# New, partially hydrolyzable synthetic analogues of lecithin, phosphatidyl ethanolamine, and phosphatidic acid

ARTHUR F. ROSENTHAL

Department of Laboratories, The Long Island Jewish Hospital, New Hyde Park, New York

ABSTRACT The synthesis of two new synthetic analogues of lecithin, two of phosphatidyl ethanolamine ("cephalin"), and one new phosphatidic acid analogue is described. They comprise one of each of the following types: the "isosteric" diether lecithin and cephalin analogues $ROCH_2CH(OR)$-$CH_2CH_2P(O)(O^-)OCH_2CH_2N^+R'_3$ ($R = C_{18}H_{37}$; $R' = H$ or $CH_3$); and the "hydrocarbon" analogues of phosphatidic acid, lecithin, and cephalin, $C_{17}H_{35}CH_2CH(C_{18}H_{37})CH_2P(O)(R) = (R')$; $[R = R' = OH; R = O^-, R' = OCH_2CH_2N^+(CH_3)_3$; and $R = O^-, R' = OCH_2CH_2N^+H_3]$. Infrared spectra and other properties of these compounds are described.

UNTIL RECENTLY the list of synthetic structural analogues of the natural glycerophosphatides has been quite small. Within the last two years, however, syntheses have been reported of a number of such analogues, which have in common the substitution of a phosphonic acid moiety for one of the phosphoric ester groups found in the natural phosphatides, with or without other structural modifications. The stimulus for this work appears to have been the finding of phosphonate-containing lipids in nature (1–4) and, coincidentally, interest in the hydrolytically stable C–P bond as a constituent of potential inhibitors of phospholipid hydrolases.

The reported phosphonate analogues may be grouped into two categories, according to which of the two P–O–C bonds of a major natural glycerophosphatide, e.g. lecithin, has been replaced: (a) glycerol derivatives of an aminoethylphosphonic acid [or (trialkylammonium)ethylphosphonic acid], and (b) derivatives of a dihydroxyalkylphosphonic acid [e.g., $CH_2(OH)CH(OH)CH_2P(O)(OH)_2$].

Known synthetic representatives of type (a) include 2-aminoethylphosphonate analogues of "cephalins" (Fig. 1, A; 5–8); of diether cephalins (Fig. 1, B; 9) and of lecithins (Fig. 1, C; 10, 11). Phosphatidic acid analogues of this type are, of course, impossible. Of type (b) are known the diether phosphatidic acid analogues 2,3-dialkoxypropylphosphonic acids (Fig. 1, D; 12) and an "isosteric" 2,3-dialkoxybutylphosphonic acid (Fig. 1, E; 13); the "cephalin" (Fig. 1, F) and lecithin (Fig. 1, G) analogues (2,3-dialkoxypropylphosphonyl)-2'-aminoethanol (8) and 2,3-dialkoxypropylphosphonyl choline[1] respectively.

This paper describes the synthesis of the diether cephalin and lecithin analogues, 2-aminoethyl 3',4'-dioctadecoxybutylphosphonate and 3,4-dioctadecoxybutylphosphonyl choline (Fig. 1, H and I respectively; $R = C_{18}H_{37}$), which are isosteric with the natural phosphatides ($-CH_2-P$ substituted for the glycerophosphoryl $-O-P$) in their ionic moieties.

The previously reported 3,4-dioctadecoxybutylphosphonate (13) was an attractive starting material for these syntheses, and therefore attention was directed toward the most satisfactory conditions for its monoesterification. If excess choline iodide in pyridine solution was used for the synthesis of the lecithin analogue, p-toluenesulfonyl chloride did not give complete esterification of the phosphonic acid. Trichloroacetonitrile (14), on the other hand, promoted complete reaction of the acid but gave a product contaminated with dark pigments which could be removed only by a tedious column chromato-

---

[1] Rosenthal, A. F., and M. Pousada, unpublished work.

