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Note

Derivatization method for the gas chromatographic–mass spectrometric characterization of aminophosphonic acids

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Our laboratories are currently involved in a project dealing with the synthesis of aminophosphonic acids for which mass spectrometric data are necessary and gas chromatographic–mass spectrometric (GC–MS) data desirable. However, aminophosphonic acids are characteristically high-melting, non-volatile compounds and thus require conversion to appropriate derivatives prior to GC–MS analysis.

The trimethylsilyl (TMS) derivative as originally prepared by Karlsson[1] for 2-aminoethylphosphonic acid (2-AEPA) has subsequently been shown not to be suitable. Harvey and Horning[2] also prepared TMS derivatives of aminophosphonic acids with functionalized amino groups, but these methods either lacked potential for use with other than primary amino groups, were inconvenient, or gave less than satisfactory derivatives for our purposes. The dimethyl ester[3] and the dibutyl ester[4] of N-trifluoroacetyl-2-aminoethylphosphonic acid have been shown to be suitable derivatives, but their preparation requires the use of diazomethane and diazobutane, respectively.

We were particularly interested in utilizing the N-trifluoroacetyl (N-TFA) esters as derivatives but wanted to avoid having to use the diazo reagents. To this end we have exploited the reaction between triethyl orthoformate and phosphonic acids[5] to prepare the ethyl esters of the N-TFA derivatives. We have applied the resulting method to 1-aminoethylphosphonic acid (1-AEPA), 2-AEPA, and α-aminobenzylphosphonic acid (BzAPA) and find it to be a convenient method for preparing N-TFA diethyl ester derivatives of aminophosphonic acids for the purpose of GC–MS analysis.

EXPERIMENTAL

Materials

1-AEPA[6] and 2-AEPA[7] were prepared by published procedures and BzAPA was prepared by a method reported elsewhere[10]. All compounds were adequately characterized on the basis of spectral data and physical constants. Trifluoroacetic acid and trifluoroacetic anhydride were purchased from Aldrich (Milwaukee, Wisc., U.S.A.), while the triethyl orthoformate was obtained from Matheson, Coleman & Bell (East Rutherford, N.J., U.S.A.).

Derivatization procedure

To 10 mg of the aminophosphonic acid was added 1 ml of trifluoroacetic acid to dissolve the sample followed by 1 ml of trifluoroacetic anhydride. The resulting solution was allowed to stand at room temperature for approximately 1 h after which the solution was concentrated to dryness under a stream of nitrogen. A 5-ml volume of triethyl orthoformate was added to the residue and the mixture was heated at reflux for 6–8 h. This solution can be utilized as such for GC–MS analyses, but we routinely evaporated the excess triethyl orthoformate under a stream of nitrogen and dissolved the residue in 5–10 ml of a solvent, usually ethyl acetate. This solution was subsequently subjected to GC or GC–MS analysis.

Gas chromatography

GC analyses were performed using either a Hewlett-Packard Model 5700A or 5720A gas chromatograph equipped with a flame ionization detector. The columns were 6 ft. × $^1/_4$ in. coiled glass packed with 3% OV-225 on 100–120 mesh Supelcoport. The nitrogen carrier gas flow-rate was 60 ml/min. The column temperature used was 130° for the diethyl N-TFA derivatives of 1-AEPA and 2-AEPA and 200° for the corresponding BzAPA derivative.

Mass spectrometry

Mass spectra were obtained on a DuPont Model 321 Dimaspec integrated gas chromatograph–mass spectrometer equipped with a DuPont Model 320 data system. The mass spectra were recorded at 70 eV and a source temperature of 220°. The accelerating voltage varied from 900–12,600 eV. Sample injection was accomplished via the injection port of the gas chromatograph using a glass jet separator. The injection port was heated at 225° and the column temperature varied depending upon the derivative under investigation (see above). The column was packed with 3% OV-225 on 100–120 mesh Supelcoport.

RESULTS AND DISCUSSION

The acylation procedure employed is essentially that already reported[4] and took advantage of the solubility of aminophosphonic acids in trifluoroacetic acid. The degree of esterification with the orthoformate depended upon the aminophosphonic acid present and the length of time heated, but, in general, sufficient esterification occurred during the time period described to allow successful analysis of the derivatives. The crude orthoformate reaction solution could be subjected directly to GC analysis, but this gave several extraneous peaks probably due to other volatile side products produced during the esterification reaction[5]. For this reason, the final reaction solution was usually freed from unwanted material by concentration under a stream of nitrogen before dissolving in a solvent for chromatographing. The N-TFA diethyl ester derivatives showed sharp, well-shaped peaks with only minimal tailing.

The mass spectra of the N-TFA diethyl ester derivatives of 1-AEPA, 2-AEPA and BzAPA are shown in Figs. 1–3. All of the derivatives studied gave discernible molecular ions, but only in the case of BzAPA (11.8%) was the molecular ion greater than 10% of that of the base peak. N-TFA diethyl ester derivatives of 1-AEPA and 2-AEPA had M^+ of 2.24% and 4.93%, respectively.

