	1555	7.4	*calibration*	$(M-1)^-$		
231	150	0.7	231.025			
291	136	0.6	291.060			
292	263	1.3	292.108			
293	178	0.9	293.086			
319	261	1.3	319.104			
321	133	0.6	321.065			

[a] Total experimental ionization = 20957 counts, total tabulated ionization = 17050. Relative intensities >1% of total ionization for $m < 120$ and >0.4% of total ionization for $m > 120$ tabulated. Calibration masses: C_2H^- (25.0078); $(M-1)^-$ (230.114). Run time = 1294 s.

which may be cleaved in producing them, and the category of the ion are included in Table 12. Table 12 also includes ions with *m/z* values above the expected quasi-molecular ion region. These may result from cationization from a trace Cu impurity.

The negative-ion spectrum given in Table 13 is uninformative. The $(M-1)^-$ ion is present in moderate abundance, which provides information about the molecular weight of the compound, but as was the case for other compounds, a very large fraction of the total ionization appears as CN^- and CNO^-. Ions with moderate intensity appear at *m/z* 115, 117, 131, and 133, but these ions are so mass deficient that they are probably produced from an inorganic impurity.

Lysyltyrosylthreonine

We have determined the positive- and negative-ion spectra of this strongly hydrophilic tripeptide, but in both the positive- and negative-ion modes the fragmentation is very extensive and no new spectral features are observed. The relative intensities of the $(M+1)^+$, $(M+Na)^+$, and $(M-1+2\ Na)^+$ ions account for 0.3, 0.4, and 0.4% of the total positive ionization, respectively. The most massive ion with an intensity equal to 1% or more of the total positive ionization is at *m/z* 142; that is, most of the ionization is in the low mass range. The $(M-1)^-$ ion has an intensity corresponding to 0.2% of the total ionization, and once again a very large fraction of the ionization involves the CN^- and CNO^- ions.

DISCUSSION

Two important conclusions to be drawn from this work are (1) the amount of fragmentation occurring in fission fragment ionization even for the relatively simple molecules included in this study is large, and (2) much of the fragmentation that occurs can be rationalized in terms of established concepts of gaseous-ion chemistry. We reiterate the point made several times previously, namely, that the amount of energy transferred to molecules undergoing ionization must be relatively large and FFI should not be looked upon only as a soft ionization method. To the extent that comparisons can be made, the spectra we have obtained by FFI differ in important ways from CI, FD, and laser desorption spectra. No direct comparisons of our spectra with the EI spectra of these compounds have been made because either the latter do not exist or they involve so much thermal decomposition in the course of volatilization that the comparisons would be specious. However, it is our opinion that the general phenomena observed in our FFI experiments are different from those observed in electron ionization. Indeed, the FFI spectra appear to be different from any other kind of spectra known to us. We emphasized above that the spectra produced (both positive and negative) contain mostly even-electron ions, and the spectra appear as though they are

produced by chemical ionization processes but with the inclusion of much more energy than is involved in conventional chemical ionization. A noteworthy aspect of the spectra is the very great tendency for the production of many ions with high intensity at low m/z values; that is, when fragmentation occurs it has a tendency to continue to very small entities. Fragmentation in mass spectra can be used to deduce structural features of the molecules producing the spectra and, indeed, it is recognized that this is a useful aspect of EI mass spectrometry. However, the fragmentation with FFI observed in this study is so extensive that it may be of limited use for structure determinations. However, our data-base for making predictions is obviously limited, and more experiments must be done to delineate this matter.

While concepts of gas-phase ion chemistry have been successfully applied to the fragmentation processes, we point out that we do not know where in the course of volatilization—ionization the fragmentation occurs. It could be in the gas phase at some appreciable distance from the surface of the sample foil, or it could be on or near the foil with the fragmentation occurring in the course of the volatilization—ionization.

Macfarlane and co-workers have accumulated an impressive body of results (see references given in Introduction) demonstrating that FFI can produce quasi-molecular ions from a variety of compounds including some that are extraordinarily large and involatile. It is clear that FFI has great utility for determining molecular weights of substances not ordinarily accessible to mass spectrometry. The implication of Macfarlane's results is that FFI is an exceedingly gentle ionization method, and our conclusion that it is not gentle is an apparent contradiction. However, this contradiction may be more apparent than real. Thus, while extensive fragmentation has been observed in the compounds we have studied, it is also true that measurable amounts of quasi-molecular ions have always been observed and our results would always enable the molecular weights of the compounds investigated to be deduced. In the papers of Macfarlane and co-workers little attention is paid to the amount and kind of fragmentation occurring, although an occasional passing reference is made to its existence. We think it possible that fragmentation (especially to produce low-mass ions) occurred in their measurements and that their results and ours are not in serious basic disagreement. While we have examined fewer compounds than Macfarlane and co-workers and have not had the opportunity to examine compounds of similar size, we believe that our present results and reasonable extrapolations based upon them tend to confirm Macfarlane's findings concerning the power and analytical utility of the FFI method.

Thus it appears that the FFI method has a dual character; namely, desorption and ionization seem to involve two modes: a gentle one producing intact quasi-molecular ions and a violent one producing, for example, the kind of fragmentation that in the case of negative ions reduces guanosine extensively to CN^- ions. This dichotomy of behavior is not observed, to our knowledge, in other high-energy ionization processes.

ACKNOWLEDGEMENTS

This work was supported by grants from the Division of Research Resources, National Institutes of Health, the National Science Foundation, and the donors of the Petroleum Research Fund, administered by the American Chemical Society. F.H.F. wishes to acknowledge with thanks the contributions made to the design and construction of the fission fragment ionization apparatus by the following former members of this laboratory: R.F. Bonner, D.V. Bowen and M. Mautner.

