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Synthesis and characterization of *rac*-1,2-bis(palmitoyloxy)-3-propyl (2-trimethylarsonioethyl)phosphonate, an arsenic-containing phosphonolipid

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Arsenic analogs of lecithins (i.e. with replacement of nitrogen by arsenic in the choline group) are probably trace constituents of phospholipids in many organisms. Attempts to synthesize 1,2-bis(palmitoyloxy)-3-propyl 2-trimethylarsonioethyl phosphate (arsenolecithin) according to well-established procedures for the synthesis of the corresponding nitrogen compound failed. However, 1,2-bis(palmitoyloxy)-3-propyl 2-trimethylarsonioethylphosphonate, an arsenic-containing phosphonolipid, was obtained in 16% yield by reacting 1,2-bis(palmitoyloxy)-3-iodopropane with silver 2-trimethylarsonioethylphosphonate in isopropanol. The precursors to the arsenic-containing phosphonolipid were obtained by quaternization of trimethylarsine with diethyl 2-bromoethylphosphonate, hydrolysis of the resulting product with concentrated hydrochloric acid, and reaction of the phosphonic acid with silver oxide to give silver 2-trimethylarsonioethylphosphonate. Quaternization and hydrolysis proceeded almost quantitatively. The silver phosphonate was not isolated but was used *in situ*.

Keywords: Arsenic-containing lipids, 2-trimethylarsonioethylphosphonic acid bromide, 1,2-bis(palmitoyloxy)-3-propyl 2-trimethylarsonioethylphosphonate, arsenic-containing phosphonolipid

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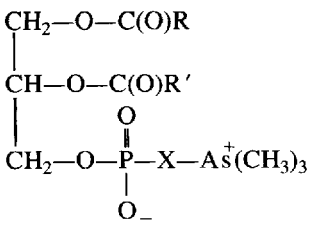
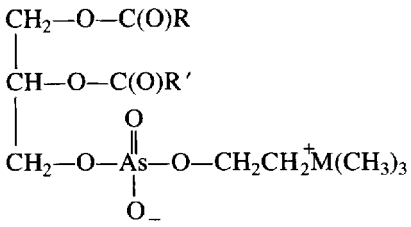
INTRODUCTION

During the past decade organic arsenic compounds with organic groups more complex than methyl were discovered in marine organisms. Carboxymethyl(trimethyl)arsonium zwitterion [arsenobetaine, $(CH_3)_3As^+CH_2COO^-$], 2-hydroxyethyl(trimethyl) arsonium salts [arsenocholine, $(CH_3)_3As^+CH_2—CH_2OH$], and dimethyl-5-ribosylarsine oxides are examples of such compounds.[1] The chemical similarity between arsenic and nitrogen and the presence of arsenocholine[2,3] in marine organisms led to the suggestion that arsenic might replace nitrogen in the choline group of lecithins and that arsenic-containing phospholipids of the lecithin-type might occur in nature.[4] An arsenic-containing phospholipid fraction was isolated by extraction of the marine unicellular alga *Tetraselmis chui* grown in artificial seawater with 10 mg dm^{-3} arsenic (as arsenate).[5] Paper chromatographic and electrophoretic experiments indicated that a large percentage of the arsenate taken up and metabolized by aquatic plants is associated with lipids.[6] Such lipids could be 1,2-bis(acyloxy)-3-propyl (2-trimethylarsonioethyl) phosphates (Scheme 1, **1**) (X = $—OCH_2CH_2—$) or the isomeric 1,3-bis(acyloxy) compounds. The replacement of the phosphate group by arsenate is also possible. However, arsenate esters are easily hydrolyzed.[7] Arsenate-containing lipids (Scheme 1, **2**), therefore, will be cleaved to bis(acyloxy)propanol during the extraction, separation, and identification procedures, unless rigorously anhydrous conditions are maintained.