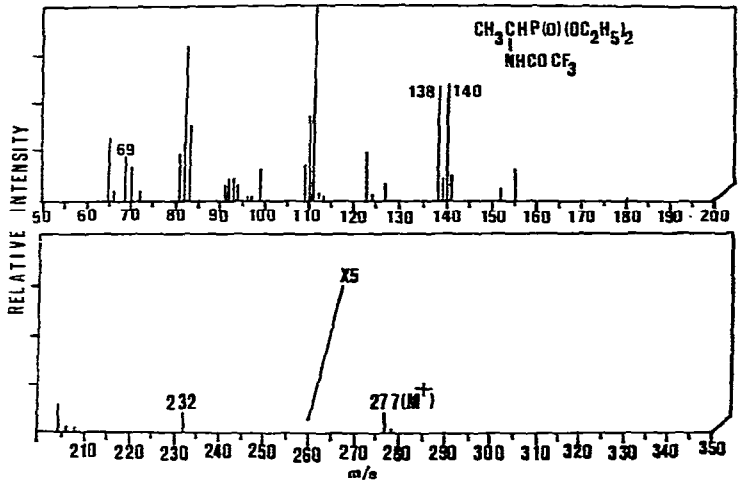

Fig. 1. Mass spectrum of diethyl N-TFA 1-aminoethylphosphonate (MW = 277).

Some tentative assignments can be made for some of the fragment ions based on analogy of this system to others[3,4,8]. Loss of CF_8 from the molecular iongives a prominent peak only with derivatized 2-AEPA giving rise to the base peak at *m/e* 208. However, the mass spectra of all three derivatives contain a peak at *m/e* 69 corresponding to a CF_3 fragment. Also appearing in the mass spectra of the N-TFA diethyl esters of 1-AEPA, 2-AEPA and BzAPA are peaks occurring at *m/e* 232, 232 and 294, respectively, corresponding to loss of C_2H_5O as well as a peak at *m/e* 138 for protonated diethyl phosphonate. One interesting aspect of the derivatives studied is that the mass spectra of the N-TFA diethyl esters of 1-AEPA and BzAPA contain prominent peaks corresponding to C–P bond cleavage with subsequent loss of $PO_3(C_2H_5)_2$ giving rise to ions at *m/e* 140 and 202, respectively; the latter being the base

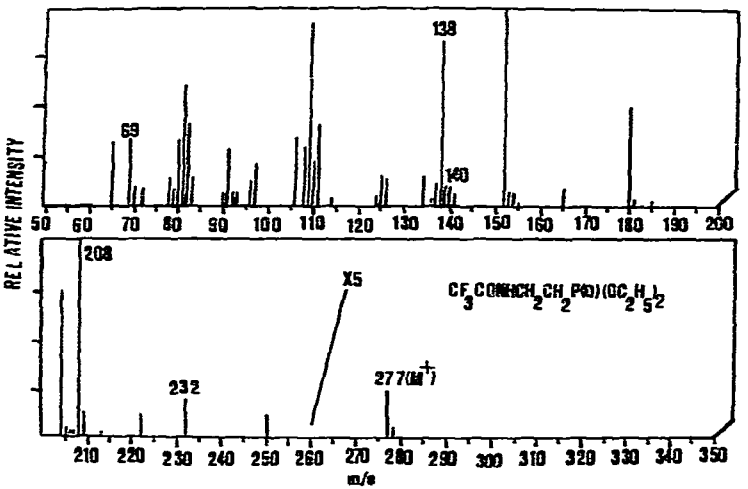

Fig. 2. Mass spectrum of diethyl N-TFA 2-aminoethylphosphonate (MW = 277).

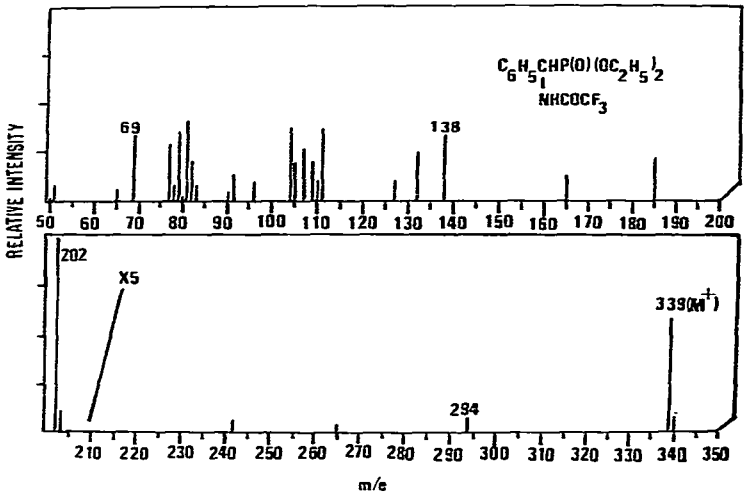

Fig. 3. Mass spectrum of diethyl N-TFA α-aminobenzylphosphonate (MW = 339).

peak in the spectrum of the BzAPA derivative. However, in the case of the 2-AEPA derivative, only a small peak (10.9%) corresponding to this loss is observed at *m/e* 140. This could conceivably be rationalized on the basis that the N-TFA diethyl esters of 1-AEPA and BzAPA are derivatives of α-amino acids and as such possess the favorable possibility of forming a resonance stabilized iminium ion from the resulting amine fragment[8]. This behavior has been observed with the N-TFA derivatives of α-aminocarboxylic acid esters[9]. In the case of the N-TFA diethyl ester of 2-AEPA, however, the parent structure is a β-amino acid, and this possibility does not exist.

In summary, a suitable method for the derivatization of aminophosphonic acids for GC–MS analysis as N-TFA diethyl esters has been developed which complements other methods available. The procedure furnishes derivatives which are stable for at least one year and whose GC characteristics are good. The mass spectra of the derivatives contain distinguishable molecular ions and show fragmentation patterns similar to related derivatives. Although the method is slightly more lengthy than some others, it has the advantage of requiring common, inexpensive, laboratory chemicals and thus obviates the necessity of using diazo compounds or other unstable derivatizing reagents. The method works satisfactorily for derivatizations carried out on a macroscale, but its utility on a microscale has not yet been investigated.

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