New synthetic phosphinate analogues of lecithin

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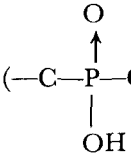
ABSTRACT The chemical syntheses of two new, completely nonhydrolyzable phosphinate analogues of lecithin are described. These have the structures $ROCH_2CH(OR)CH_2CH_2P(O)(O^-)CH_2CH_2\overset{+}{N}(CH_3)_3$ and $ROCH_2CH(OR')CH_2P(O)(O^-)CH_2CH_2CH_2\overset{+}{N}(CH_3)_3$, where R = $C_{18}H_{37}$ and R′ = $C_{16}H_{33}$; each is thus isosteric with lecithin on either side of the phosphorus function. The infrared spectra of these compounds undergo unexpected changes under mild acid, base, or adsorptive treatment. These are discussed and compared with related lecithin analogues, including the simple phosphinate $C_{18}H_{37}P(O)(O^-)CH_2CH_2\overset{+}{N}(CH_3)_3$, whose synthesis is also reported.

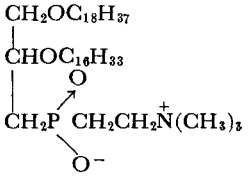
SUPPLEMENTARY KEY WORDS isosteric · nonhydrolyzable · ethers · infrared spectra

THE PHOSPHINIC ACID  group contains two carbon–phosphorus bonds, and therefore a lecithin analogue containing this moiety would contain no phosphorus ester groups. Since in the absence of special structural features the two carbon–phosphorus bonds of a phosphinic acid are hydrolytically stable, a phosphinate-containing lecithin analogue can also be expected not to contain hydrolyzable phosphorus functions.

No examples of phospholipids containing the phosphinic acid group have as yet been isolated from natural sources. The chemical synthesis of one such lecithin analogue has, however, recently been reported from this laboratory (1, 2). This substance, a 2,3-dialkoxypropyl-[2′-(trimethylammonium)ethyl]phosphinate (I), is a compound in which ether groups substitute for the lecithin ester groups and which thus contains no hydrolyzable groups at any position. Compared with natural phosphate lecithin, however, this phosphinate analogue contains one less atom between phosphorus and nitrogen and between phosphorus and the long-chain functional groups.

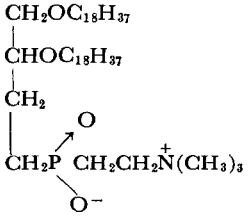
The two phosphinate-containing lecithin analogues whose synthesis is reported below represent structures isosteric (—CH_2— in place of —O—) with the natural lecithins on either side of the phosphorus atom. 3,4-Dioctadecoxybutyl[2′-(trimethylammonium)ethyl]phosphinate (II) is isosteric at the "glycerol" chain, while 2-hexadecoxy-3-octadecoxypropyl[3′-(trimethylammonium)propyl]phosphinate (III) shows a similar structural feature at the nitrogenous base portion of the molecule.

PHOSPHINATE ANALOGS OF LECITHIN



I

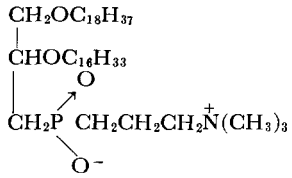
2-Hexadecoxy-3-octadecoxypropyl[2′-(trimethylammonium)-ethyl]phosphinate



II

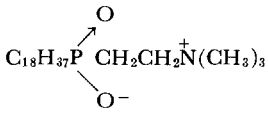
3,4-Dioctadecoxybutyl[2′-(trimethylammonium)ethyl]phosphinate

Abbreviations: TLC, thin-layer chromatography.



