platinum and mercury electrodes and also spectroscopic data has been required to elucidate the nature of the species present in solution. On evaporation of solutions of $[Hg_3(Et_2dtc)_4]^{2+}$, the solid isolated has been shown to be $[Hg_5(Et_2dtc)_8](ClO_4)_2$. The crystallization of this particular complex is, we believe, almost accidental and depends solely upon relative solubility factors in this complex solution, and in solution it dissociates to reform $[Hg_3(Et_2dtc)_4]^{2+}$ and $Hg(Et_2dtc)_2$. Nevertheless, its formation does give some indication of the great complexity of the range of species in solution which are undergoing exchange which is rapid on the synthetic and electrochemical time scales and of intermediate rate on the NMR time scale. Thus $[Hg_3(Et_2dtc)_4]^{2+}$ (or multiples of this) is an average description of the thermodynamically stable range of complexes in solution generated after the apparently simple process of one-electron oxidation of $Hg(Et_2dtc)_2$.

Oxidative chemistry in situations where the ligand is readily oxidized and also stabilizes high oxidation states is always likely to have the capacity to generate complex oxidative redox chemistry as the present example illustrates.

Registry No. $Hg(Et_2dtc)_2$, 14239-51-1; $[Hg_5(Et_2dtc)_8](ClO_4)_2$, 105762-81-0; $Hg_2(ClO_4)_2$, 65202-12-2; $[Hg_3(Et_2dtc)_4]^{2+}$, 105729-53-1; $[Hg_5(Et_2dtc)_8]^{2+}$, 105729-52-0; $Bu_4N[Et_2dtc]$, 71195-45-4; Et_4tds, 97-77-8; Bu_4NClO_4, 1923-70-2; Et_4NI, 68-05-3; ^{199}Hg, 14191-87-8; Hg, 7439-97-6; Pt, 7440-06-4.

Supplementary Material Available: Tables S-I–S-III listing hydrogen coordinates, anisotropic thermal parameters, and equations of least squares (7 pages); listing of structure factor data (10 pages). Ordering information is given on any current masthead page.

Aspects of the Production of FAB and SIMS Mass Spectra

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Received September 26, 1986

Abstract: It is shown that reduction is a common process in FAB mass spectra and that these processes are frequently consistent with one-electron reductions. The model previously presented by Cooks et al. to rationalize features of SIMS (and, by analogy, FAB) spectra obtained from a matrix is developed in terms of qualitative energetic considerations. It is shown that the population of ions observed is consistent with the formation of gas-phase ions whose energies are in the approximate range 0–3 eV (and not >8 eV). From an analysis of the ratio of singly to doubly charged ions in FAB spectra, it is deduced that in the positive ion spectra of highly basic compounds (e.g., arginine–containing peptides), comparable amounts of the analyte are desorbed as ions and neutrals. In contrast, it is concluded that polyhydroxy compounds lacking a highly basic site are largely desorbed as neutral species, with a consequent loss in sensitivity.

The advent of the particle bombardment methods of mass spectrometry, e.g., SIMS,[1] FAB,[2] and fission fragment ionization,[3] has revolutionalized our ability to obtain molecular weight information on relatively large polar molecules. In many respects, the spectra obtained by the above techniques are rather similar, e.g., abundant MH^+ ions in most positive ion spectra and abundant $(M - H)^-$ ions in negative ion spectra, with relatively little fragmentation.[4] Yet the physicochemical phenomena which are responsible for these features are far from clear. In this paper, we report some observations from FAB mass spectra and consider plausible explanations for our observations. It is hoped that these observations and explanations, which are presented with a brief summary of relevant conclusions from prior work, will facilitate the most efficient exploitation of particle bombardment methods in subsequent research in bioorganic chemistry.

The translational energies of the particles used in plasma desorption mass spectrometry are of the order of 100 MeV, whereas for SIMS and FAB mass spectrometry the energies are in the region of 10 keV. It is considered that at the higher translational energies, the dominant initial absorption of energy by the target occurs via electronic excitation. At the lower translational energies, the target initially absorbs energy predominantly by recoil of the nuclei of its atoms or molecules.[5] While sputtering from the target is a direct consequence of the latter event, it can readily be seen[6] how electronic excitation can subsequently also deposit a few electronvolts of kinetic energy into nuclear motion; if any repulsive excited states are generated, these will decompose with release of translational energy. Additionally, positively charged ions generated within the particle track can promote translational energy release via their charge–charge repulsions. Even in the case of keV particles, it is clear both from prior work and data which we present that ionization events will compete with direct translational excitation of the nuclei. We commence our discussion with chemical evidence for such ionization by keV particles. Later, we present a model which relates the energies of the collisions undergone by desorbed species to the approximate range of ΔH_f values of ions observed in FAB. The essence of this model is that although a desorbed species does not undergo sufficient collisions following its initial translational excitation (by atom or ion impact) to generate a Maxwell–Boltzman distribution, sufficient collisions occur so that the final excitation energies of the desorbed species are largely determined by their relative translational energies at the end of the collision cascade. We conclude that these relative translational energies, and final excitation energies, are only ca. 0–3 eV. A final excitation energy of ca 0–3 eV means, for example, that if two colliding species present at the end of the collision cascade are represented in their most stable forms by A and B, then modifications of these forms (A′, B′, ..., etc.) can

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(1) Benninghoven, A.; Jaspers, D.; Sichterman, W. *Appl. Phys.* **1976**, *11*, 35. Busch, K. L.; Cooks, R. G. *Science (Washington, DC)* **1982**, *218*, 247.

(2) Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *Nature (London)* **1981**, *293*, 270.

(3) Macfarlane, R. D. *Acc. Chem. Res.* **1982**, *15*, 268.

(4) See, for example, a recent review: Field, F. H., manuscript presented at the International Conference on Mass Spectrometry, Swansea, U.K., September, 1985.

(5) See, for example: Sigmund, P. *Top. Appl. Phys.* **1981**, *47*, 9.

(6) Johnson, R. E.; Sundqvist, B. *Int. J. Mass Spectrom. Ion Phys.* **1983**, *53*, 337.

