# ANALYSIS OF COMPOUNDS CONTAINING PHOSPHATE AND PHOSPHONATE BY GAS-LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

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Summary. Trimethylsilyl derivatives of phosphorylethanolamine and phosphonoethylamine have been separated by gas-liquid chromatography and subsequently analyzed by mass spectrometry. The results indicate that this method is superior to earlier methods for the identification of natural phosphoric and phosphonic acid derivatives.

Some years ago phosphonate-containing organic substances were discovered in lower animals (for a review, see ref. 1), some of them bound in lipids, especially sphingolipids (1). Animal phosphosphingolipids may be hydrolyzed (2-5) by phospholipase to ceramide and a water soluble part, which may be phosphorylcholine (from sphingomyelin), phosphorylethanolamine (2), phosphorylglycerol (6), 1-phosphono-2-aminoethane (1), N-methyl-1-phosphono-2-aminoethane (7, 8), N-acyl-1-phosphono-2-aminoethane (9), N-acyl-N-methyl-1-phosphono-2-aminoethane (9), and possibly 2-amino-3-phosphonopropionic acid (1), N-dimethyl-1-phosphono-2-aminoethane (1), and N-trimethyl-1-phosphono-2-aminoethane (1). Methods used to differentiate between these polar groups include paper chromatography (9-11), ion-exchange chromatography (10, 12), electrophoresis (13), infrared (11, 13) and nuclear magnetic resonance (13) spectroscopy, and survival of organic phosphorus of phosphonates but not phosphates after prolonged time of acid hydrolysis (11, 13-15). However, none of these methods alone is sufficient for identification of individual compounds, and often relatively large amounts of material are needed. The present paper demonstrates a conclusive identification on a microscale of some of the mentioned substances, using combined gas-liquid chromatography and mass spectrometry (GLC-MS).

Materials and methods. O-phosphorylethanolamine, O-phosphorylserine and O-phosphorylthreonine were obtained from Sigma Chemical Company, St. Louis, U.S.A. 1-phosphono-2-aminoethane and 2-amino-3-phosphonopropionic acid were gifts from J. S. Kittredge (1) and were synthesized by A. F. Isbell (13). GLC was performed on an F and M model 402 apparatus (Hewlitt-Packard) equipped with a flame ionization detector and with a 6 feet glass column (i. d. 3 mm) packed with 3% OV-1 on 100-120 mesh Gas-Chrom Q (Applied Science Laboratories, U. S. A.). The column temperature was 150° and the carrier gas (argon) had a flow of 20 ml/min. GLC-MS was performed on a LKB 9000 instrument, (LKB-Produkter, Sweden), with an electron energy of 70 eV and a trap current of 60 μA. Peaks below m/e 40 were not recorded. Silylation was done with suitable amounts of bis (trimethylsilyl) trifluoroacetamide-pyridine-trimethylchlorosilane, 10:5:2, v/v/v, and heating at 60° for 30 min. (16) in a test tube with a teflon-faced screw cap. Part of this solution was injected into the instrument.

Results and discussion. The GLC separation and mass spectra of trimethylsilyl (TMS) derivatives of O-phosphorylethanolamine (I) and 1-phosphono-2-aminoethane (II) are reproduced in Fig. 1-3. A similar separation is obtained for TMS derivatives of O-phosphorylserine (III) and 2-amino-3-

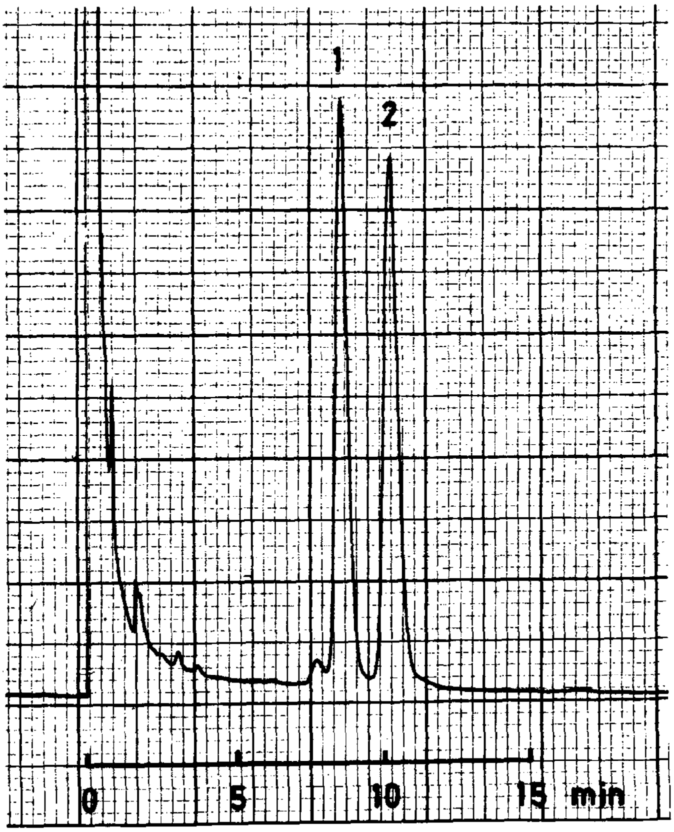

Fig. 1. Gas chromatogram of TMS derivatives of 1-phosphono-2-aminoethane (1) and O-phosphorylethanolamine (2). Conditions, see text.