III

2-Hexadecoxy-3-octadecoxypropyl[3′-(trimethylammonium)-propyl]phosphinate

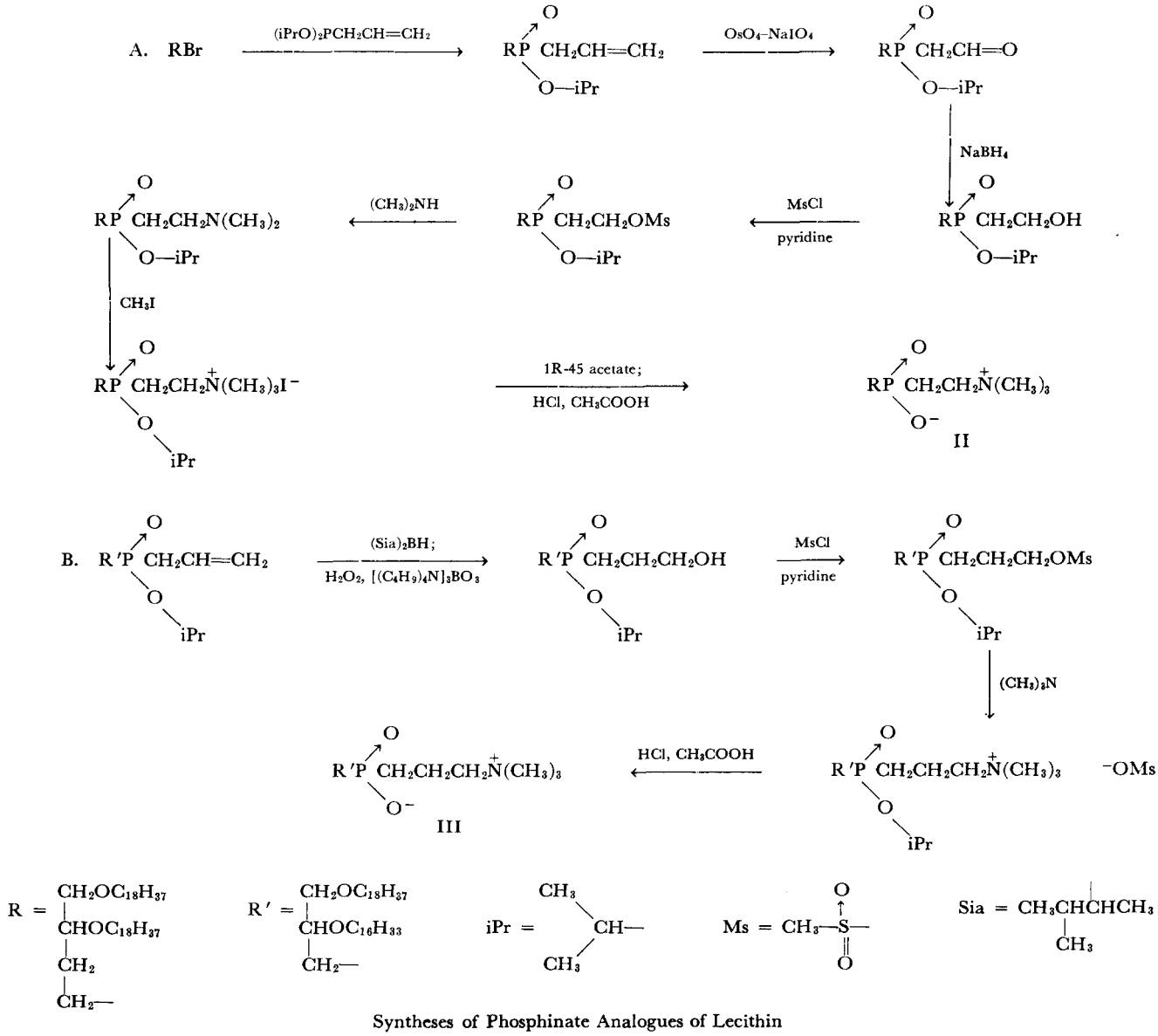


IV

Octadecyl[2′-(trimethylammonium)ethyl]phosphinate

The synthesis of the analogue II was very similar to that of the previously reported 2-hexadecoxy-3-octadecoxypropyl[2′ - (trimethylammonium)ethyl]phosphinate (1, 2), while the synthesis of the 3-carbon base analogue III followed a quite different route, starting from 2 - octadecoxy - 3 - octadecoxypropyl(allyl)phosphinate. The synthetic schemes are outlined below.

Both diether-phosphinate lecithin analogues proved to be hygroscopic (though not deliquescent) and each appeared to form several hydrates, so that analyses for the hydrates of the compositions reported could be obtained only by careful drying under specified conditions. This hydrophilicity was also shown by the much greater ease with which these analogues formed almost clear dispersions by sonication in water compared to the corresponding phosphonate and phosphate analogues.



Syntheses of Phosphinate Analogues of Lecithin

The infrared spectra of these and similar compounds showed a number of unexpected transformations when they were exposed to mild acidic, basic, or adsorptive conditions.

MATERIALS AND METHODS

2 - Hexadecoxy - 3 - octadecoxypropylphosphonylcholine, mp 193.5–194.5°C (dec) (3), and 2-hexadecoxy-3-octadecoxypropyl[2′ - (trimethylammonium)ethyl]phosphinate, mp 202–203°C (dec) (1, 2), were synthesized as previously described. These substances were used only for comparison of their infrared spectra (see below).

1,2-*O*-Dihexadecylglycerophosphorylcholine was obtained from Schwarz/Mann, Orangeburg, N.Y. Infrared spectra were obtained on KBr pellets or chloroform solutions, using a Perkin–Elmer 337 infrared spectrometer. Elemental microanalyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y. Thin-layer chromatography in all cases was performed on 5 × 20 cm Silica Gel G-coated plates, using unlined cylindrical tanks.

Synthetic Route

The syntheses of the phosphinate analogues II and III are outlined in the scheme above. It should be noted that both 2- and 3-carbon base analogues were prepared via the versatile allyl intermediates. In the first case, a 3,4-dialkoxybutyl bromide (4) on reaction with diisopropyl allylphosphonite gave the isopropyl 3,4-dialkoxybutyl(allyl)phosphinate, the allyl group of which was oxidatively cleaved by osmate–periodate. Borohydride reduction, mesylation, and reaction with aqueous dimethylamine produced the 2-dimethylaminoethyl phosphinate, which was quaternized with methyl iodide. After conversion of the iodide salt to the acetate to avoid the formation of hydriodic acid during acid hydrolysis, reaction with hydrochloric acid removed the isopropyl ester group to give the lecithin analogue II as a sesquihydrate.

For the synthesis of analogue III, hydroboration of isopropyl 2-octadecoxy-3-octadecoxypropyl(allyl)phosphinate with di-*sec*-isoamylborane, then oxidation with hydrogen peroxide in a homogeneous buffered solution, gave the 3′-hydroxypropyl phosphinate. Mesylation, reaction with trimethylamine, and, finally, acid hydrolysis gave the lecithin analogue III as the monohydrate.