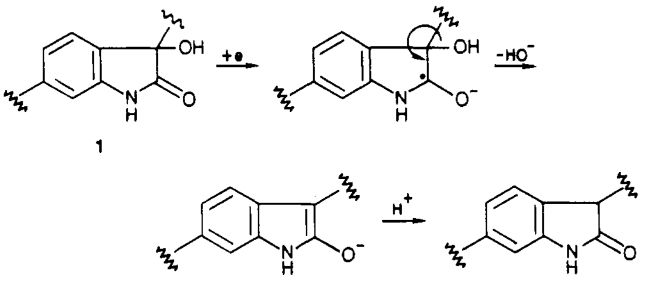
0002-7863/87/1509-1980$01.50/0

be populated only if the sum of their energies is $\lesssim 3$ eV in excess of the sum of the energies of A and B.

Discussion

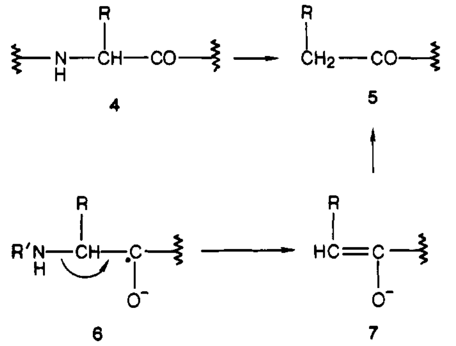
1. Observation of Reduction Products in FAB Mass Spectra. Ionization of a target molecule (matrix or sample) will initially produce an electron and a cation radical. Since the electron mass is much less than that of the molecules, its response time (ca. 10^{-16} s) is much less, and it will move more rapidly from the site of its production than other particles. The initial translational energies of at least some of these electrons will be reduced toward thermal energies via collisions. When thermal or near thermal energies are reached, the electrons can be captured by sample molecules in the matrix. The processes effected will be one-electron reductions, with the requirement that any sample to be reduced must have available relatively low-lying unoccupied orbitals in which to initially capture the electron. In summary, one-electron reduction will provide evidence for ionization as a consequence of Xe atom bombardment. In support of such ionization, we now list some chemical reductions which occur in FAB mass spectrometry (using glycerol or diglycerol/thioglycerol (1:1) as matrix), all of which can reasonably be accommodated as one-electron reductions.

(i) A peptide of molecular weight 1020, obtained by oxidation of the naturally occurring peptide moroidin, was deduced to contain the unit **1**.[7] Its FAB mass spectrum[7] showed the formation of a comparable amount of a reduction product with MH^+ at 16 mu below that of the original peptide. We formulate this as a one-electron reduction of an α-hydroxyamide (**1** → **2** → **3**).



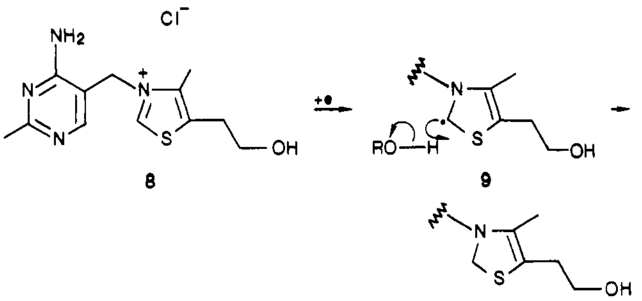
1 **2** **3**

(ii) In analogy to the above process, it is observed that many peptides show peaks 15 mu below MH^+, which arise due to net loss of NH. Additionally, fragment ions (which are useful in sequence studies) corresponding to the overall change **4** → **5** are frequently observed.[8] Both these phenomenon can be accommodated as one-electron reductions (**6** → **7** → **5**). It is noteworthy that these processes are otherwise very difficult to understand; e.g., direct loss of NH (the apparent loss when R = H in **6**) is far too high in its energy requirement.



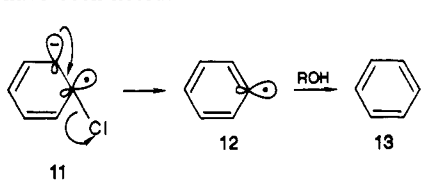
4 **5** **6** **7**

(iii) The thiazolium salt **8** shows in its FAB mass spectrum not only an abundant ion associated with the intact cation (*m/z* 265) but also a dihydro product (*m/z* 267) of approximately 35% of the relative abundance of *m/z* 265. Imminium cations should be ready acceptors of electrons, and the resulting radicals can then abstract a hydrogen atom from the matrix to produce the dihydro derivative **10** (which will be analyzed as its protonated form in the usual way).



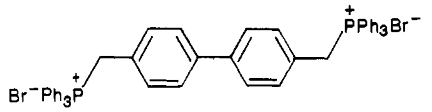
8 **9** **10**

(iv) The antibiotic vancomycin contains two chlorines attached to aromatic rings. When its FAB mass spectrum is obtained, the antibiotic is partially reduced by a process in which one Cl is replaced by H. This process can be formulated as a one-electron addition to the aromatic ring followed by the sequence **11** → **13**[9] and is analogous to reductions which occur on pulse radiolysis. Other dehalogenation reactions occurring in FAB mass spectrometry have been noted.[10]

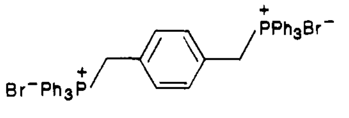


11 **12** **13**

(v) In the spectra of **14** and **15**, and other bis(phosphonium) salts which we have examined, abundant singly charged ions are formed by a reductive process. For example, **15** affords an abundant ion due to $Ph_3P^+CH_2C_6H_4CH_3$. Such a reduction can occur via capture of a slow electron (in the vacant d-orbitals of phosphorus), followed by loss of PPh_3. The reduction would then be completed by hydrogen radical abstraction (by the benzyl radical) from the matrix.