A.
$$CH_2OCOR$$
$$CHOCOR$$
$$CH_2O\overset{O}{\underset{O^-}{P}}—CH_2CH_2\overset{+}{N}H_3$$

B.
$$CH_2OR$$
$$CHOR$$
$$CH_2O\overset{O}{\underset{O^-}{P}}CH_2CH_2\overset{+}{N}H_3$$

C.
$$CH_2OCOR$$
$$CHOCOR$$
$$CH_2O\overset{O}{\underset{O^-}{P}}—CH_2CH_2\overset{+}{N}(CH_3)_3 \quad \cdot H_2O$$

D.
$$CH_2OR$$
$$CHOR$$
$$CH_2\overset{O}{P}(OH)_2$$

E.
$$CH_2OR$$
$$CHOR$$
$$CH_2CH_2\overset{O}{P}(OH)_2$$

F.
$$CH_2OR$$
$$CHOR$$
$$CH_2\overset{O}{\underset{O^-}{P}}—OCH_2CH_2\overset{+}{N}H_3$$

G.
$$CH_2OR$$
$$CHOR$$
$$CH_2\overset{O}{\underset{O^-}{P}}—OCH_2CH_2\overset{+}{N}(CH_3)_3 \quad \cdot H_2O$$

H.
$$CH_2OR$$
$$CHOR$$
$$CH_2CH_2\overset{O}{\underset{O^-}{P}}—OCH_2CH_2\overset{+}{N}H_3$$

I.
$$CH_2OR$$
$$CHOR$$
$$CH_2CH_2\overset{O}{\underset{O^-}{P}}—OCH_2CH_2\overset{+}{N}(CH_3)_3 \quad \cdot H_2O$$

J.
$$CH_2CH_2C_{16}H_{33}$$
$$CHCH_2C_{17}H_{35} \quad \text{or} \quad C_{18}H_{37}CHCH_2\overset{O}{P}(OH)_2$$
$$CH_2\overset{O}{\underset{OH}{\underset{OH}{P}}} \qquad C_{18}H_{37}$$

K.
$$C_{18}H_{37}CHCH_2\overset{O}{P}—OCH_2CH_2\overset{+}{N}H_3$$
$$\underset{C_{18}H_{37}}{} \quad O^-$$

L.
$$C_{18}H_{37}CHCH_2\overset{O}{P}—OCH_2CH_2\overset{+}{N}(CH_3)_3$$
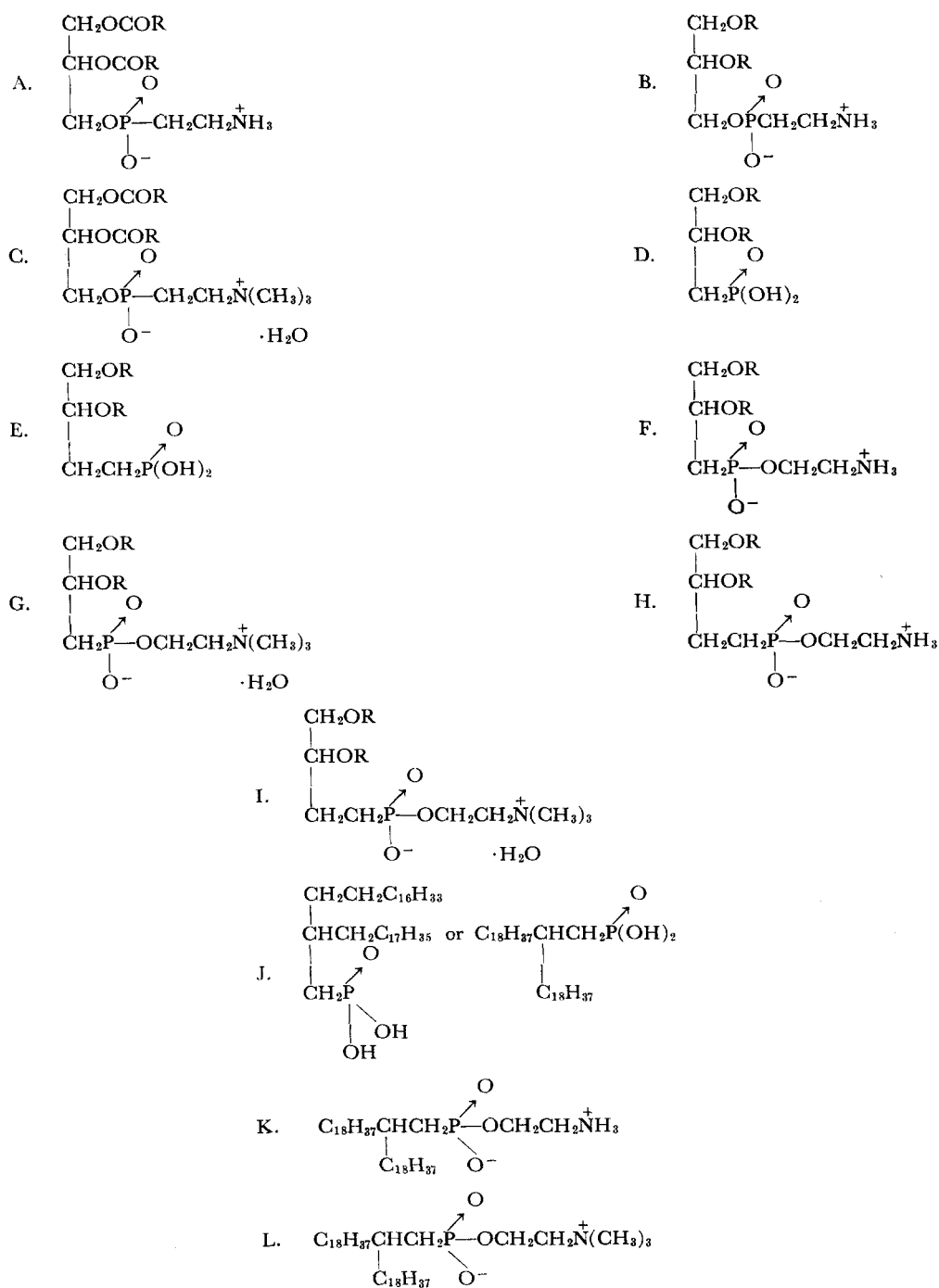$$\underset{C_{18}H_{37}}{} \quad O^-$$