EXPERIMENTAL

1. DL-*3,4-Dioctadecoxybutyl[2′-(trimethylammonium)-ethyl]phosphinate (II)*

DL-*Isopropyl 3,4-Dioctadecoxybutyl(allyl)phosphinate.* 3,4-Dioctadecoxybutyl bromide (10.0 g, 0.015 mole [4]) and freshly distilled diisopropyl allylphosphonite (5 g, 0.035 mole [5]) containing a trace of hydroquinone were heated under nitrogen at 120 ± 2°C for 40 hr. The cooled mixture in ether was washed with dilute HCl and water. It was then dried over $MgSO_4$, filtered, and the solvent was evaporated. To the residue was added acetonitrile at 10°C, and the precipitated product was filtered, washed with cold acetonitrile, and dried in vacuo. The yield of crude product was 9.8 g.

Crude isopropyl 3,4-dioctadecoxybutyl(allyl)phosphinate (9.0 g) in 100 ml of methylene chloride was applied to a column (50 mm I.D.) containing Mallinckrodt SilicAR CC-7 (450 g), previously activated by heating at 110°C for 16 hr and washing with 1 liter of methanol and 2.5 liters of methylene chloride. The column was eluted with methylene chloride (1 liter) and 1 liter each of 3%, 5%, and 6% ethanol in methylene chloride, all of which eluted only low-polarity impurities. The product was eluted with 8 and 10% ethanol in methylene chloride (1 liter each), successively. The product obtained by evaporation of the solvent weighed 5.5 g (61% yield). When chromatographed by TLC there were two essentially identical spots, R_F 0.47–0.49, when the Silica Gel G plates were developed in 8% ethanol in methylene chloride. Recrystallization of the product from chloroform–acetone and from absolute ethanol at 5°C yielded an analytically pure product, mp 35–36° C, presumably an approximately equal mixture of the two racemic diastereoisomers.

Analysis: $C_{46}H_{93}O_4P$ (mol wt 741.22);
calculated: C, 74.54; H, 12.62; P, 4.18
found: C, 74.24; H, 12.37; P, 3.89

The infrared spectrum showed a rather weak but sharp C═C band at 1625 cm^{-1} in addition to the P → O (1245 cm^{-1}), ether (1130 and 1110 cm^{-1}), and P—O—C (955 cm^{-1}) absorptions.

DL-*Isopropyl 3,4-Dioctadecoxybutyl(2′-acetaldo)phosphinate.* The intermediate aldehyde, alcohol, mesylate, dimethylaminoethyl, and (trimethylammonium)ethyl isopropyl phosphinate esters were not isolated in analytically pure form before conversion to the phosphinate lecithin.

2 g of sodium metaperiodate in 43 ml of water and 500 ml of 95% ethanol were added to 1 g (0.00135 mole) of the purified allylphosphinate in 117 ml of absolute ethanol. While the mixture was stirred vigorously, a freshly prepared solution of osmium tetroxide (60 mg) in absolute ethanol (50 ml) was added. After stirring 2 hr at room temperature the mixture was evaporated in vacuo at 35°C to about 50 ml. The residue was extracted with chloroform and water, and after the chloroform layer was dried with $MgSO_4$ the solvent was removed in vacuo. The residue, which consisted of several spots on

thin-layer plates, weighed 0.99 g and showed a strong, sharp aldehyde absorption at 1710 cm^{-1}.

DL-*Isopropyl 3,4-Dioctadecoxybutyl(2'-hydroxyethyl)phosphinate.* Reduction of the aldehyde was accomplished by addition of sodium borohydride (450 mg) to a solution of the crude aldehyde (0.99 g) in absolute ethanol (65 ml); the mixture was stirred at room temperature for 16 hr. It was cooled to 5°C and 12 N HCl was added dropwise until no hydrogen was evolved. The mixture was evaporated to dryness in vacuo at 50°C. The crude alcohol (0.97 g) showed only a very slight absorption in the 1700 cm^{-1} region.

The entire product in 20 ml of chloroform was applied to a column (20 mm I.D.) containing 100 g of SilicAR CC-7 (previously activated at 110°C and then washed with methanol and chloroform). The product was eluted in several fractions with 1 and 2% methanol in chloroform. The main fraction (0.70 g) was homogeneous (R_F 0.27) as judged by TLC in chloroform–methanol–58% aqueous ammonia 95:4:0.4.

DL-Isopropyl *3,4-Dioctadecoxybutyl(2'-mesyloxyethyl)phosphinate.* The crude alcohol (0.70 g) in dry pyridine (12 ml) was cooled to 5°C while methanesulfonyl chloride (1.2 ml) was added dropwise during 5 min of vigorous stirring. After stirring at 5°C for 10 min more and at room temperature for 20 min, the mixture was extracted with ether (100 ml) and water (100 ml). The aqueous layer was reextracted with 2 × 50 ml of ether. The combined ether extracts were washed successively with 2 × 50 ml of water, 1 × 50 ml of 2 N H_2SO_4, 2 × 50 ml of water, 1 × 50 ml of 3 M Na_2CO_3, and finally with 1 × 100 ml of water. The ether solution was dried over $MgSO_4$, filtered, and evaporated in vacuo to give 0.75 g of the crude product. There was one main spot (R_F 0.79) by TLC (8% ethanol in methylene chloride). Bands at 1345 and 1163 cm^{-1}, which are characteristic of sulfonate esters, were observed.

