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Fast-atom Bombardment Mass Spectrometry of Mono- and Difluorophosphonate Analogues of Glycerol-3-phosphate†

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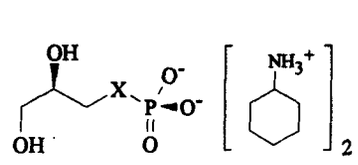
The mass spectrometric behaviour of the cyclohexylammonium salts of mono- and difluorophosphonate analogues of *sn*-glycerol-3-phosphate has been studied by means of positive- and negative-ion mass spectrometry. A direct comparison is made with that of *sn*-glycerol-3-phosphate itself. In both positive- and negative-ion modes a lower stability of the phosphate is observed, resulting in an increase in decomposition channels.

Phosphonates (**a**) are a pharmacologically important class of organophosphorus compounds, being employed as hydrolytically stable phosphate mimics in bioorganic chemistry.[1] The CH_2 group of the phosphonate replaces the bridging oxygen of the phosphate group and thus renders it resistant to phosphatase hydrolysis. However such a replacement results in removal of the electronegative oxygen atom, so that the CH_2-phosphonate is less acidic than the original phosphate. The subsequent introduction of fluorine atom(s) onto the methylene group introduces only a small steric change but leads to phosphonates which increase in acidity owing to the electron withdrawing properties of the fluorine atom(s). It has been proposed[2a] that such fluorinated phosphonates should be better phosphate mimics than CH_2-phosphonates in biological systems and, recently, a comparative study of CH_2-, CHF- and CF_2- phosphate analogues of *sn*-glycerol-3-phosphate as substrates for *sn*-glycerol-3-phosphate dehydrogenase has been carried out.[2b] Also the X-ray structures of the CH_2-, CHF- and CF_2-phosphonate moieties have been compared to the phosphate group to assess geometric trends in the series.[3]

Organophosphonates have been the object of many mass spectrometric investigations.[4–11] Recently, tandem mass spectrometry[12, 13] and atmospheric pressure ionization tandem mass spectrometry[14, 15] proved to be particularly effective in the structural elucidation of phosphonates. The



a : X = CH_2
1 : X = O
2 : X = CHF
3 : X = CF_2

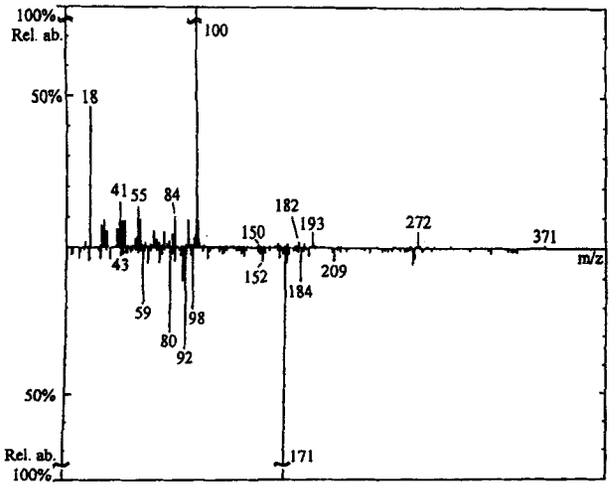


Figure 1. *up*: positive-ion FAB mass spectrum of compound **1**; *down*: negative-ion FAB mass spectrum of compound **1**.

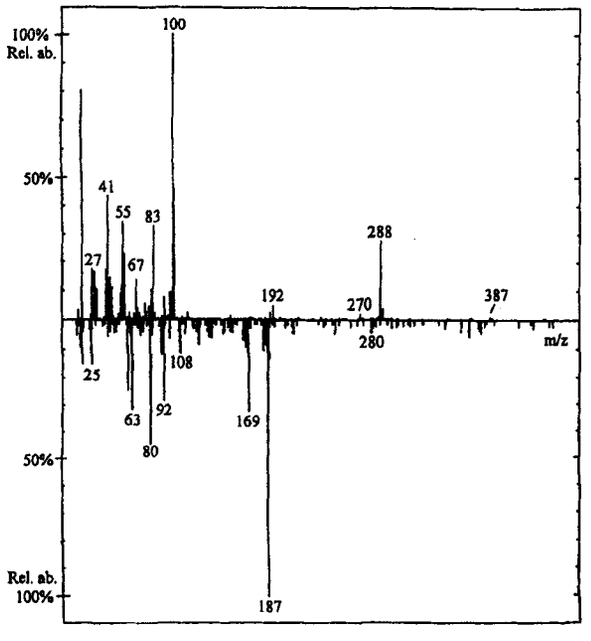


Figure 2. *up*: positive-ion FAB mass spectrum of compound **2**; *down*: negative-ion FAB mass spectrum of compound **2**.