14



15

The above examples, taken in conjunction with previously reported work pointing to electron-capture processes occurring in FAB mass spectrometry[11] and SIMS[12] can leave little doubt that keV particles produce electrons in the matrix via ionization of target molecules and that these electrons can then be effective as reducing agents.

2. Model for Desorption from a Volatile Matrix. In the introductory section it was pointed out that previous work indicates that translational excitation of nuclei, and ionization, caused by bombarding particles results in desorption of target molecules and

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[7] Leung, T.-W. C.; Williams, D. H.; Barna, J. C. J.; Foti, S. *Tetrahedron* **1986**, *42*, 3333.

[8] Williams, D. H.; Bradley, C. V.; Santikarn, S.; Bojesen, G. *Biochem. J.* **1982**, *201*, 105.

[9] Andrieux, C. P.; Blocman, C.; Dumas-Bouchiat, J. M.; M'Italla, F; Saveant, J. M. *J. Am. Chem. Soc.* **1980**, *102*, 3806.

[10] Sethi, S. K.; Nelson, C. C.; McCloskey, J. A. *Anal. Chem.* **1984**, *56*, 1975.

[11] Clayton, E. C.; Wakefield, A. J. C. *J. Chem. Soc., Chem. Commun.* **1984**, 969. Cooper, R.; Ungar, S. *J. Antibiot.* **1985**, *38*, 24.

[12] Pelzer, G.; De Pauw, E.; Dung, D. V.; Marien, J. *J. Phys. Chem.* **1984**, *88*, 5065.

ions. In developing a quantitative approach to the energetics of desorption by FAB and SIMS, we follow a model for the desorption process which has been presented by Busch et al.[13] and by Cooks and Busch.[14] Our description of this model, elaborated to include our semiquantitative consideration of the energetics, is as follows.

(i) The large amounts of energy which are initially deposited by particle bombardment initially give a wide range of translational energies to particles in the matrix. Although we cannot define this range with any confidence, we propose it is considerably higher than 0–3 eV. Due to the very high energies associated with the initial events, particles which formerly were bonded in their environments by a wide range of energies are dislodged from their environments. Hence, particles (ions and neutrals) with a wide range of ΔH_f values ($\gg$0–3 eV) can be translationally excited. Since we shall not be dealing with absolute ΔH_f values, we define the translationally excited particles as having high ΔH_f values when this ΔH_f is greater than that of the species in its original environment by >3 or $\gg$3 eV. Species with high ΔH_f values would therefore include metal ions and atoms (in dry SIMS),[14] large polar molecules stripped of large numbers of solvent molcules (before the large molecule has had time to undergo a change in conformation), and cation radicals. The last group of ions clearly qualifies since the ΔH_f values of organic molecules are typically 7–10 eV lower than those of the corresponding cation radicals.

(ii) Following the earlier model,[13,14] it is assumed that the flux of particles leaving the matrix is such that numerous collisions will occur between them before they become isolated in the gas phase and then analyzed. The number of these collisions, while insufficient to cause thermal equilibration, is sufficient to transform, to more stable species, those which have the highest ΔH_f values. To illustrate this last point, we refer to the thermochemistry of the $H_3O^+ (H_2O)_n$ system.[15] The ion H_3O^+ is taken to typify a species of high ΔH_f which may be desorbed from a matrix due to the high energies associated with this process. Suppose this undergoes an encounter with a water dimer, for which $\Delta H_f \simeq -110$ kcal mol^{-1} (since $\Delta H_f(H_2O) = -58$ kcal mol^{-1}, and the hydrogen bond strength in the water dimer is calculated to be ca. 6 kcal mol^{-1}). The most exothermic exit channel for the transiently formed complex is as follows (where $H_3O^+(H_2O)_2$ is the collision complex):

$$H_3O^+(H_2O)_2 \rightarrow H_3O^+{\cdot}H_2O + H_2O$$

The final combination is more favored than the initial state by 26 kcal mol^{-1}, i.e., by $\Delta H_f(H_3O^+{\cdot}H_2O) + \Delta H_f(H_2O) - \Delta H_f(H_3O^+) - \Delta H_f([H_2O]_2)$. Thus, approximately 1 eV of energy is made available to provide a mixture of vibrational and translational excitation and with the result that the initially produced species of high ΔH_f does not survive to be recorded. It is clear from the above example that the more collisions an initially isolated ion of high ΔH_f makes with oligomers of low ΔH_f, the more products of intermediate ΔH_f may be generated.

The collisions remove the "hot" species, but it is important to know qualitatively what range of ΔH_f values can still be populated. This will be determined by the range of translational energies of the particles which pertain at the end of the collision cascade. We propose in the light of the experimental data which are presented later that the relative translational energies following the collisions lie in the range 0–3 eV. It follows that these relative translational energies are sufficient to maintain a population of particles whose ΔH_f values spread over a similar range. This is essentially a consequence of the Boltzmann equation but with the proviso that the number of collisions is so limited that the available energy has not equilibrated into internal modes. Thus, among the population of ions in the particles, species of high ΔH_f have clearly been quenched; these will include cation radicals and multiply charged ions that cannot be effectively solvated (either intramolecularly or intermolecularly).

(iii) The final step of relevance to our discussion may be desolvation of an ion or neutral following the above collisions.[13,14] Where desolvation is endothermic (as it will be unless it is replaced by intramolecular solvation, associated with a conformational change in the desolvated molecule or ion), it provides a mechanism for converting vibrational energy of the reactant into electronic energy of products. By this, we imply that energy of bonding (in the solvated ion) has been removed by using the available vibrational energy to produce separated, and less stable, products. It is thus a mechanism to reduce fragmentation relative to the situation found in the absence of a matrix.[13,14]

In closing this discussion of the model, we note that the generation of particles with a wide range of ΔH_f values (0–x eV, where $x \gg 3$) at a time closely following atom (ion) impact will clearly include some particles of low ΔH_f values. The analysis under i–iii above implies that the population of particles of low ΔH_f values will be swelled as the collision cascade proceeds.