DL-*Isopropyl 3,4-Dioctadecoxybutyl[2'-(trimethylammonium)ethyl]phosphinate Iodide.* To the mesylate (750 mg) in 43 ml of tetrahydrofuran was added 40% aqueous dimethylamine (17 ml) and water (7 ml) and the mixture was left at room temperature for 4 days. Volatile material was removed in vacuo at 50°C and the residue was dehydrated by addition of ethanol and by evaporation of the solvent to dryness. This procedure was carried out twice. The product (a single spot by TLC, R_F 0.24, 4% ethanol in methylene chloride), was dissolved in ether (70 ml) and swirled, without vigorous shaking, with a solution of 10 g of Na_2CO_3 in 31 ml of 0.1 N NaOH. The ether layer was separated and to it was added 7.5 ml of methyl iodide; the solution was kept in the dark at room temperature for 5 days. The mixture was cooled to 16–17°C for 1 hr and then filtered rapidly and isothermally; the precipitate was washed with minimal quantities of cold ether and dried in vacuo. The product (720 mg) was homogeneous by TLC (R_F 0.67, chloroform–methanol–water 65:25:4).

DL-*3,4-Dioctadecoxybutyl[2'-(trimethylammonium)ethyl]phosphinate (II).* The crude methiodide (720 mg) in 40 ml of ethanol–water–chloroform 7:3:1 was passed through a jacketed column 1 cm in diameter containing Amberlite 1R-45 acetate (20 g, previously equilibrated with the above solvent) at 30–35°C. The column was washed with 200 ml of additional solvent and the combined eluates were evaporated to dryness in vacuo at 50°C. The residue, after dehydration by reevaporation with isopropanol three times, weighed 700 mg and was iodide-free. This acetate salt was dissolved in 150 ml of acetic acid and 15 ml of 6 N HCl and kept at 80°C for 24 hr. The mixture was evaporated in vacuo at 50–55°C, and the product was precipitated with cold acetonitrile. The filtered and washed precipitate was dissolved in a minimal amount of warm chloroform, 0.5 ml of pyridine was added, and the product was again precipitated with cold acetonitrile. The yield was 370 mg, or 36% over-all from the allylphosphinate. The lecithin analogue was homogeneous as judged by TLC in chloroform–methanol–water 65:25:4 (R_F 0.34). Recrystallization from chloroform–acetone yielded a pure product, mp 204–205°C (dec). Due to the tendency of the compound to retain water tenaciously with the apparent formation of various hydrates, it was found very difficult to obtain a correct carbon analysis. However, redrying just prior to analysis at 40°C in vacuo and then at 100°C and 150°C in a nitrogen atmosphere permitted a correct analysis for a sesquihydrate.

Analysis: $C_{45}H_{94}NO_4P + 1^1/_2H_2O$ (mol wt 771.25);
calculated: C, 70.08; H, 12.68; N, 1.81; P, 4.02
found: C, 70.36; H, 12.77; N, 1.86; P, 3.94

The infrared spectrum of this compound is discussed below.

2. *2-Hexadecoxy-3-octadecoxypropyl-[3'-(trimethylammonium)propyl]phosphinate (III)*

DL-*Isopropyl 2-Hexadecoxy-3-octadecoxypropyl(3'-hydroxypropyl)phosphinate.* A solution of di-*sec*-isoamylborane was prepared by adding 12.0 g (0.17 mole) of 2-methyl-2-butene dropwise to 100 ml of 0.85 M borane in tetrahydrofuran (1 M solution from Ventron Corp.) during 30 min of stirring under nitrogen at 18°C.

To isopropyl 2-hexadecoxy-3-octadecoxypropyl(allyl)-phosphinate (5.5 g, 0.0079 mole) in 30 ml of anhydrous peroxide-free tetrahydrofuran the above borane solution was added, and the clear solution was left 1 hr at room temperature and then overnight at 3°C. The mixture was evaporated to dryness in vacuo at 30°C and the residue was dissolved in tetrahydrofuran (80 ml). To

this was slowly added an organic buffer prepared by dissolving 2.37 g of boric acid in a mixture of 38 ml of 25% methanolic tetrabutylammonium hydroxide and 190 ml of tetrahydrofuran (evolution of hydrogen). The mixture was cooled to 5°C, 15% aqueous hydrogen peroxide (50 ml) was added dropwise with stirring during 1 hr, and the mixture was stirred for 30 min more at room temperature. It was evaporated in vacuo at 50°C to half its volume, poured into a mixture of 2 liters of ice-water and 80 ml of 12 N HCl, and then kept overnight at room temperature. The mixture was filtered and the precipitate was washed thoroughly with water. The wet material was dissolved in chloroform and washed with water; the chloroform layer was dried over $MgSO_4$, filtered, and evaporated in vacuo at 45°C. The crude alcohol (5.6 g) in chloroform (50 ml) was applied to a column 50 mm in diameter containing Mallinckrodt SilicAR CC-7 (activated at 105°C and by washing with methanol and then with chloroform). Chloroform (1 liter) and 2% absolute ethanol in methylene chloride eluted low-polarity impurities. The product was eluted with 4% and then 6% absolute ethanol in methylene chloride (1 liter each). Evaporation of the solvent yielded a product (3.45 g) which consisted of two components in approximately equal amounts. The R_F's on thin-layer plates developed in 4% methanol in chloroform were 0.43 and 0.60. One diastereoisomer (R_F 0.43) could be separated when the column was eluted only with 6% absolute ethanol in chloroform.

DL-*Isopropyl 2-Hexadecoxy-3-octadecoxypropyl(3'-mesyloxypropyl)phosphinate*. Methanesulfonyl chloride (2.2 ml, 3.3 g, 0.029 mole) was added dropwise to a solution of the above purified alcohol (1.29 g) in dry pyridine (40 ml) during 5 min of stirring vigorously at 5°C. After 10 min at 5°C and 20 min at room temperature, the mixture was stirred at 10°C with ether (60 ml) and water (60 ml). The aqueous phase was reextracted twice with ether, and the combined ether extracts were washed successively with water (2×), 60 ml of 2 N sulfuric acid, 60 ml of 3% aqueous sodium carbonate, and finally with 100 ml of water. The ether solution was dried over $MgSO_4$, filtered, and evaporated to dryness at 30°C.