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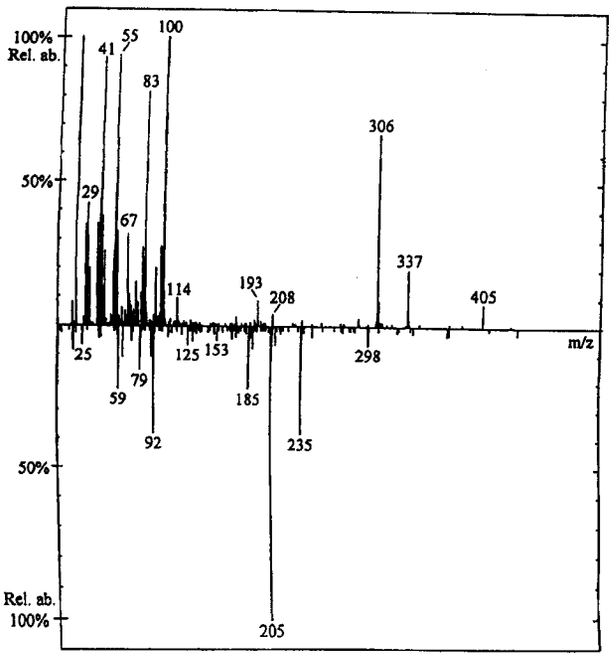


Figure 3. *up*: positive-ion FAB mass spectrum of compound **3**; *down*: negative-ion FAB mass spectrum of compound **3**.

analysis of phosphorus containing pesticides by high-performance liquid chromatography (HPLC) coupled to mass spectrometry has been also reported.[16] However, to date, no mass spectrometric investigations have been carried out on the title compounds and, due to the increasing role of CHF- and CF_2-phosphonates in medicinal and bio-organic chemistry, we report here the results obtained by positive- and negative-ion fast atom bombardment (FAB) on compounds **1–3**, with the aid of metastable-ion studies.