Phospholipids containing arsenocholine could have special functions as membrane lipids, might also





1: X = —O—CH_2—CH_2, —CH_2—CH_2— **2**: M = N, As

Scheme 1

perhaps be the molecular entities that are required for the optimal functioning of some biochemical reactions, and could thus make arsenic an essential element. Evidence for the essentiality of arsenic has been accumulating over the past two decades.[8,9] To explore the properties of arsenic-containing lipids, sufficient quantities of these compounds of known composition must be available. Their extraction from organisms is an unpleasant, labor-intensive and expensive operation. These compounds are best identified and purified by high-pressure liquid chromatography (HPLC) using arsenic-specific detectors such as graphite furnace atomic absorption (GF AA) spectrometers or plasma atomic emission (AE) spectrometers.[10] The identification is based on retention times. Unequivocal identification requires standards that are presently not available.

To make available arsenic-containing phospholipids in sufficient quantities for chemical, biochemical, and toxicological testing and to provide fully characterized standards for the chromatographic identification of arsenolipids in extracts from organisms, experiments were carried out to find methods suitable for the preparation of 1,2-bis(acylocy)-3-propyl (2-trimethylarsonioethyl) phosphates. These experiments were not successful. However, a phosphonolipid, in which the 2-trimethylarsonioethyl moiety is linked to the phosphorus atom with a phosphorus—carbon (P—C) bond was successfully prepared.

EXPERIMENTAL

Materials and instrumentation

Trimethylarsine,[11] diethyl 2-bromoethylphosphonate,[12] isopropylidene DL-glycerol,[13] and 1-hydroxy-2-propene oxide (glycidol)[14] were prepared according to literature procedures. All solvents were reagent-grade. Diethyl ether was dried over sodium wire. Silver nitrate, silver oxide, and palmitoyl chloride were purchased from Aldrich, USA. Solvents were evaporated from the reaction mixtures in a rotary evaporator. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee, USA. FAB mass spectra were obtained on a VG 70S analytical high-resolution, double-focusing magnetic-sector mass spectrometer equipped with a VG analytical 11/250 J data system using 3-nitrobenzyl alcohol as the medium and argon (Ar) ions (6—8 keV) as bombarding ions. The proton NMR spectra of the compounds in D_2O, deuterobenzene, or deuterochloroform solutions were recorded on a Varian XL-200 NMR spectrometer.

[Diethyl (2-trimethylarsonioethyl) phosphonate] bromide

Diethyl 2-bromoethylphosphonate (Scheme 3, **4**) (15.0 g, 61.2 mmol) and trimethylarsine (7.35 g, 61.2 mmol) were transferred into a nitrogen-flushed, heavy-walled tube. The tube was sealed under an atmosphere of nitrogen with a natural gas/oxygen torch and then heated at 80°C for 8 h. During this time the two liquids reacted to form a solid. The tube was cooled in dry ice, broken open, and then evacuated with an oil pump protected by a dry ice/acetone trap to remove any unreacted trimethylarsine. The solid was dissolved in methanol (100 cm^3). The solution was filtered. Methanol was distilled from the filtrate at 60°C under an aspirator vacuum. The solid residue was triturated with diethyl ether (100 cm^3). The mixture was filtered and the filter cake dried under an oil pump

vacuum. The hygroscopic product weighed 21.0 g (94% yield) and melted at 102–104°C (Scheme 3, **5**).

Analysis for $C_9H_{23}AsBrO_3P$ (365.09), found (calcd): C 29.25 (29.61), H 6.57 (6.35)%.

[(Trimethylarsonioethyl)phosphonic acid] bromide

[Diethyl (2-trimethylarsonioethyl)phosphonate] bromide (10.0 g, 27.4 mmol) and concentrated hydrochloric acid (60 cm^3) were placed into a 250 cm^3 round-bottomed flask equipped with a magnetic stirrer and a reflux condenser. The mixture was gently refluxed for 48 h. Excess hydrochloric acid was removed at 70°C under an aspirator vacuum. The pale yellow oil crystallized after addition of a seed crystal or after refrigeration for several days. The resulting solid was crushed and the powder triturated with diethyl ether overnight. The mixture was filtered and the filter cake dried at room temperature under an oil-pump vacuum. The highly hygroscopic product (7.4 g, 87% yield) melted at 159–161°C (Scheme 3, **6**).

Analysis for $C_5H_{15}AsBrO_3P$ (308.98), found (calcd): C 19.91 (19.44), H 4.94 (4.89)%.