The product (1.28 g) consisted primarily of one component with an R_F of 0.47 when TLC was carried out using 6% ethanol in methylene chloride. The infrared spectrum showed bands characteristic of sulfonate esters at 1345 and 1175 cm^{-1}.

DL-*Isopropyl 2-Hexadecoxy-3-octadecoxypropyl[3'-(trimethylammonium)propyl]phosphinate Methanesulfonate Salt*. The sulfonic ester (1.28 g) was dissolved in 40 ml of 25% methanolic trimethylamine and the solution was left at room temperature for 5 days. Evaporation in vacuo at 35°C left a product which was almost homogeneous (R_F 0.57, chloroform–methanol–water 65:25:4). Recrystallization twice from acetone yielded a pure white product (1.1 g) which proved difficult to analyze because of its hygroscopicity. Redrying at 50°C just prior to analysis gave values correct for a monohydrate.

Analysis: $C_{47}H_{100}NO_7PS + H_2O$ (mol wt 872.350);
calculated: C, 64.71; H, 11.79; N, 1.60; S, 3.67
found: C, 65.06; H, 11.73; N, 1.83; S, 3.75

If the compound was dried at 150°C in a nitrogen atmosphere just prior to analysis, the anhydrous material was formed.

Analysis: $C_{47}H_{100}NO_7PS$ (mol wt 854.335);
calculated: C, 66.08; H, 11.80; N, 1.64; S, 3.75
found: C, 65.91; H, 11.87; N, 1.83; S, 3.76

The sulfonic ester regions at 1175 and 1345 cm^{-1} showed little absorption. After successive drying at 40°C, 60°C, 100°C, and 120°C in vacuo, the salt melted at 156–156.5°C.

DL-*2-Hexadecoxy-3-octadecoxypropyl[3'-(trimethylammonium)propyl]phosphinate* (*III*). The methanesulfonate salt (800 mg, 0.917 mmole) was dissolved in a warm mixture of acetic acid (120 ml) and 6 N hydrochloric acid (12 ml), and the solution was kept at 80–85°C for 24 hr. Evaporation of the mixture to dryness at 50°C followed by removal of residual acid (in vacuo < 1 mm) for 20 min gave a white product. The material was dissolved in 2 ml of chloroform; 0.5 ml of pyridine was added, and the product was precipitated with cold acetonitrile. The precipitate was filtered and washed with cold acetonitrile and acetone and dried in vacuo. The product at this point weighed 700 mg (44% yield from the allylphosphinate); it was almost homogeneous (R_F 0.32, chloroform–methanol–water 65:25:4). The material dissolved in warm ethanol–water–chloroform 7:3:1 was passed through a column (1 cm I.D.) of 20 g of 1:1 mixture of Amberlite 1R-120(H^+) and 1R-45 (OH^-) at 40–45°C. The column was washed thoroughly with the same solvent, and the combined eluates were evaporated in vacuo at 45°C. The residue was dehydrated by reevaporation three times with isopropanol. The product was recrystallized from boiling chloroform–acetone; the yield at this point was 510 mg, or 32% over-all yield from the allylphosphinate. The compound melted sharply with decomposition at 192–194°C. The material was very difficult to dry adequately for analysis. After redrying at 150°C in a stream of nitrogen and in vacuo, a correct analysis for the monohydrate was obtained.

Analysis: $C_{43}H_{90}NO_4P + H_2O$ (mol wt 734.18);
calculated: C, 70.35; H, 12.63; N, 1.91; P, 4.22
found: C, 70.11; H, 12.83; N, 1.80; P, 3.91

The infrared spectrum is discussed below.

The synthesis could also be accomplished if the initial hydroboration of the allyphosphinate was performed with borane or 9-borabicyclo[3,3,1]nonane (6); in either case, however, the succeeding intermediates were much more difficult to purify than when disiamylborane was used.

3. Octadecyl[2′-(trimethylammonium)ethyl]-phosphinate (IV)

The synthesis of this single long-chain hydrocarbon phosphinate analogue was very similar to that of the dioctadecoxybutyl analogue, except that none of the intermediates were purified extensively or characterized.

Isopropyl octadecyl(allyl)phosphinate was prepared in 62% yield from 1-bromooctadecane and diisopropyl allylphosphonite according to the method used for the corresponding dioctadecoxybutyl compound (see above). The product, however, was somewhat soluble in acetonitrile at room temperature, so that precipitation was performed at −10°C. The material as isolated contained several components on TLC.

The crude allylphosphonate was oxidized by the periodate–osmate procedure as in the dioctadecoxybutyl series discussed above. The crude isopropyl octadecyl(2-acetaldo)phosphinate showed a very strong aldehyde band at 1710 cm^{-1}. Reduction as above with sodium borohydride yielded isopropyl octadecyl(2-hydroxyethyl)phosphinate, as evidenced by loss of the aldehyde absorption. The mesylate was similarly prepared as described above for the dioctadecoxybutyl series. It showed strong bands at 1345 and 1120 cm^{-1}, characteristic of sulfonate esters.