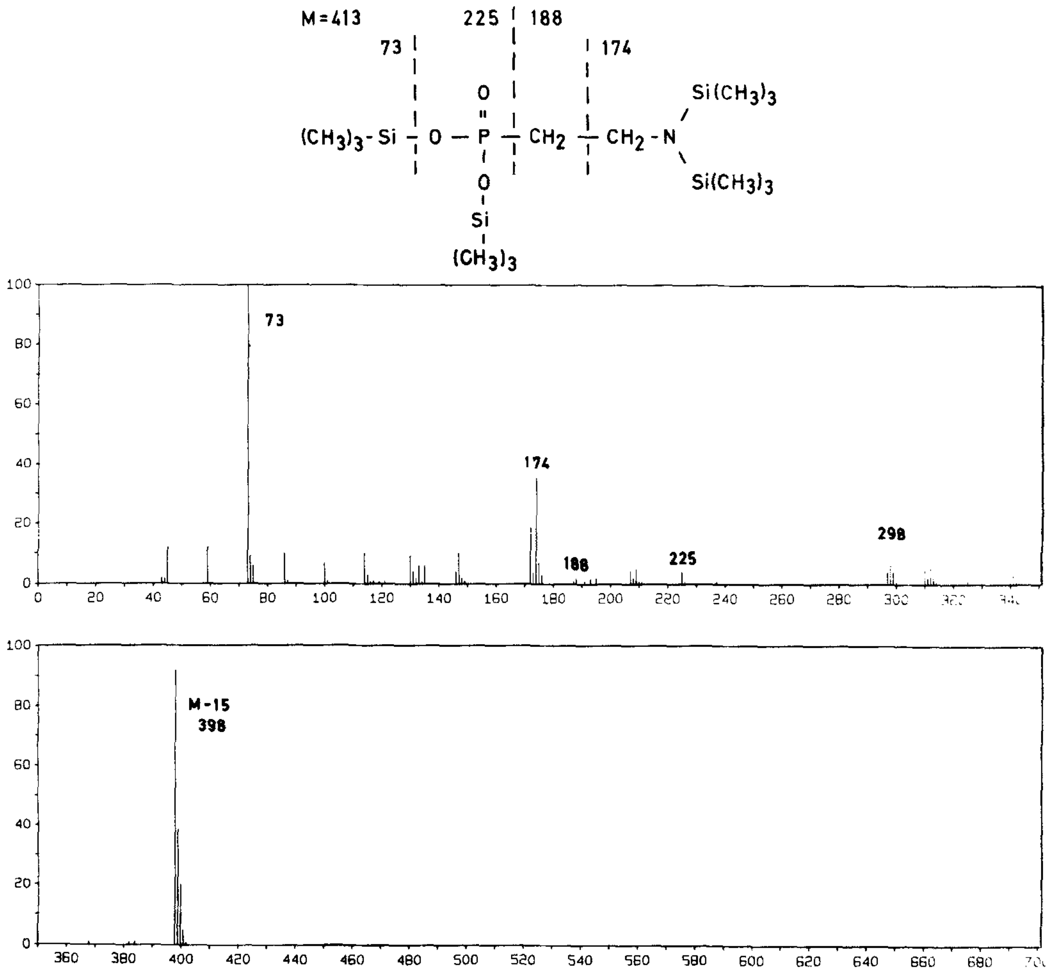

Fig. 2. Mass spectrum of the TMS derivative of 1-phosphono-2-aminoethane. Some fragments have been indicated in the top formula.