[(2-Trimethylarsonioethyl)phosphonic acid] nitrate

A solution of [(2-trimethylarsonioethyl)phosphonic acid] bromide (6.0 g, 19.4 mmol) in distilled water (50 cm^3) was heated in a 250 cm^3 Erlenmeyer flask to approximately 80°C on a hot plate. A solution of silver nitrate (3.3 g, 19.4 mmol) in distilled water (30 cm^3) was added slowly to the magnetically stirred solution of the bromide. The precipitated silver bromide was allowed to settle for 5 min. The supernatant was checked for the absence of silver ions and bromide ions. The mixture was then filtered and the filter cake washed with distilled water (10 cm^3). The filtrate and the washings were combined and evaporated to dryness on a rotary evaporator under an aspirator vacuum at 70°C. The residual solid was triturated overnight with absolute diethyl ether (50 cm^3). The mixture was filtered. The filter cake was dried at room temperature under an oil-pump vacuum. The hygroscopic product (5.4 g, 96%) melted at 128–130°C.

Analysis for $C_5H_{15}AsNO_6P$ (291.14), found (calcd): C 20.72 (20.63), H 5.07 (5.19)%.

1,2-Bis(palmitoyloxy)-3-iodopropane

3-Iodo-DL-glycerol (18.4 g, 90.9 mmol) — prepared from isopropylidene-DL-glycerol[13] or from glycidol[14] — and pyridine (14.4 g, 182 mmol) were dissolved in dichloromethane (100 cm^3). The solution was placed into a 500 cm^3 round-bottomed flask equipped with a magnetic stirrer. The flask was immersed in an ice/water bath. Freshly distilled palmitoyl chloride (50.0 g, 182 mmol) was added dropwise during 30 min. Then the cooling bath was removed. The stoppered flask was kept at room temperature for 12 h. Pyridinium chloride was removed by filtration. The filtrate was evaporated at 50°C under an aspirator vacuum. Acetone (50 cm^3) was added to the residual oil. The mixture was kept in the refrigerator for crystallization. The crystals were collected by filtration and recrystallized from acetone. The moderately heat- and light-sensitive compound (57.0 g, 92%) was stored at 0°C in the dark.

rac-1,2-Bis(palmitoyloxy)-3-propyl (2-trimethylarsonioethyl)phosphonate

[(2-Trimethylarsonioethyl)phosphonic acid] bromide (0.68 g, 2.2 mmol), silver oxide (0.76 g, 3.3 mmol), and 2-propanol (10 cm^3) were placed into a 50 cm^3 round-bottomed flask equipped with a magnetic stirrer and a reflux condenser. The mixture was gently refluxed for 30 min to produce the silver salt (Scheme 3, **7**). Then 1,2-bis(palmitoyloxy)-3-iodopropane (0.75 g, 1.1 mmol) was added at once. The mixture was refluxed for an additional 30 min. The solvent was evaporated at 60°C under an aspirator vacuum. The residue was extracted with two 10 cm^3 portions of dichloromethane. The combined extracts were kept overnight at room temperature. Finely divided silver that had formed overnight was separated by centrifugation. The supernatant was evaporated at 60°C under an aspirator vacuum. The combined residue from two reactions was heated with hexane (10 cm^3) at 60°C on a water bath. The mixture was filtered. The filtrate on standing at room temperature precipitated a white solid that was collected by centrifugation, washed twice with hexane (5 cm^3), and recrystallized twice from acetone (5 cm^3 each).

The product (0.15 g, 16%), after drying at room temperature under an oil-pump vacuum, melted at 89–91 °C (Scheme 3, **8**).

Analysis for $C_{40}H_{80}AsO_7P$ (778.98), found: C 60.95, H 10.61.
Calculated for anhydrous compound: C 61.67, H 10.35.
Calculated for hemihydrate: C 60.97, H 10.36%.