REFERENCES

1 H.W. Schmitt, W.E. Kiker and C.W. Williams, Phys. Rev., 137 (1965) B837.
2 D.F. Torgerson, R.P. Skowronski and R.D. Macfarlane, Biochem. Biophys. Res. Commun., 60 (1974) 616.
3 R.D. Macfarlane and D.F. Torgerson, Science, 191 (1976) 920.
4 R.D. Macfarlane and D.F. Torgerson, Int. J. Mass Spectrom. Ion Phys., 21 (1976) 81.
5 C.A. Hassell, ^{252}Cf-Plasma Desorption Mass Spectrometry of Dipeptides and Tripeptides. Doctoral Dissertation 76-3643, University Microfilms International, Ann Arbor, MI, 1977.
6 R.D. Macfarlane, in G.R. Waller and O.C. Dermer (Eds.), Biomedical Applications of Mass Spectrometry, First Supplementary Volume, Wiley, 1980, Ch. 38.
7 R.D. Macfarlane, in Trace Organic Analysis: A New Frontier in Analytical Chemistry, Proc. 9th Materials Research Symposium, April 10—13, 1978, Gaithersburg, MD, Natl. Bur. Stand. Spec. Publ. 519 (1979).
8 H. Kasai, K. Nakanishi, R.D. Macfarlane, D.F. Torgerson, Z. Ohashi, J.A. McCloskey, H.J. Gross and S. Nishimura, J. Am. Chem. Soc., 98 (1976) 5044.
9 C.J. McNeal and R.D. Macfarlane, presented at the 25th Ann. Conf. on Mass Spectrometry and Allied Topics, Washington, DC, May 29—June 3, 1977, Pap. RC6.
10 C.J. McNeal and R.D. Macfarlane, presented at the 26th Ann. Conf. on Mass Spectrometry and Allied Topics, St. Louis, MO, May 28—June 2, 1978, Pap. FB8.
11 C.J. McNeal, S.A. Narang, R.D. Macfarlane, H.M. Hsiung and R. Brousseau, Proc. Natl. Acad. Sci. U.S., 77 (1980) 735.
12 R.D. Macfarlane, D. Uemura, K. Ueda and Y. Hirata, J. Am. Chem. Soc., 102 (1980) 875.
13 J.E. Hunt, R.D. Macfarlane, J.J. Katz and R.C. Dougherty, Proc. Natl. Acad. Sci. U.S., 77 (1980) 1745.
14 O. Becker, N. Fürstenau, F.R. Krueger, G. Weiss and K. Wien, Nucl. Instrum. Methods, 139 (1976) 195.
15 O. Becker, N. Fürstenau, W. Knippelberg and F.R. Krueger, Org. Mass Spectrom., 12 (1977) 461.
16 B. Schueller and F.R. Krueger, Org. Mass Spectrom., 14 (1979) 439.
17 R.D. Macfarlane and D.F. Torgerson, Phys. Rev. Lett., 36 (1976) 486.
18 R.D. Macfarlane, Biophys. J., 24 (1978) 375.
19 N. Fürstenau, W. Knippelberg, F.R. Krueger, G. Weiss and K. Wien, Z. Naturforsch., 32a (1977) 711.
20 F.R. Krueger, Z. Naturforsch., 32a (1977) 1084.
21 L.E. Sieberling, J.E. Griffith and T.A. Tombrello, A Thermalized Ion Explosion Model for High Energy Sputtering and Track Registration, Band Aid Preprint in Atomic and Applied Physics, California Institute of Technology, BAP-21.
22 P. Dück, W. Treu, W. Galster, H. Fröhlich and H. Voit, Nucl. Instrum. Methods, 168 (1980) 601

23 S. Pauker and N.H. Steiger-Shafrir, Nucl. Instrum. Methods, 91 (1971) 557.
24 C.J. McNeal, R.D. Macfarlane and E.L. Thurston, Anal. Chem., 51 (1979) 2036.
25 R.D. Macfarlane, D.F. Torgerson, Y. Fares and C.A. Hassel, Nucl. Instrum. Methods, 116 (1974) 381.
26 R.F. Bonner, D.V. Bowen, B.T. Chait, A. Lipton, F.H. Field and W.F. Sippach, Anal. Chem., 52 (1980) 1923.
27 H.U. Winkler and H.D. Beckey, Org. Mass Spectrom., 6 (1972) 655.
28 G.W.A. Milne, T. Axenrod and H.M. Fales, J. Am. Chem. Soc., 92 (1970) 5170.
29 M. Meot-Ner and F.H. Field, J. Am. Chem. Soc., 95 (1973) 7207.
30 Eight-Peak Index of Mass Spectra, Mass Spectrometry Data Centre, AWRE, Aldermaston, Reading, U.K., 1974.
31 A.L.C. Smit and F.H. Field, J. Am. Chem. Soc., 99 (1977) 6471.
32 D.F. Hunt, J. Shabanowitz, F.K. Batz and D.A. Brent, Anal. Chem., 49 (1977) 1160.
33 R.J. Beuhler, E. Flanigan, L.J. Greene and L. Friedman, J. Am. Chem. Soc., 96 (1974) 3990.
34 M.A. Posthumus, P.G. Kistemaker, H.L.C. Meuzelaar and Ten Noever de Brauw, Anal. Chem., 50 (1978) 985.
35 G.D. Daves, Jr. and G. Doyle, Acc. Chem. Res., 12 (1979) 359.
36 H.R. Schulten and H.D. Beckey, Org. Mass Spectrom., 7 (1973) 861.
37 M. Mudgett, D.V. Bowen, F.H. Field and T.J. Kindt, Biomed. Mass Spectrom., 4 (1977) 159.