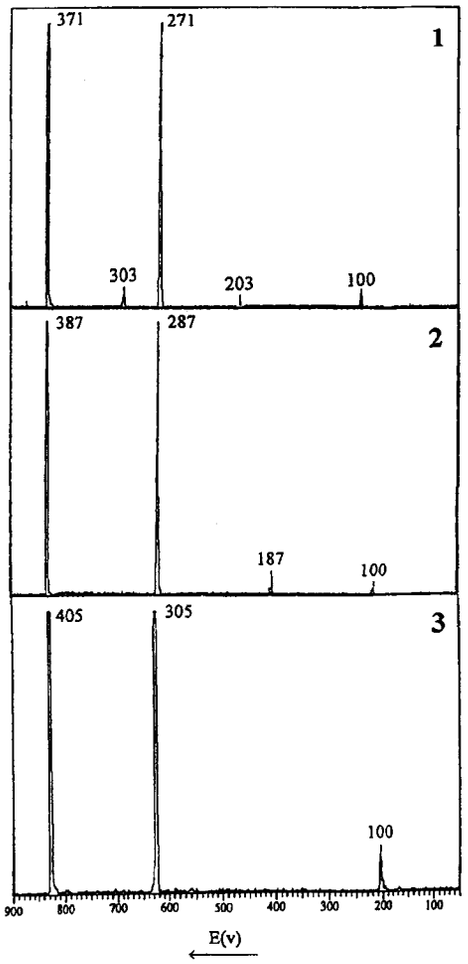
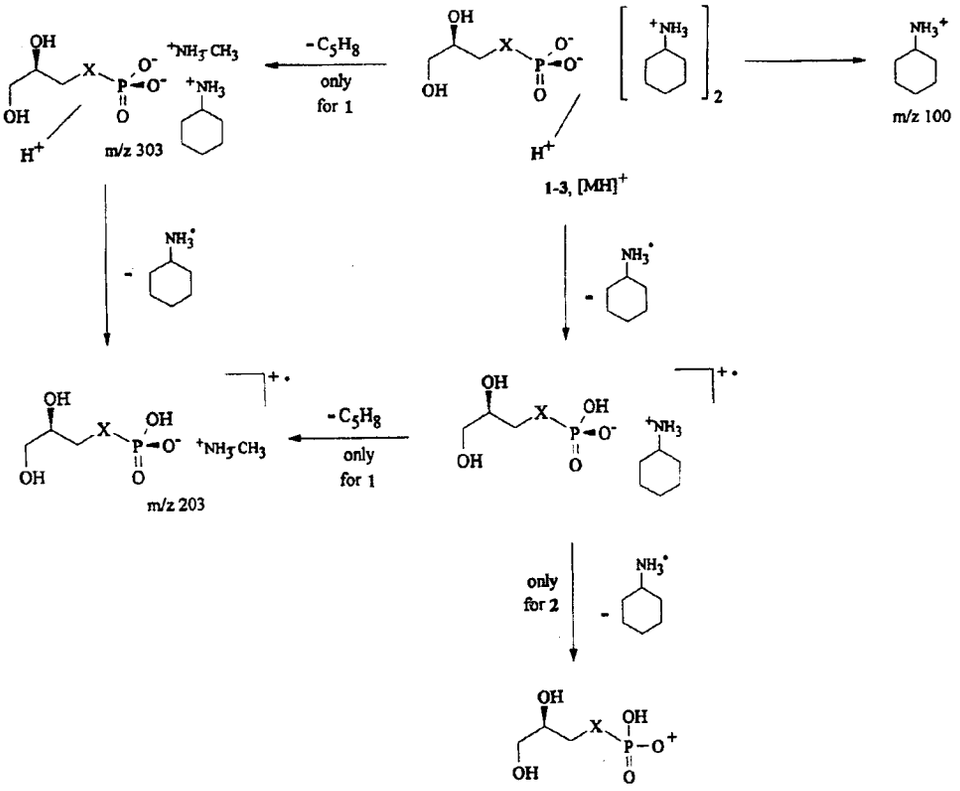
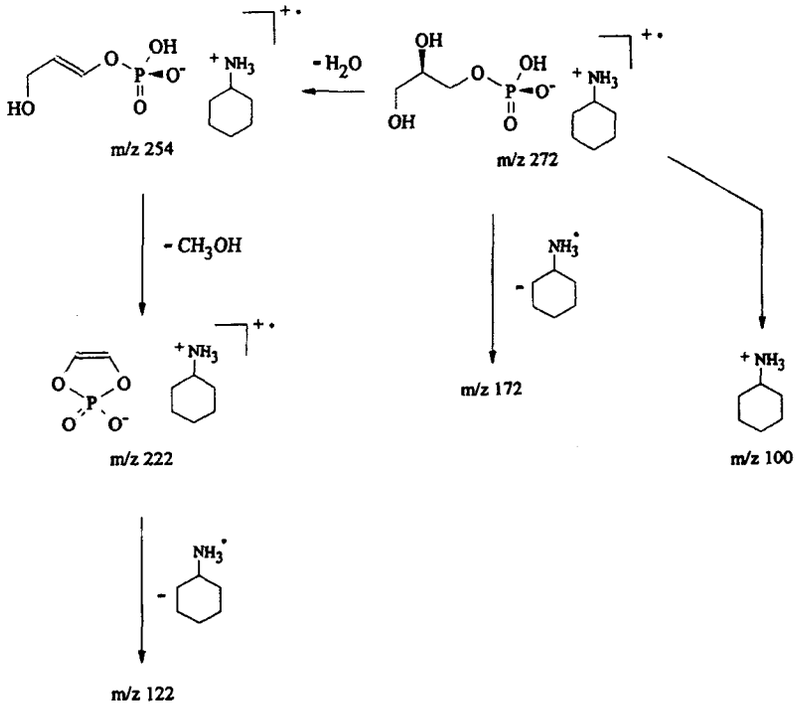


Figure 4. MIKE spectra of FAB-generated $[MH]^+$ ions of compounds **1–3**.