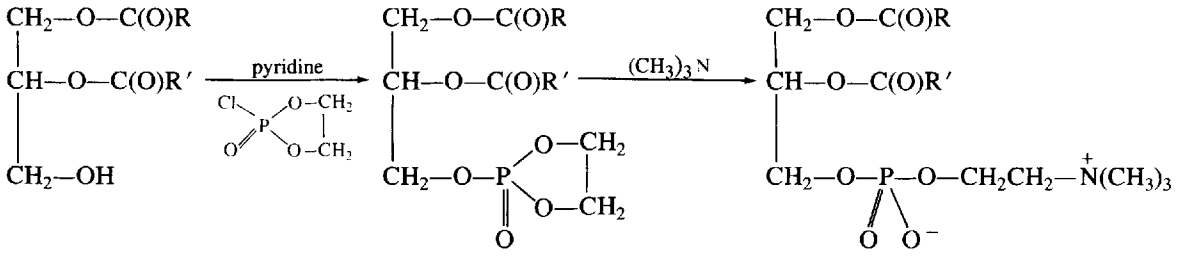
RESULTS AND DISCUSSION

2-Hydroxyethyl(trimethyl)arsonium bromide is easily prepared from trimethylarsine and 2-bromoethanol. The bromide can be exchanged for a variety of other anions. Attempts to prepare 1,2-bis(acyloxy)-3-propyl-2-trimethylarsonio-1-ethyl phosphate (Scheme 1, **1**) (X = $-OCH_2CH_2-$) (phosphatidyl arsenocholine) by well-established routes for phosphatidyl cholines[16,17] failed. The condensation of 1,2-bis(palmitoyloxy)-3-hydroxypropane with ethylene chlorophosphate and the subsequent cleavage of one of the cyclic ester bonds in the resulting 1,2-bis(palmitoyloxy)-3-propyl ethylene phosphate with trimethylamine is one of the most elegant preparations of phosphatidyl cholines **3** (Scheme 2). When this reaction was performed with trimethylarsine instead of trimethylamine, the cyclic ester bond was not broken and no arsenic-containing lipid was formed. To investigate the conditions under which a cyclic phosphate ester will react with trimethylarsine, ethyl ethylene phosphate — a cyclic phosphate ester thermally more stable than ethylene chlorophosphate — was prepared. Ethyl ethylene phosphate did not react with trimethylarsine when heated in a sealed tube without solvent at temperatures up to 70 °C. At 90 °C only polymeric materials were formed. Although these materials contained arsenic, the reaction is not suited for the synthesis of arsenolecithins.

1,2-Bis(palmitoyloxy)-3-propyl 2-bromoethyl hydrogen phosphate was then explored as an alternate starting material. Quaternization of trimethylarsine with the phosphate ester should produce phosphatidyl arsenocholine. However, no reaction occurred when the reagents were heated at 70 °C in a sealed tube for long periods under an atmosphere of nitrogen. At higher temperatures the phosphate ester decomposed. Similar experiments with the neutral triester, 1,2-bis(palmitoyloxy)-3-propyl 2-bromoethyl ethyl phosphate, were also unsuccessful.

The condensation of 1,2-bis(acyloxy)-3-iodopropane with monosilver 2-trimethylarsonioethyl phosphate would be a third way to prepare arsenolecithins. However, 2-trimethylarsonioethyl dihydrogen phosphate could not be obtained from arsenocholine and phosphorus oxychloride, although the corresponding nitrogen compound was prepared in this manner. The product of the reaction between 2-hydroxyethyl(trimethyl)arsonium bromide and phosphorus oxychloride was a 2-chloroethyl (trimethyl)arsonium salt[15] and not the expected ester. Phosphorus trichloride reacted similarly.

Esterification of arsenocholine with phosphoric acid at 165 °C produced a mixture of compounds, from which 2-trimethylarsonioethyl dihydrogen phosphate was isolated as the barium salt.[15] The high temperatures required for this reaction led to considerable decomposition, formation of polyphosphates, and a low yield of the desired product.