There is clear experimental evidence to support postulates ii and iii. Spectra obtained by dry SIMS may exhibit species of high ΔH_f, but addition of a matrix removes these species.[16] Additionally, it has been shown that the addition of *p*-toluenesulfonic acid to solutions of zwitterionic substances not only increases dramatically the abundance of MH^+ (due to the desorption of preformed ions) but also markedly decreases fragmentation relative to MH^+.[17]

3. Experiments to Support Energetic Aspects of the Model. The important consequences of the model, from the viewpoint of energy, are that species of energy 0–3 eV can be populated and that species having the highest values will be populated to the smaller extent (a *partial* equilibration of energy having occurred). Experiments which support these assertions are now presented.

(i) Pelzer et al.[12] have shown that in SIMS and FAB spectra obtained from a glycerol matrix, the following partial reductions may occur: $Cr^{3+} \rightarrow Cr^{2+}$; $Cu^{2+} \rightarrow Cu^+$; and $Fe^{3+} \rightarrow Fe^{2+}$. In a system which is partially equilibrated through a limited number of collisions, these reactions should be reversible. When $Cr(NO_3)_3$ is bombarded in a glycerol matrix, Cr^{3+} is partially reduced to Cr^{2+}; the data of Pelzer et al.[12] show that the abundance of $[Cr^{3+}(G - 2H)^{2-}G]^+$ is approximately twice that of $[Cr^{2+}(G - H)^-G]^+$, and the abundance of $[Cu^{2+}(G - H)^-G]^+$ is approximately half that of $[Cu^+G_2]^+$. The redox potential for Cr^{3+}/Cr^{2+} is −0.41 V and for Cu^{2+}/Cu^+ is 0.16 V; the spectra apparently reflect the less thermodynamically favorable nature of the former reduction. The extent of the $Fe^{3+} \rightarrow Fe^{2+}$ reduction is measured by using the corresponding ion cluster as used for the Cr^{3+}/Cr^{2+} pair, $[Fe^{3+}(G - 2H)^{2-}G]^+/[Fe^{2+}(G - H)^-G]^+$ ($\sim$1:4). Again, the greater extent of the $Fe^{3+} \rightarrow Fe^{2+}$ reduction reflects the more favorable thermodynamic nature of this process (the redox potential for Fe^{3+}/Fe^{2+} is 0.7 V). The precise values are of course unimportant, and we appreciate that the energy differences utilized represent the ions in aqueous solution at unit activity. Nevertheless, the above ion clusters were considered because the ions are extensively solvated, and since each glycerol molecule contains three hydroxyl groups, the situation should not be grossly different from an aqueous medium. The important point is that species differing in energy by values in the range 0–1 eV are populated to comparable extents, in accord with available energies of 0–3 eV, and not an order of magnitude more.

(ii) Radical cations are not normally observed in FAB or liquid SIMS experiments. According to the present proposal, this is because their energies will usually be 7–10 eV in excess of the ground-state molecule. Conversely, it is a consequence of the

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[13] Busch, K. L.; Unger, S. E.; Cooks, R. G. In *Ion Formation from Organic Solids*; Benninghoven, A., Ed.; Proceedings of the First International Conference, Munster, Federal Republic of Germany, October, 1980.

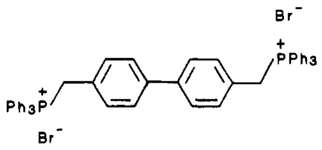
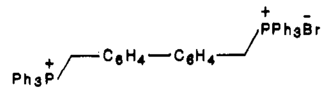
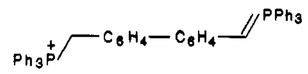
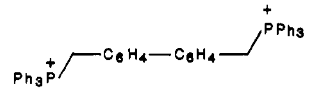
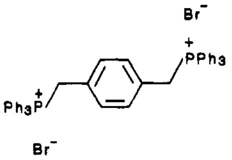
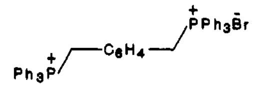
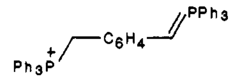
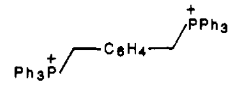
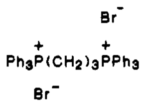
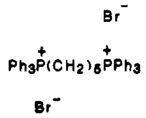
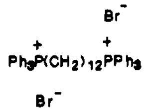
[14] Cooks, R.; Busch, K. L. *Int. J. Mass. Spectrom. Ion Phys.* **1983**, *53*, 111.

[15] Kebarle, P. *Annu. Rev. Phys. Chem.* **1977**, *28*, 445.

[16] Busch, K. L.; Hsu, B. H.; Xie, Y.-X.; Cooks, R. G. In *Ion Formation from Organic Solids*; Benninghoven, A., Ed.; Proceedings of the Second International Conference, Munster, Federal Republic of Germany, Sept. 7–9, 1982, p 138. Renner, D.; Spiteller, G. *Biomed. Env. Mass Spectrom.* **1986**, *13*, 401.

[17] Busch, K. L.; Unger, S. E.; Vincze, A.; Cooks, R. G.; Keogh, T. *J. Am. Chem. Soc.* **1982**, *104*, 1507.

Table I. Some Relative Ion Abundances for Compounds Forming Doubly Charged Ions in FAB Mass Spectra