Scheme 1



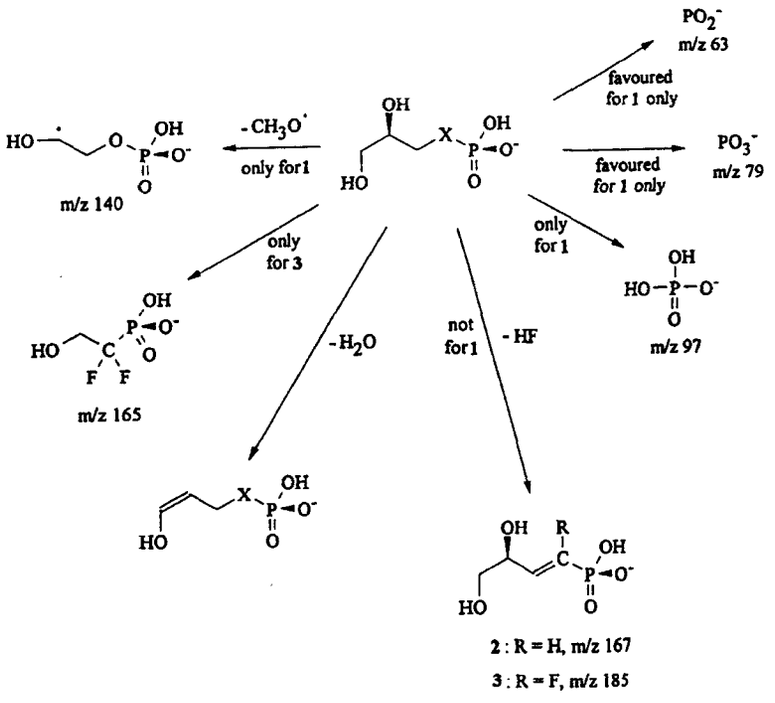
Scheme 2

EXPERIMENTAL

Compounds **1–3** were analytically pure samples. Full synthetic details have recently been published.[3] All mass spectrometric measurements were performed by a VG ZAB 2F[17] (VG, Altrincham, UK) instrument operating under FAB[18] conditions (8 keV xenon atoms bombarding glycerol solutions of the samples). Metastable ions were studied by mass-analyzed ion kinetic energy (MIKE) spectrometry.[19]

RESULTS AND DISCUSSION

The positive- and negative-ion FAB spectra of compounds **1–3** are reported in Figs 1–3 respectively. For all of the examined compounds, the base peak in the positive-ion spectra is the cyclohexylammonium ($C_6H_{11}NH_3^+$) cation at *m/z* 100. However, the protonated salts are also always detectable. The common fragmentation pattern of the $[MH]^+$ species of compounds **1–3**, as obtained from MIKE spectra (see Fig. 4), is reported in Scheme 1. Metastable ions show that the formation of the $C_6H_{11}NH_3^+$ ion, as cited



Scheme 3

above, is not only due to its desorption from the liquid phase but, at least partially, a gas-phase decomposition of $[MH]^+$ species. This result provides good evidence for a persistent electrostatic interaction between the phosphate anion and the cyclohexylammonium cations.

A common primary decomposition channel in MIKE spectra is the loss of a cyclohexylammonium radical, which is responsible for the most abundant peak. Only in the case of compound **2** is the concomitant loss of the two $C_6H_{11}NH_3^{\bullet}$ radicals observed, giving rise to the ion at *m/z* 187. The $[MH]^+$ ion of the phosphate **1** exhibits specific behaviour, generating an ion at *m/z* 303, detectable only under MIKE conditions. This ion corresponds to the loss of C_5H_8, which must originate from the cleavage of the cyclohexyl ring with hydrogen rearrangement to leave a methyl ammonium cation. The phosphonates **2** and **3** do not behave in this way, suggesting that the electrostatic interaction between the phosphate anion and ammonium cation is stronger than that between the phosphonate anion and ammonium cation. The $[MH - C_5H_8]^+$ cation at *m/z* 303, generated from **1**, shows a further loss of $C_6H_{11}NH_3^{\bullet}$, giving rise to a prominent ion at *m/z* 203 in the MIKE spectrum.