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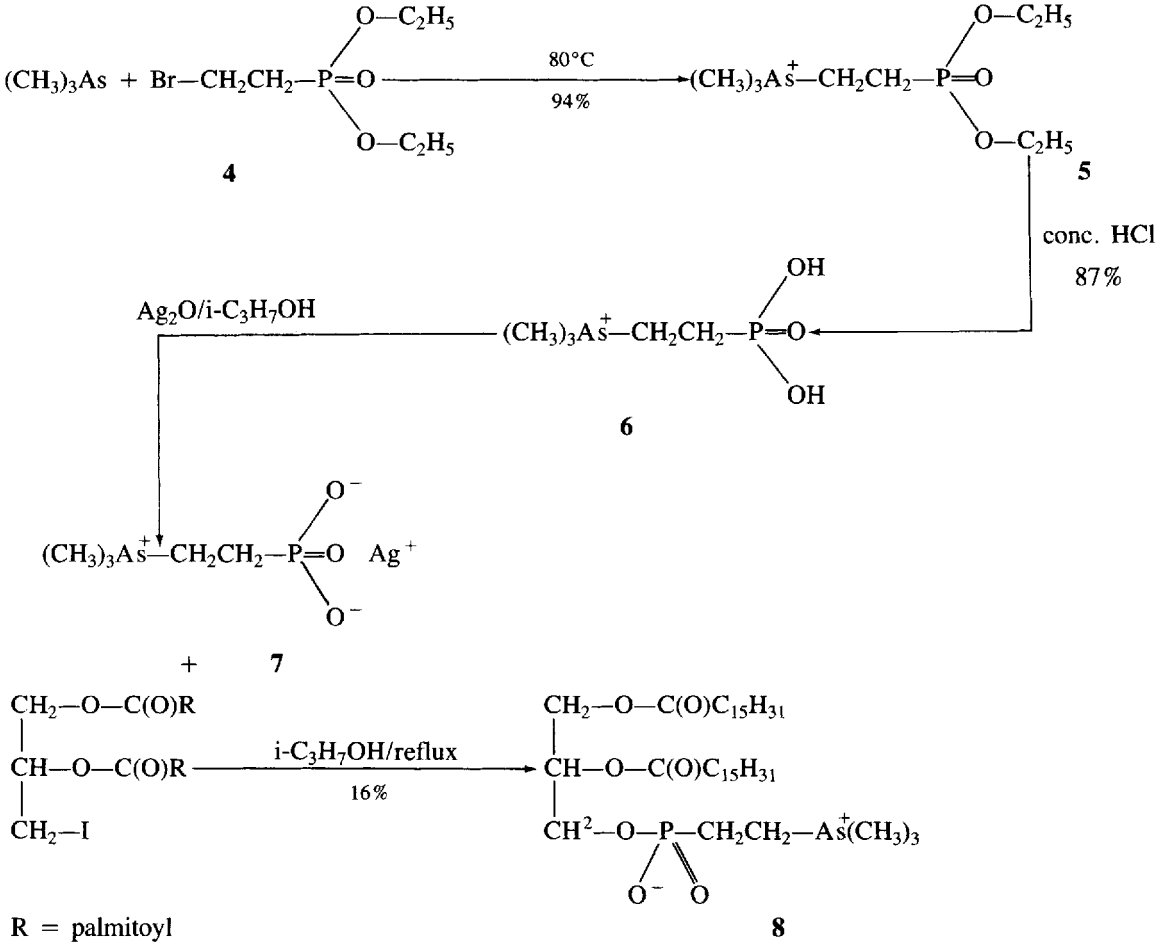
Scheme 2

Attempts to condense the phosphorylated arsenocholine with 1,2-bis(palmitoyloxy)-3-iodopropane were unsuccessful. No suitable solvent could be found that would dissolve the barium salt of the phosphorylated arsenocholine and the iodopropane derivative. Addition of phase-transfer catalysts brought no improvement. Various attempts to condense 1,2-bis(palmitoyloxy)-3-hydroxypropane with 2-trimethylarsonioethyl hydrogen phosphate in the presence of dicyclohexylcarbodiimide also ended in failure.

These unexpected difficulties that can be tracted to the differences in chemical behavior between nitrogen — an atom without the possibility of expanding its valence shell — and arsenic, an atom that can expand its valence shell, led to experiments with phosphonates. 2-Trimethylarsonioethylphosphonic acid (Scheme 3) a compound with a phosphorus–carbon bond replacing the ester bond in the corresponding phosphoric acid, has fewer possibilities to react, could serve as a model compound, and should lead to arsenic-containing phosphonolipids. Nitrogen-containing phosphonolipids are known.[16]