compd	ion	*m/z*	ratio
 **14**		783/785	0.3
		703	1.2
		352	1.0
 **15**		707/709	4.0
		627	2.0
		314	1.0
 Br^- $Ph_3\overset{+}{P}(CH_2)_3\overset{+}{P}Ph_3$ Br^- **16**	$Ph_3P^+(CH_2)_3P^+Ph_3Br^-$	645/647	4.4
	$Ph_3P^+(CH_2)_2CH{=}PPh_3$	565	0.7
	$Ph_3P^+(CH_2)_3P^+Ph_3$	283	1.0
 Br^- $Ph_3\overset{+}{P}(CH_2)_5\overset{+}{P}Ph_3$ Br^- **17**	$Ph_3P^+(CH_2)_5P^+Ph_3Br^-$	673/675	3.4
	$Ph_3P^+(CH_2)_4CH{=}PPh_3$	593	1.1
	$Ph_3P^+(CH_2)_5P^+Ph_3$	297	1.0
 Br^- $Ph_3\overset{+}{P}(CH_2)_{12}\overset{+}{P}Ph_3$ Br^- **18**	$Ph_3P^+(CH_2)_{12}P^+Ph_3Br^-$	771/773	3.0
	$Ph_3P^+(CH_2)_{11}CH{=}PPh_3$	691	1.2
	$Ph_3P^+(CH_2)_{12}P^+Ph_3$	346	1.0
EGMIGTLTS*KR*I*K*Q-NH_2	MH^+	1543	1.0
21	MH_2^{2+}	772	1.0
GIG*K*FLHSA*K*	MH^+	1057	1.7
22	MH_2^{2+}	529	1.0
	MH_3^{3+}	353	≤0.014
GIG*K*FLHSAG*K*FG	MH^+	1318	2.3
23	MH_2^{2+}	659.5	1.0
*K*IA*K*VGLQGLMQP*K*	MH^+	1510	3.2
24[a]	MH_2^{2+}	744.5	1.0
	MH_3^{3+}	504	0.04
*K*IA*K*VGL*KE*LIQP*K*	MH^+	1564	2.4
25[a]	MH_2^{2+}	782.5	1.0
	MH_3^{3+}	522	0.05
dApdCpdGpdT	MH^+	1174	38
26[b]	MH_2^{2+}	587.5	1.0
	$(M - H)^-$	1172	12
	$(M - 2H)^{2-}$	585.5	1.0

[a] Peptides **24** and **25** were run as a mixture with the components present in approximately equimolar proportions. [b] Run as its triammonium salt.

model that if radical cations are unusually stable with energies lying only ~2 eV above the ground-state species, then they should be readily detectable in the resulting mass spectrum.

When chlorophyll absorbs light in the visible region, the light absorbed has an energy of ca. 2 eV, and this corresponds to the energy for the following conversion:

$$2Chl \rightarrow Chl^{\bullet+} + Chl^{\bullet-}$$

The charge separation of the ionic species is ca. 0.4 nm.[18] The facility of this process is of course due to the high-lying HOMO, and low-lying LUMO, associated with the extended chromophore of chlorophyll. The energy associated with the electron transfer is probably somewhat higher in the less polar environment where collisions occur following desorption, and there will be an additional requirement to separate fully the charges of the radical cation and radical anion before they are recorded. However, it is striking that the positive ion FAB spectrum of chlorophyll *b* is highly unusual insofar as $M^{\bullet+}$ and MH^+ are of approximately the same abundance and $M^{\bullet-}$ is approximately one-third and the abundance of $[M - H]^-$.[19] The implication is that the upper part of the range 0–3 eV is the energy required to populate $M^{\bullet+}$ along with $M^{\bullet-}$. It is also the energy range required to populate, to a similar extent,

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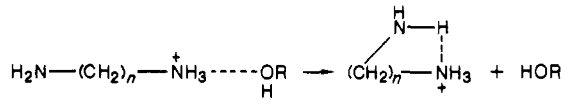
(18) See, for example: Shipman, L. L.; Cotton, J. M.; Norris, J. R.; Katz, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1791.

(19) Brereton, R. G.; Bazzaz, M. B.; Santikarn, S.; Williams, D. H. *Tetrahedron Lett.* **1983**, *24*, 5775.

MH^+ along with $[M - H]^-$. Furthermore, since $M^{\bullet -}$ and $M^{\bullet +}$ are only exceptionally observed in FAB and liquid SIMS, whereas MH^+ and $[M - H]^-$ are almost always observed, we need to show that production of these separated organic ion pairs will in general require energies approximated by the range 2–3 eV.

(iii) Production of MH^+ and $[M - H]^-$. In this section, we consider the work which must be done to separate an ion pair and thus produce isolated ions in the gas phase. If, in an ion pair, we have charges separated by 0.5–0.3 nm, then the enthalpy of this species is ca. 3–5 eV less than that of the isolated (fully separated) ions (taking the point charge approximation for ions in vacuo). The stabilization that isolated ions derive from partial solvation is known. For example, carbocation molecules solvated by formaldehyde are stabilized by ca. 18 kcal mol^{-1}[20] and Na^+ or K^+ by 24 or 17 kcal mol^{-1}, respectively, by a single H_2O molecule.[15] As examples of anion stabilization, isolated Cl^- is stablized by 13 or 27 kcal mol^{-1} in solution by one H_2O or one phenol molecule, respectively.[15] It is evident that the work done in separating an ion pair corresponding to a water-soluble salt can be completely compensated for by solvating each of the ionic products by ca. three or four H_2O molecules. As a guide, we therefore approximate that gas-phase ion-pair products in FAB will be more stable than the separated ions by a maximum of ca. 3 eV (when the separated ions are poorly solvated) and a minimum of ca. 0–1 eV (when the products are relatively well solvated). We consider that these approximations support the appropriateness of the energetic aspects of the model.

(iv) Observation of Protonated Glycerol Oligomers $(C_3H_8O_3)_nH^+$. It is remarkable that a glycerol matrix, when not protected by surface layers of a substrate, produces oligomers of the formula $(C_3H_8O_3)_nH^+$ (readily detectable up to $n = 12$), whereas extensive solvation of an analyte A (e.g., as $(C_3H_8O_3)_nAH^+$) is the exception rather than the rule. Possible reasons for this observation are that (i) glycerol cannot associate as strongly with AH^+ as with $(C_3H_8O)_nH^+$ and/or (ii) initially generated $(C_3H_8O_3)_nAH^+$ clusters lose glycerol molecules as the conformation of A changes so that the charge in AH^+ is stabilized by intramolecular solvation. This kind of effect is illustrated by the following reaction, which can be judged to be exothermic on the basis of thermochemical data presented in ref 15:



The available thermochemical data[15] indicate that the observation of $(C_3H_8O_3)_nH^+$ clusters is consistent with the availability of relative translational energies toward the end of the collision cascade in the range 0–3 eV.