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-phosphonopropionic acid (IV), eluted somewhat slower. Mass spectrometry allows an easy identification of the two types of phosphorus compounds. Phosphoric acid derivatives contain an intense peak at m/e 299, proposed (17) to originate, through loss of methane, in a rearrangement ion at m/e 315 ($C_9H_{28}O_4Si_3P$). A peak at m/e 298 in the spectra of phosphonic acid derivatives may be composed of $C_9H_{27}O_3Si_3P$. The parent ion minus 15 (M-15) is stronger in the spectra of phosphonate compared with phosphate compounds. The amino group is disubstituted in I and II, but monosubstituted in III and IV, and also in the TMS derivative of O-phosphorylthreonine. Quarternary amine compounds, like phosphorylcholine and its corresponding phosphonate compound, are not analyzable directly but may first

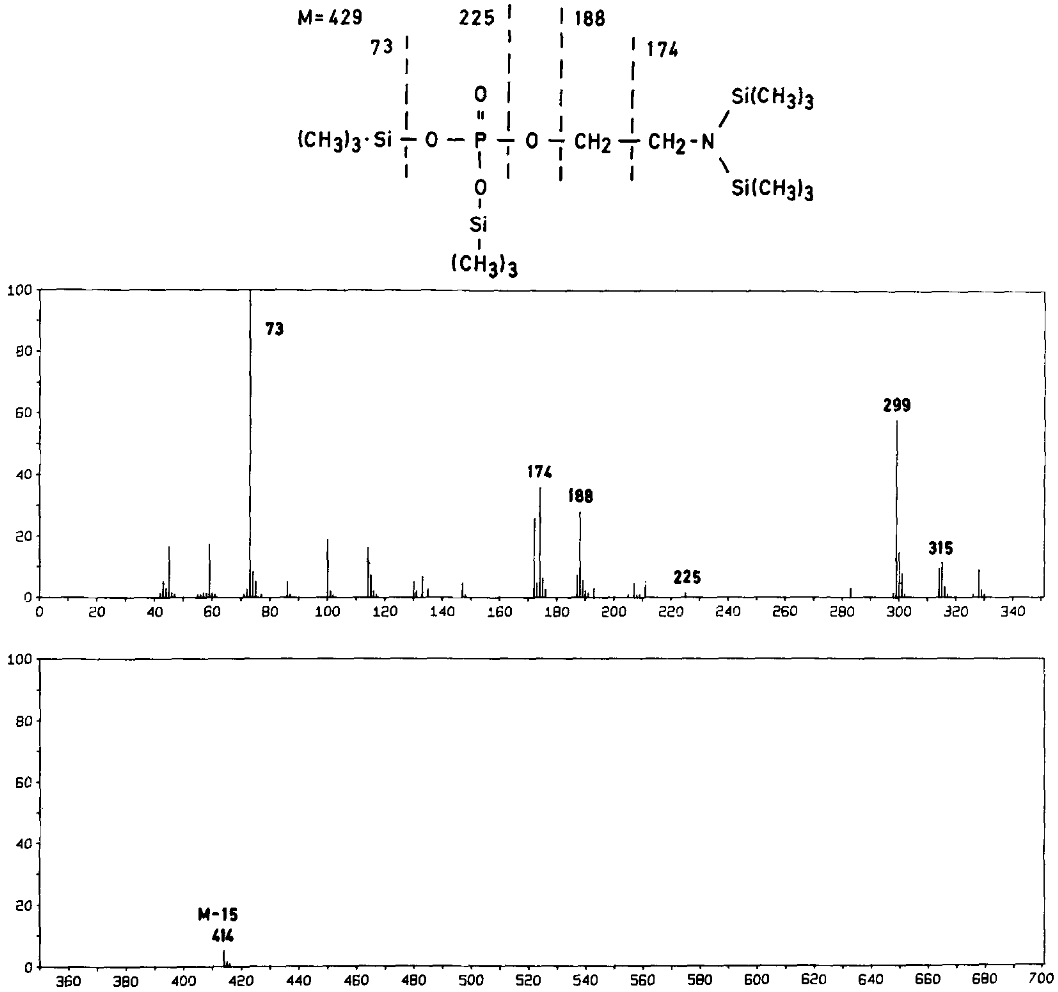

Fig. 3. Mass spectrum of the TMS derivative of O-phosphorylethanolamine. Some fragments have been indicated in the top formula.

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be demethylated to N-dimethyl compounds, as shown before for e. g. acetylcholine (18, 19).

Using the present method we have confirmed the structure (11, 20) of the polar part (after phospholipase hydrolysis) of the major phosphonate-containing sphingolipid of the sea anemone, Metridium senile, as 1-phosphono-2-aminoethane, with small amounts of N-methyl-1-phosphono-2-aminoethane. The original study (11) was done on the species Anthopleura elegantissima, which contains sphingosine (1, 3-dihydroxy-2-amino-4-trans-octadecene) as the major long-chain base. In the present case the base is 1, 3-dihydroxy-2-amino-4, 8-trans, trans-octadecadiene, an earlier unknown base (5). Mass spectrometry of the phosphosphingolipid without preceeding hydrolysis will be reported elsewhere.

Mass spectra of TMS derivatives of phosphoric acid (21) and of nucleotides (16, 17) have been reported. Mass spectrometry is thus useful for the analysis of several types of phosphorus-containing substances. The present method may be of help in identifying phosphonate-containing compounds (1), and in structure elucidation of phosphorus-containing complex lipids of membrane origin.

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