Diethyl 2-trimethylarsonioethylphosphonate bromide (Scheme 3, **5**) was prepared in 94% yield from diethyl 2-bromoethylphosphonate (Scheme 3, **4**) and trimethylarsine at 80°C. Subsequent hydrolysis with concentrated hydrochloric acid produced the phosphonic acid (Scheme 3, **6**) a very hygroscopic solid. Reaction of the silver salt of this acid (Scheme 3, **7**), prepared by refluxing a mixture of silver oxide and the acid in 2-propanol, with 1,2-bis(palmitoyloxy)-3-iodopropane produced 1,2-bis(palmitoyloxy)-3-propyl 2-(trimethylarsonio)ethylphosphonate (Scheme 3, **8**), the first example of a synthetic, arsenic-containing lipid. The yield of the isolated pure product **8** was 16%. The same lipid was obtained when



Scheme 3

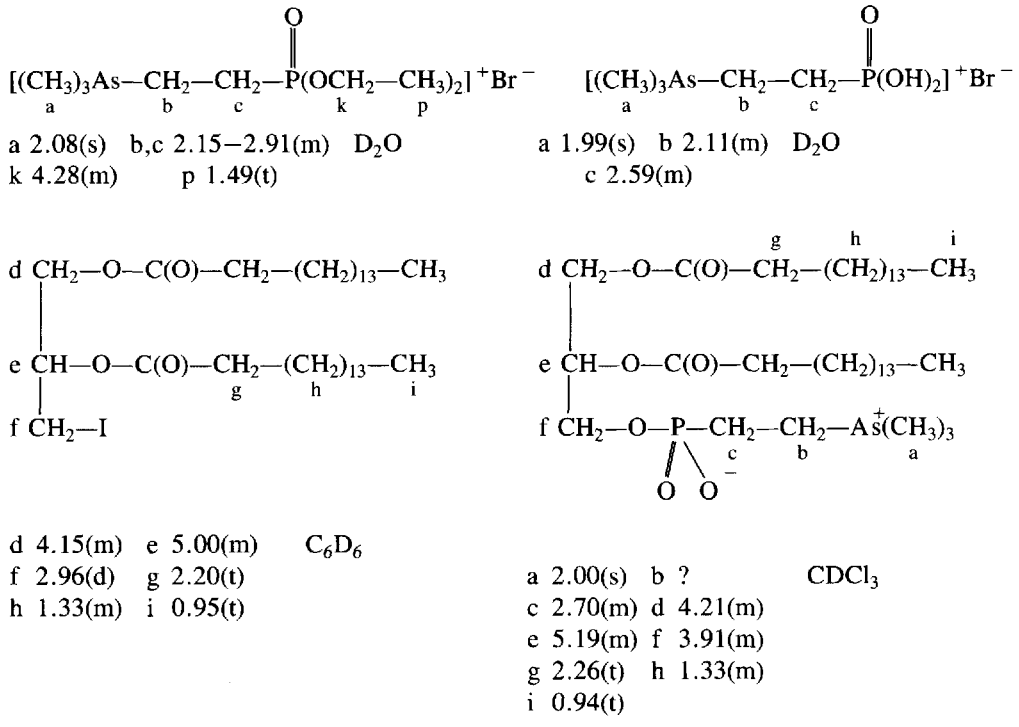


Figure 1 Proton NMR shifts for 1,2-bis(palmitoyloxy)-3-propyl 2-trimethylarsonioethylphosphonate and precursor compounds.

1,2-bis(palmitoyloxy)-3-propyl 2-bromoethylphosphonate and trimethylarsine were heated for 5 h in a sealed tube at 70°C. An ^1H NMR spectrum of this lipid indicated that the 1,2-diacyl compound was contaminated by the 1,3-diacyl derivative formed by isomerization during the reaction with trimethylarsine. Attempts to quaternize trimethylarsine with the corresponding phosphoric acid derivative were unsuccessful.

The arsenic-containing phosphonolipid was characterized by elemental analyses, proton-NMR spectrocopy, and mass spectroscopy. The experimental carbon/hydrogen percentages agreed with the percentages calculated for the lipid hemihydrate. The compound should be stored in a refrigerator. At room temperature slow decomposition occurred in the course of several weeks as indicated by additional signals in the proton NMR spectrum.