(v) Ratio of Ions to Neutrals Obtained in FAB. We have argued above that the separation of organic ion pairs will normally require a maximum of ca. 3 eV, considerably less if the separated ions are extensively delocalized and/or solvated. It is, therefore, a consequence of our model that separated ions of the latter type should be populated to an extent that is comparable to neutrals or ion pairs. Since the mass spectrometer does not record neutral species, it might be thought difficult to obtain a guide to this ratio from mass spectrometric data. However, the following approach suggests that this is not so. Suppose a molecule A can also exist in its protonated form. It may therefore be detected by the mass spectrometer as AH^+, although desorbed A or the ion pair AH^+X^- will not be detected. If the concentrations of species which become isolated in the gas phase are represented by these symbols in square brackets, and if the ratio of neutrals to ions is x, then

$$x = \frac{[A] + [AH^+X^-]}{[AH^+]}$$

This ratio would be measured by a mass spectrometer if A itself were a charged species. If the charge were retained in a portion B^+ covalently attached to A, then the corresponding ratio x' is given by

$$x' = \frac{[B^+A] + [B^+AH^+X^-]}{[BAH^{2+}]}$$

i.e., by a ratio of singly to doubly charged ions. Moreover, if the ionic probe is introduced in such a manner as to affect minimally the propensity of A to leave the matrix as A, AH^+, or AH^+X^-, then x' would be a reasonable approximation of x. To try to meet this condition, the following two criteria should be followed.

First, the charge separation in the doubly charged ion BAH^{2+} should be relatively large in order to avoid significant destabilization by charge–charge repulsion. If the charges are designed to be ca. 10 Å apart in a relatively rigid system, then the charge–charge repulsion is still large (ca. 33 kcal mol^{-1}) in a vacuum ($\epsilon = 1$) but only ca. 1 kcal mol^{-1} in glycerol ($\epsilon = 43$). It is difficult to know the effective dielectric constant when a sputtered particle last makes a collision that may determine its charged vs. neutral state, but a value of ~5 seems a pragmatic estimate, giving a destabilization of ca. 6 kcal mol^{-1}. On the basis of our previous arguments, this destabilization should have only a small effect on relative ion abundances.

Second, if the charge in AH^+ is stabilized by internal solvation, then the charge within BAH^{2+} should also be capable of being solvated to a similar extent without removing important solvation of B^+. The importance of this effect can be seen by considering hypothetical peptides $X^-(ArgH^+—ArgH^+)X^-$, where the solid line represents say two or eight intervening neutral amino acids. The charge in the singly charged ion $ArgH^+—Arg$ in the gas phase would be expected to be considerably stabilized by internal "solvation" (e.g., intramolecular hydrogen bonding) in the case of two or eight intervening amino acids, but there should be considerable discrimination against $ArgH^+—ArgH^+$ when only two intervening residues are available as sources of intramolecular hydrogen bonds to stabilize both charges.

With the above considerations in mind, we have obtained the FAB mass spectra of some series of compounds. Relevant data are represented in Table I. Although Table I records the actual relative ion abundances observed in the spectra, we recognize that in view of the dynamic nature of the FAB analysis, the values will be subject to some variation from experiment to experiment. Therefore, we interpret the qualitative trends only. We discuss first those compounds (**14–18**) necessarily existing as doubly charged ions in solution.

Compound **14** satisfies the criterion i already presented. The charges are well separated, and the same charge-removal mechanisms are open at each charged site, that is via formation of the ion pair or via phosphorane formation. It is evident from the data (Table I) that in a singly charged phosphonium salt the number of neutrals becoming free in the gas phase should be roughly comparable to the number of charged phosphonium ions. The data for **15** are presented in support of our application of criterion i above regarding charge separation. Since the positive charges are now closer together, there is discrimination against the dication. The energy difference associated with this discrimination should be ≤0.5 eV (relative to the situation pertaining in **14**). Note that the data support our contention that if a given ion is observed in a FAB spectrum, then an alternative ion associated with the same analyte can be competitively formed in the gas phase if the energy differences between the ions lie in the region of 1 eV or so.

The corresponding data for some more flexible aliphatic bis(phosphonium) salts (**16–18**) are also given Table I. Note that in all cases the ion abundances correspond to analysis of considerable amounts of material as ion pairs and phosphoranes, even when the charge probe is greatly distant (in **18**) from the other phosphonium center. In all these cases, ion-pair formation may be favored by bridging[21] (see **19**).

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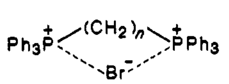
(20) Bowen, R. D.; Williams, D. H. *Int. J. Mass Spectrom. Ion Phys.* **1979**, *29*, 47.

(21) Yamdagni, R.; Kebarle, P. *J. Am. Chem. Soc.* **1973**, *95*, 3504.



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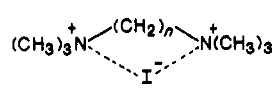

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A number of bis-quaternary ammonium iodides of the general formula $(CH_3)_3N^+(CH_2)_nN^+(CH_3)_32I^-$ have also been examined.[23,24] It is noticeable that in these cases, where the positive charge is less electronically shielded than in phosphorus and is additionally not stabilized by intramolecular hydrogen bonding, external solvation of the charge is observed, either by I^- or glycerol or by both. The relative abundances of the singly charged ion pairs $(G)_nM^{2+}I^-$ (where G is a glycerol molecule and M^{2+} represents $(CH_3)_3N^+(CH_2)_nN^+(CH_3)_3)$ to the corresponding doubly charged ions $(G)_n(M^{2+})$ are 15, 12, 5, 4, and 5 for n = 1, 2, 3, 4, and 5, respectively. In these cases, the ion pair will be favored due to bridging (**20**)[21] and the dication discriminated against by Coulombic repulsion for low values of n. Note that as expected for higher values of n, the abundance of the doubly charged ions relative to the singly charged ion pair increases.