Treatment of the sulfonate ester with aqueous dimethylamine, washing the isolated crude product with base to free the tertiary amine, and allowing the latter to react with methyl iodide in the dark for several days as above gave isopropyl octadecyl[2-(trimethylammonium)ethyl]phosphinate iodide, isolable as a precipitate from cool ether at 10–15°C.

The methiodide was hydrolyzed directly, without conversion to the acetate salt, by hydrochloric acid in acetic acid solution at 80°C for 17 hr. The product was isolated as for the above two lecithin analogues, but with precipitation by acetonitrile at −15°C. The product in chloroform was purified by adsorption on SilicAR CC-7 (50 g per 150 mg of product) in a 20-mm (I.D.) column. Chloroform (200 ml), and 5, 15, and 25% methanol in chloroform (150 ml each), eluted impurities, which were present only in small quantities. 200 ml of methanol–chloroform 1:3 eluted a small quantity of an intermediate fraction containing both the desired product and impurities, and the pure product was eluted with 180 ml of chloroform–methanol–water 65:25:4. For analysis the product was recrystallized twice from chloroform–acetone.

Before chromatography the yield of product was 30% over-all from the allylphosphinate; about 25% of the applied material was lost during the chromatographic purification. The pure product melted at 212–213°C with decomposition. Although the product appeared to be hygroscopic, careful drying at 100°C in a nitrogen stream and in vacuo gave a correct analysis for an *anhydrous* product.

Analysis: $C_{23}H_{50}NO_2P$ (mol wt 403.63);
calculated: C, 68.44; H, 12.49; N, 3.47; P, 7.62
found: C, 68.51; H, 12.49; N, 3.61; P, 7.35

The infrared spectrum of this substance is discussed below.

Infrared Spectra of Lecithin Analogues

A curious and unexpected phenomenon was observed (2) in the infrared spectrum of the previously reported phosphinate lecithin analogue, 2-hexadecoxy-3-octadecoxypropyl[2′-(trimethylammonium)ethyl]phosphinate. As ordinarily obtained after acid hydrolysis, the compound does not possess any strong absorptions around 1000–1050 cm^{-1}, and the P → O absorption centered around 1280 cm^{-1} is rather broad and shallow and merges with the broader and much stronger absorption at 1190–1120 cm^{-1}. When this compound in any of a variety of solvents (chloroform–methanol mixtures, aqueous tetrahydrofuran, chloroform–ethanol–water, etc.) was adsorbed and then eluted from silicic acid, or passed through ion-exchange resins or even Sephadex LH-20, the reisolated compound showed dramatically different infrared absorptions in the regions noted. The strong absorption around 1100 cm^{-1} became much weaker and was now bracketed by two much stronger and moderately sharp absorptions at 1040 and 1160 cm^{-1}.

We have now found that this change is more simply effected by warming the material in aqueous pyridine for a few hours or in tetrahydrofuran–aqueous ammonia for several hours at room temperature. Furthermore, it was freely reversible; by allowing material containing these new peaks to stand overnight in acetic acid containing a little hydrochloric acid, removing the acids in vacuo, adding pyridine, and reprecipitating with cold acetonitrile, the original material was obtained again.

The same transformation was observed for the dialkoxybutyl analogue II. The infrared spectrum (Fig. 1, *A*) of this compound was not altered after redissolving it in warm chloroform–pyridine 3:1, adding 5 volumes of cold acetonitrile, and reisolating the reprecipitated product by filtration. Nonetheless, the following treatment is an example of a procedure which effected the

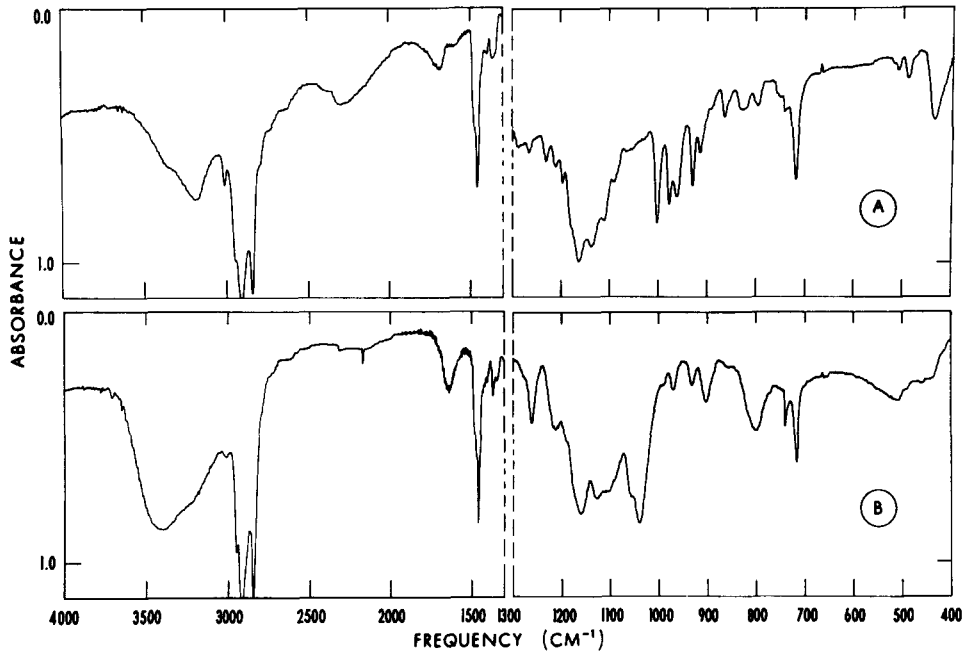


FIG. 1. *A*, spectrum of 3,4-dioctadecoxybutyl[2′-(trimethylammonium)ethyl]phosphinate (II) after acidic treatment; *B*, spectrum after basic treatment. Conditions are given in text.