It is worth noting that while a primary $C_6H_{11}NH_3^{\bullet}$ loss is the favoured process in MIKE spectra, the loss of $C_6H_{11}NH_2$ as a neutral molecule is observed predominantly in the FAB spectra. Thus $C_6H_{11}NH_3^{\bullet}$ loss appears to be favoured for the ions of lower internal energy.

The behaviour of the $[MH - C_6H_{11}NH_3]^+$ cations was investigated by MIKE spectra and a clear difference was found between the behaviour of the phosphate **1** (for which X=O) and the fluorinated phosphonates **2** and **3** (for which X=CHF- and CF_2- respectively). For **1**, a series of decomposition pathways related to the glycerol moiety are apparent (due to H_2O loss and sequential losses of H_2O and CH_3OH, see Scheme 2), whereas for **2** and **3** the only decomposition products are those due to the formation of $C_6H_{11}NH_3^+$ cations and the subsequent loss of $C_6H_{11}NH_3^{\bullet}$ radicals.

The negative-ion mass spectra of the compounds **1–3** exhibit a base peak due to the $[M - C_{12}N_2H_{27}]^-$ anion (Scheme 3). The primary fragmentation patterns of the MIKE spectra (Fig. 5) show individual behaviour for the different samples. Thus, for compound **1** the formation of PO_3^- anions is favoured and phosphorus-containing ions are also detected at *m/z* 63 (PO_2^-) and 97 (HPO_4^-). The less-favoured decomposition pathways are due to $CH_3O^{\bullet}$ and H_2O losses. For compounds **2** and **3** the formation of phosphorus-containing anions is suppressed and the most abundant fragments are those due to competing H_2O and HF losses. Elimination of HF is favoured over that of H_2O in compound **2**, but this situation reverses for compound **3**.

In conclusion, the mass spectrometric data are in agreement with the condensed phase behaviour of the title compounds. In the case of the phosphate **1** (X=O) the molecule is more labile, while for the phosphonates **2** and **3** (where X=CHF or $X=CF_2$) a lower number of decomposition channels is activated.

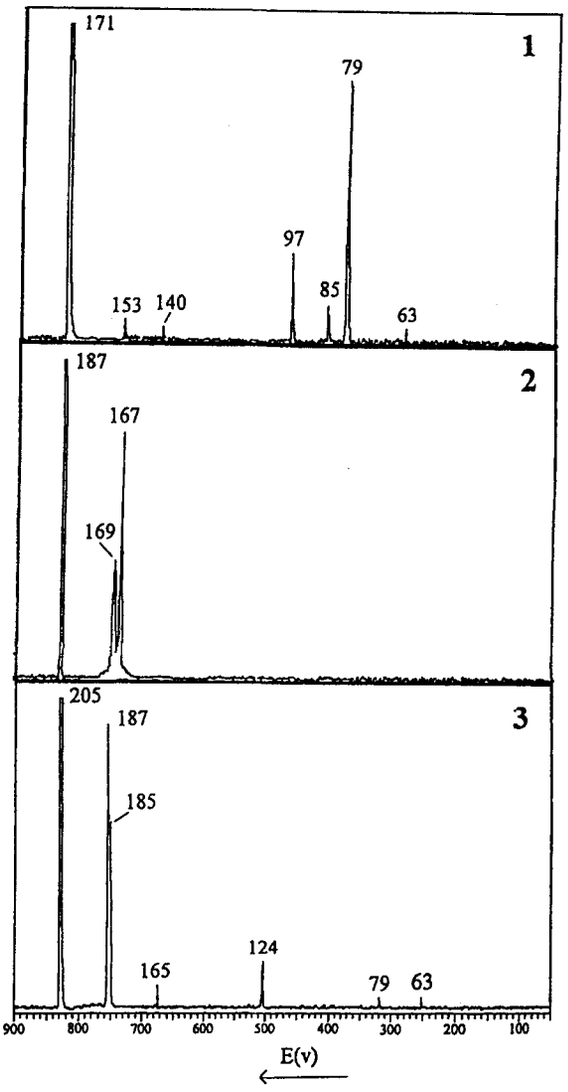


Figure 5. MIKE spectra of FAB-generated $[M - C_{12}N_2H_{27}]^-$ anions of compounds **1–3**.

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