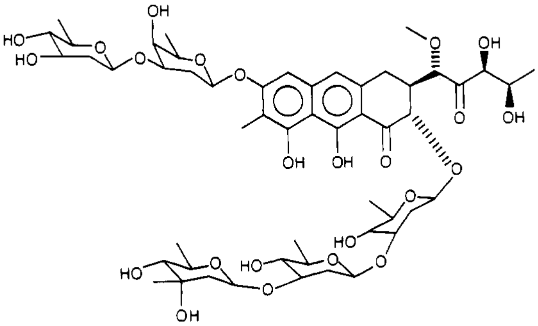
Data for some basic peptides are also given (Table I). The amino acids are represented by the single letter code, and residues bearing a charge at neutral pH are given in italics (*K* = lysine, *R* = arginine, and *E* = glutamic acid). A general feature of the FAB spectra of peptides is that basic residues do not retain their counter anions (in those cases where this ion pair could, in principle, be identified by virtue of a net positive change in the rest of the molecule). Nor are glycerol adduct ions commonly observed. These are clear indications that external solvation of the positive charge, in order to increase the stability of the desorbed species, is not necessary in these cases. This fact clearly points to charge stabilization now occurring via formation of intramolecular hydrogen bonds between atoms bearing charge and electronegative atoms of the peptide (e.g., to oxygen in C=O groups). Thus, since glycerol will initially be hydrogen bonded to many of the charged groups, conformational changes occur in the peptides (to stabilize the desolvated state) before the lapse of a time beyond which the ion could not be recorded at its appropriate molecular mass.

The data for the peptides (Table I) indicate, in the light of the earlier discussion, that in the presence of basic residues such as lysine and/or arginine, the concentrations of peptides reaching the gas phase as neutrals or ions are comparable. Thus, in peptides of this type, when run under acidic conditions, losses in sensitivity due to desorption of neutrals are not large.

There is considerable discrimination against the formation of triply charged ions even when the peptides (**24** and **25**) might form such ions due to the presence of 3 or more basic residues. However, this discrimination can be readily understood in terms of the greater difficulty in (i) stabilizing three charges by suitable hydrogen bonding within one molecule and (ii) accommodating three charges without unfavorable Coulombic repulsions.

The data from the negative ion spectrum of the tetranucleotide triphosphate **26** indicate that considerable amounts of nucleotides will be carried into the gas phase as neutrals if they are analyzed as their ammonium salts. This conclusion is reached on the basis of using the negative charge on one phosphate to monitor the fraction ($\sim{}^1/_{12}$) of a second phosphate reaching the gas phase as $(RO)_2P(O)O^-$ vs. $(RO)_2P(O)OH$. This large discrimination against the ionic form (relative to the situation for, say, arginine in a peptide) may be due to (i) a more unfavorable ΔH_f change for a phosphate anion in passing to a partially solvated environment and/or (ii) a poorer ability of the remainder of an oligonucleotide to intramolecularly solvate $(RO)_2P(O)O^-$. As might be expected, the relative abundance of singly to doubly charged ions in the positive ion spectrum is even greater (Table I). Since nucleotides have no strongly basic site, a larger fraction of the nucleotide will appear in the gas phase as the neutral molecule.

It follows from the above arguments that polyhydroxy compounds lacking highly basic sites will give doubly charged ions of low or negligible abundance in their positive ion FAB spectra. As a corollary, such compounds are mainly desorbed as neutral species, with a consequent loss in sensitivity. Experimental data support the first consequence. Thus, both mithramycin (**27**) and tribulosin (**28**) show no detectable M^{2+} ions in their FAB spectra, in the former case even after addition of 1 μL of 0.1 M HCl to the glycerol matrix (2 μL containing 5% H_2O).



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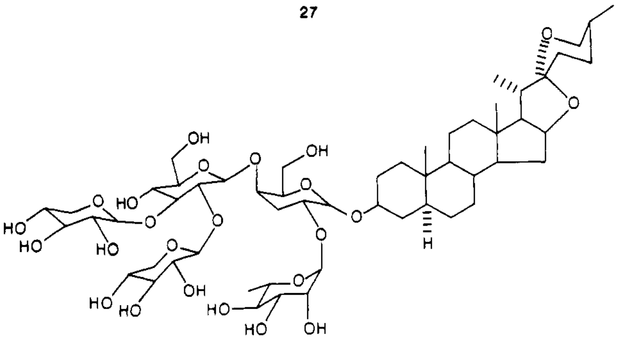

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Conclusion

Evidence is presented to support the concept that one-electron reductions occur in the matrix as a consequence of Xe atom bombardment. Furthermore, a model is developed based on the work of Cooks[13,14] in which Xe atom bombardment results in the initial formation of molecules of high translational energies, but following a collisional cascade, only combinations of species having energies ca. 0–3 eV in excess of the most stable combinations of product energies exit into the mass spectrometer. The model allows an understanding as to why radical cations are rarely observed in FAB and matrix SIMS experiments and can indicate the kinds of compounds for which sensitivity will be adversely affected by the production of mainly neutral sample molecules following the collision cascade.

The conclusions reached here are in accord with those recently published by de Pauw et al.[25] They conclude that the internal energy distribution of ions emitted in liquid SIMS lies in the approximate range 1–3.5 eV.

Experimental Section

Vancomycin as vancocin hydrochloride was obtained from Eli Lilly and mithramycin from Pfizer, U.K. All were used without further purification.

Mass Spectra. Mass spectra were recorded on a Kratos MS-50 spectrometer fitted with a high-field magnet (mass range of 10 000 daltons at an 8-keV accelerating voltage). Solutions of samples for FAB–MS analysis (2–10 nmol) were evaporated on a stainless steel probe tip from an appropriate solvent. The matrix (2 μL) was added to the probe tip, thoroughly mixed with the sample, and then subjected to bombardment with xenon atoms. The atom beam was provided by an

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[22] Maercker, A. *Org. React.* (*N.Y.*) **1965**, *14*, 271.

[23] For a general preparation and purification of the bis-quaternary ammonium salts, see: Sommer, H. Z.; Lipp, H. I.; Jackson, L. J. *J. Org. Chem.* **1971**, *36*, 824.