transformation: The lecithin analogue II (unrecrystallized; 125 mg) in 15 ml of methylene chloride–methanol 19:1 was applied to a 20-mm (I.D.) column containing 20 g of SilicAR CC-4 previously washed with the same solvent. Methylene chloride–methanol 19:1 (40 ml) and methylene chloride–absolute ethanol 9:1 (100 ml) eluted only trace impurities. Chloroform–methanol–water 65:25:4 (120 ml in 10-ml fractions) eluted, successively, 14 mg of impurities, 14 mg of unchanged analogue, and 90 mg of material of altered infrared spectrum (Fig. 1, *B*).

Alternative procedures for producing the form of spectrum *B*, Fig. 1, were aqueous ammonia (30°C for 3 hr in tetrahydrofuran–58% ammonia 9:1) and aqueous pyridine (50°C overnight in tetrahydrofuran–pyridine–water 5:3:2), as mentioned above. In either case the product was isolated by evaporation of solvents in vacuo. Passage of the spectrum *A* form of analogue II in chloroform–ethanol–water 1:7:3 through an equimolar mixture of Amberlite 1R-120 (H^+) and 1R-45 (free base), followed by elution with the same solvent, evaporation in vacuo, reevaporation with isopropanol, and drying of the product gave the spectrum *B* form.

Spectra of this and the other analogues studied were initially taken in KBr pellets. The essential spectral differences between two forms of the same analogue were, however, preserved when the spectra were examined in chloroform solution.

Approximately the same effects were seen in the case of 2-hexadecoxy-3-octadecoxypropyl[3′-(trimethylammonium)propyl]phosphinate (analogue III). The spectra without and with peaks are seen in Fig. 2, *C* and *D*, respectively. Some indication was observed that the transformation may not be quite as facile with the 3-carbon base analogue as with the above 2-carbon base compounds, since fractions which were intermediate in spectral characteristics between the two forms were obtained from a silica column. Nevertheless, the transformation between the two forms took place quite readily.

The elemental analysis given above for this lecithin analogue was obtained on the silica-treated form, which showed peaks at 1045 and 1150 cm^{-1}. This compound, on reconversion to the spectrum *C* form, nonetheless had the same elemental composition.

Analysis: $C_{43}H_{90}NO_4P + H_2O$ (mol wt 734.18);
calculated: C, 70.35; H, 12.63; N, 1.91; P, 4.22
found: C, 70.11; H, 12.83; N, 1.80; P, 3.91

Some differences in the melting points were observed; form *C* melted with decomposition at 204–206°C, while form *D* melted with decomposition at 192–194°C. The TLC behavior of both was identical; the two forms could not be separated from a mixture.

The possibility was considered that the ether groups were in some way involved in these changes, since the region around 1100 cm^{-1}, which shows considerable difference between the two forms, is in a range where ether absorptions occur. For this reason the hydrocarbon

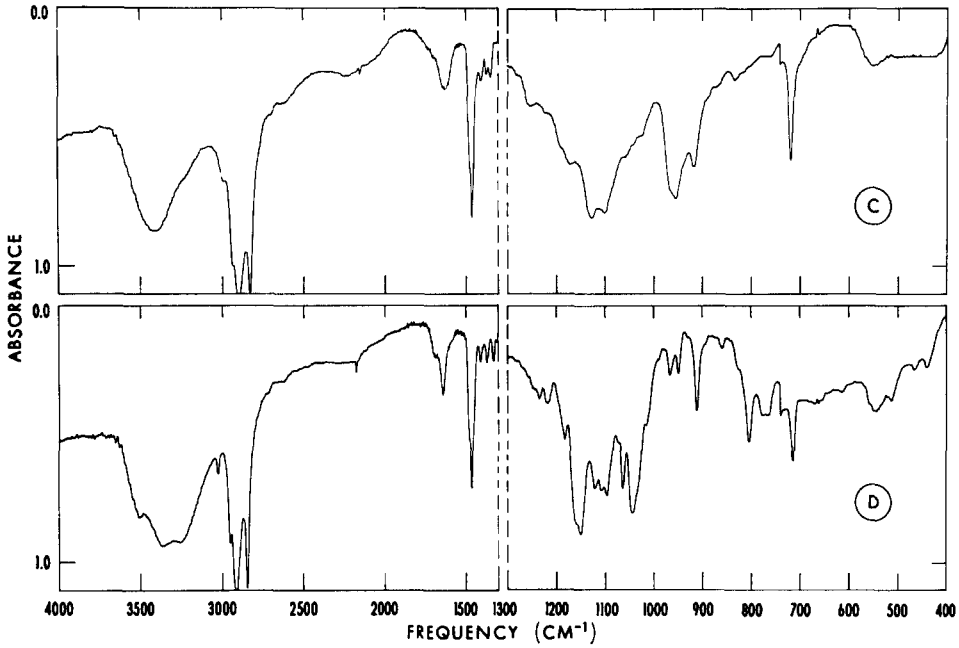


FIG. 2. *C*, spectrum of 2-hexadecoxy-3-octadecoxypropyl[3′-(trimethylammonium)propyl]phosphinate (III) after acidic treatment; *D*, spectrum after basic treatment. Conditions are given in text.