The proton NMR spectra of the 1,2-bis(palmitoyloxy)-3-propyl (2-trimethylarsonioethyl)phosphonate (Scheme 3, **8**) and its precursors are summarized in Fig. 1. The sharp singlets from the methyl groups bonded to arsenic are in the range 1.99–1.08 ppm in the 2-trimethylarsonioethylphosphonates. The two methylene groups joining the arsenic to the phosphorus atom produce complex multiplets in the range 2.11–2.91 ppm. In the lipid the methylene groups bonded to the carboxyl carbon atoms resonate in the same region and make exact assignments difficult. The protons of the glycerol group in the phosphonolipid produce signals that are downfield from the resonances of the corresponding protons in 1,2-bis(acyloxy)-3-iodopropane. The exchange of iodine for the phosphonate group shifts the methylene signals from 2.96 ppm to 3.91 ppm.

The FAB mass spectrum (Fig. 2) has the M+H peak at *m/z* 780 with the C-13 signals at 781 of correct intensity. The protonated molecular ion releases $(CH_3)_3AsCH_2CH_2PO_3H$ producing the fragment with *m/z* 552, which in turn loses $C_{15}H_{31}CO$, followed by C_2H_3O and finally CH_2O to give $C_{15}H_{31}CHO$ (*m/z* 239). The signals at *m/z* 229 ascribable to $(CH_3)_3As$-$CH_2CH_2PO_3H_2$ and 211 $[(CH_3)_3AsCH_2CH_2PO_2]$ are the base peaks in the spectrum. The trimethylarsine peak at *m/z* 120 is the next most intense signal. The molecular ion and the fragments are in agreement with the formulation of the lipid as 1,2-bis(palmitoyloxy)-3-propyl 2-trimethylarsonioethylphosphonate.

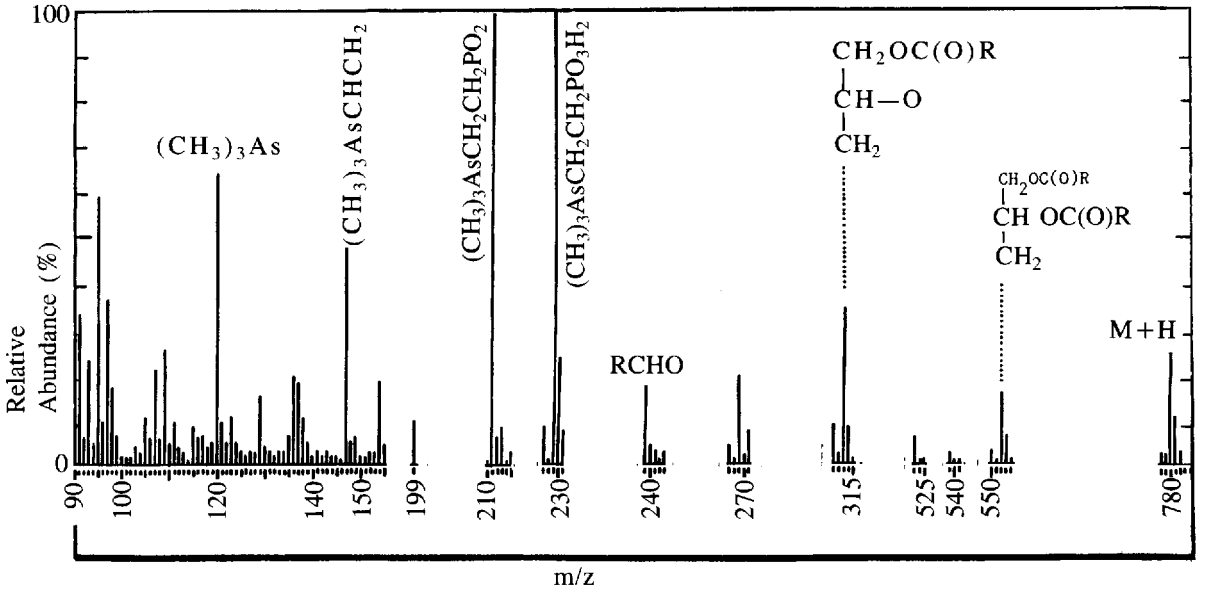


Figure 2 The FAB mass spectrum of 1,2-bis(palmitoyloxy)-3-propyl 2-trimethylarsonioethylphosphonate (R = $C_{15}H_{31}$).

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