[24] For prior work on the SIMS spectra of diquaternary ammonium compounds, including evidence for their reduction, see: Ryan, T. M.; Day, R. J.; Cooks, R. G. *Anal. Chem.* **1980**, *52*, 2054.

[25] de Pauw, E.; Pelzer, G.; Natalis, P. In *Ion Formation from Organic Solids*; Benninghoven, A., Ed.; Proceedings of the Third International Conference, Springer Proceedings in Physics; Springer-Verlag: West Berlin, 1986; Vol. 9.

Ion Tech FAB gun operating at 8 keV with a current of 30-40 μA. The magnet was typically scanned at a rate of 30-100 s/decade. All matrices were vacuum distilled before use.

Bis(phosphonium) Salts. All bis(phosphonium) salts (**14**-**18**) were prepared and purified as described in the literature.[22]

Acknowledgment. We thank Dr. L. Poulter for the amphibian basic peptides, Dr. S. Salisbury for the oligonucleotide sample, and Dr. J. Waltho for the sample of tribulosin. We also thank Churchill College and the SERC (U.K.) for financial assistance.

Registry No. 8, 59-43-8; **14**, 106835-92-1; **15**, 40817-03-6; **16**, 7333-67-7; **17**, 22884-31-7; **18**, 22884-33-9; **21**, 106820-85-3; **22**, 106820-86-4; **23**, 106820-87-5; **24**, 106835-93-2; **25**, 106820-88-6; d(ApCpGpT), 106835-94-3; Xe, 7440-63-3; vancomycin hydrochloride, 1404-93-9; glycerol, 56-81-5.

Olation and Structure

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Abstract: The ability of cis hydroxoaqua-metal complexes to undergo olation and form the corresponding diols is correlated with their dimeric structure, having two μ-(H_3O_2) bridging ligands between the two metal atoms. A hypothesis is put forward that olation may take place only if the cis complex has this dimeric structure. The hypothesis is tested by means of the only cis hydroxoaqua complex that does not have a dimeric structure, $[Cr(pico)_2(H_2O)OH]S_2O_6$, compound **1** (pico = 2-picolylamine). This complex does not olate. Replacement of the dithionate counter ion by iodide yielded the complex $[(pico)_2Cr(H_3O_2)_2Cr(pico)_2]I_4{\cdot}2H_2O$, compound **2**. The structure of **2** was determined by a single-crystal X-ray study, which established that it had the normal dimeric structure. Compound **2** olates, as predicted by the hypothesis, and produces the diol $[(pico)_2Cr(OH)_2Cr(pico)_2]^{4+}$. The structure of this ion was determined by X-ray study of $[(pico)_2Cr(OH)_2Cr(pico)_2](S_2O_6)_2{\cdot}2^1/_2H_2O$, compound **3**. A two-step mechanism of olation, in the solid state, is proposed. A mono-ol is produced in the first step, followed by the formation of the diol. This mechanism predicts retention of the racemic $\Lambda\Lambda,\Delta\Delta$ configuration of compound **2** in the product **3** and was confirmed by the structures of **2** and **3**. The well-known fundamental feature of olation, namely that only cis isomers olate and trans isomers do not, is explained, for the first time, by the proposed mechanism and is correlated with the structure of the reagents and products.

Formation of μ-(OH) bridging between metal ions, also known as "olation" (reaction 1), is the first step in the processes of polymerization of metal aqua ions in solution and "aging" of metal hydroxides in the solid state.[3] Therefore, the mechanism of reaction 1 is a key to the understanding of these important processes.

$$M(OH_2)^{n+} + (HO)M^{(n-1)+} \rightarrow M(OH)M^{(2n-1)+} + H_2O \qquad (1)$$

Although olation was investigated and discussed since the beginning of this century,[2-4] no firm experimental evidence has yet been furnished for the mechanism of reaction 1 in the solid state. Pfeiffer, Werner, Dubski, and their contemporaries[2] who investigated olation reactions of hydroxoaqua-metal complexes such as

$$2\,cis\text{-}[Cr(en)_2(H_2O)(OH)]X_2 \xrightarrow{100\ °C} [(en)_2Cr(OH)_2Cr(en)_2]X_4 + 2H_2O \qquad (2)$$

noted that only cis hydroxoaqua ions olate to form diols, while their trans isomers do not. This notable difference was used in their classical structure determinations to distinguish between cis and trans configurations. However, no satisfactory explanation was given, to date, for the reactivity of the cis and inertness of the trans isomers, i.e., for the fact that cis isomers form di-μ-OH binuclear ions while trans isomers do not form μ-OH bridged polynuclear chains. The present investigation was undertaken in order to explain this fundamental difference in reactivity. Investigations conducted since the late fifties, and especially in the last decade,[3c,d,4] furnished some valuable insight into the mechanism of olation in aqueous solution. In his studies on the reverse of reaction 2, Springborg[4] proved that olation in solution proceeds by two consecutive steps

$$2[L_4M(H_2O)(OH)]^{2+} \rightleftharpoons [(HO)L_4M(OH)ML_4(H_2O)]^{4+} + H_2O \qquad (3)$$

$$[(OH)L_4M(OH)ML_4(H_2O)]^{4+} \rightleftharpoons [L_4M(OH)_2ML_4]^{4+} + H_2O \qquad (4)$$

The intermediate (product of reaction 3) was isolated, and its structure was determined.[4b]

Recent investigations of hydroxoaqua metal ions by single crystal X-ray studies revealed that these ions are not mononuclear, as their classical formulation implies, but *binuclear* or *polynuclear*.[5] A distinction between hydroxo and aqua ligands is usually impossible in these ions because they merge into a symmetrical $H_3O_2^-$ bridging ligand by means of a very short (<2.5 Å), strong, and centered hydrogen bond. As a result of this bridging, cis isomers have a dimeric structure with two $H_3O_2^-$ bridges between the metal atoms (form a), and trans isomers consist of infinite

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(1) (a) The Hebrew University of Jerusalem. (b) University of Copenhagen.

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