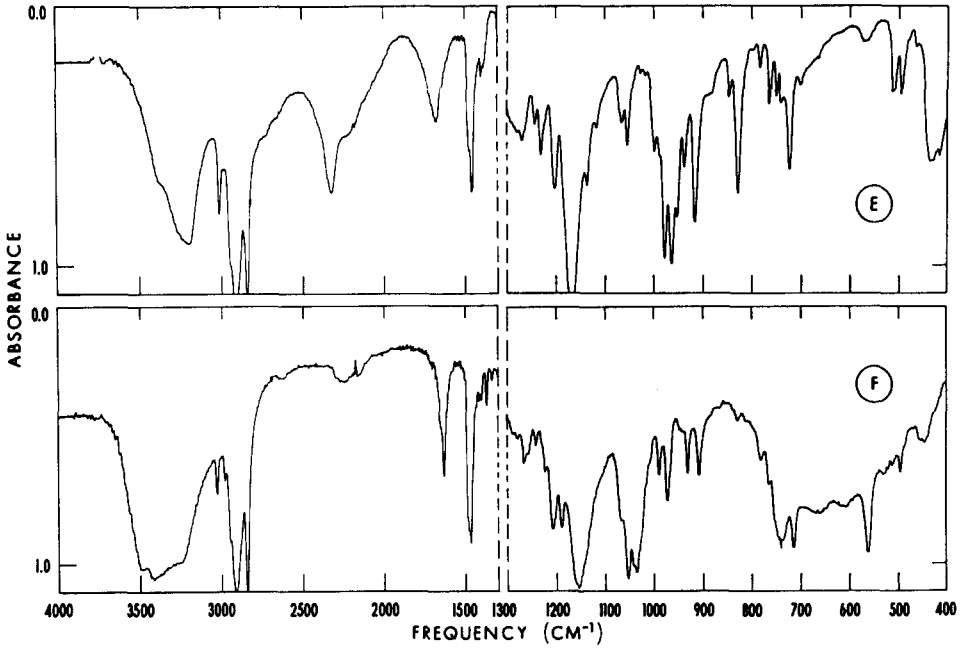


FIG. 3. *E*, spectrum of octadecyl[2′-(trimethylammonium)ethyl]phosphinate (IV) after acidic treatment; *F*, spectrum after basic treatment. Conditions are given in text.

analogue octadecyl[2′-(trimethylammonium)ethyl]phosphinate was synthesized. The changes in its infrared spectrum were studied after its isolation under the different conditions outlined above. Essentially the same spectral alterations were observed as with the ether-containing phosphinate analogues. The two spectra of the hydrocarbon analogue are shown in Fig. 3, *E* and *F*.

A commercial diether phosphate lecithin, 2,3-dihexadecylglycerylphosphorylcholine, showed some variation in its absorption peaks at 970 and 1245 cm^{-1} after the acid treatment; a sharper peak at 1265 cm^{-1} appeared while the 970 cm^{-1} absorption became much weaker. These changes, however, are much less pronounced than those seen in the phosphinate analogues.

Intermediate in structure between the phosphinate and phosphate analogues is the phosphonate analogue (2-hexadecoxy-3-octadecoxy)propylphosphonylcholine (3). Its acid-induced spectral transformation was also more or less intermediate between the phosphinate and phosphate analogues. The strong absorption at 1210 cm^{-1} virtually disappeared after acid treatment, while the complex medium-width absorption at 1060–1100 cm^{-1} was replaced by a broader absorption in the same region with three distinct peaks at 1055, 1097, and 1125 cm^{-1}. The original sharp, strong peak at 970 cm^{-1} became stronger but broader. As in the case of all the lecithin-type structures studied, these alterations were completely reversible.

Various explanations were considered for these transformations, which at least in the case of the phosphinates were remarkable in degree. The most obvious possibility was that the acid-treated compounds were simply the free phosphinic acid–chloride salt forms. This seems unlikely in view of the careful removal of excess hydrochloric acid at 20 mm and then below 1 mm, followed by addition of excess pyridine to a concentrated chloroform solution of the products before isolation by precipitation with acetone or acetonitrile. Moreover, the spectral shifts observed are not in accord with the changes seen in simple acid–salt interconversions of phospholipids, which have been studied in detail by Abramson, Norton, and Katzman (7). These authors stress that P—OH absorptions occur not only in the 2700 cm^{-1} region but also at 1000–1040 cm^{-1} and perhaps at 1250–1220 cm^{-1}. In the salt forms, the $P—O^-$ absorption occurs at 1110–1090 cm^{-1}. In our compounds the single most remarkable change is the appearance of a strong, rather sharp band at about 1040 cm^{-1}, where little absorption was seen before, after treatment with weak bases (which should ionize any P—OH groups present) or adsorbents. In other words, the spectral shift in this region is just opposite to that which would be predicted if simple ionization were taking place. Differences between the two forms in the 2700 cm^{-1} region, moreover, are very minor, while variations in the 1250–1220 cm^{-1} region are not consistent from compound to compound (but in compound II are again opposite to the predicted variation).

We tentatively suggest that these forms represent different conformational structures, possibly involving different orientations of hydrogen-bonded hydration water. Conceivably, one form may be a quasi-cyclic structure analogous to the *gauche* form proposed by Sundaralingam (8) for phosphate lecithins, and the other may be a more or less "*trans*" or open-chain structure. Differences in hydrogen bonding is suggested because the greatest changes occur in the absorption region of $P \rightarrow O$, whose position is known to be very susceptible to shifts produced by hydrogen bonding (9). Further physical studies are of course necessary to clarify these alterations.

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