FIG. 1. Phosphonate-containing phosphatide analogues.

graphic separation. Substitution of choline p-toluenesulfonate, however, for the iodide yielded a much lighter colored product with trichloroacetonitrile as condensing agent.

The tosylate salt had the additional advantage that it is much more soluble in pyridine than is the iodide. Further, residual pigments could be almost completely removed by passage of the product in aqueous tetrahydrofuran solution through the monobed resin Amberlite MB-3. The pure lecithin analogue could then be obtained in good yield by simple recrystallization of the decolorized product.

The preparation of the cephalin analogue was even more straightforward; the intermediate phthalyl cephalin, prepared by condensation of 2-hydroxyethylphthalimide with the phosphonic acid in the presence of trichloroacetonitrile, showed no tendency to retain pigments. Removal of the protective phthalyl group with

hydrazine in isopropanol proceeded smoothly to give the cephalin analogue in over 70% over-all yield from the starting acid.

In the course of another investigation the need arose for a phosphatidic acid analogue containing both a phosphonate group and completely nonpolar bridges to the long-chain moieties. Thus the synthesis of the "hydrocarbon" phosphatidate analogue 2-octadecyleicosylphosphonic acid (Fig. 1, J) was undertaken.

The known 2-octadecyleicosanoic acid (15) was smoothly reduced by diborane (16) to 2-octadecyleicosanol, whose tosylate ester was also readily prepared. Reaction of the tosylate with sodium diethyl phosphite, followed by acid hydrolysis produced the desired phosphonic acid (Fig. 1, J), but in only moderate yield. No significant improvement in yield was obtained by the use of potassium diethyl phosphite, and reaction of a crude 2-octadecyleicosyl iodide with triethyl phosphite gave even lower yields.

2-Octadecyleicosylphosphonic acid served as a convenient starting material for the syntheses of the corresponding cephalin (Fig. 1, K) and lecithin (Fig. 1, L) analogues, in which the long-chain groups contain no oxygen functions. These "hydrocarbon" cephalin and lecithin analogues were prepared by methods very similar to those used in the synthesis of the corresponding "isosteric" analogues. The cephalin and lecithin analogue were obtained in 68 and 87% yields, respectively, from the starting phosphonic acid, or 21 and 27% overall for the six and five steps, respectively, from 2-octadecyleicosanoic acid.

It is of interest that, unlike most lecithins and their analogues, 2-octadecyleicosylphosphonyl choline could be obtained either as a monohydrate, or, by careful drying, in an anhydrous form. This suggests that the "HOH" of the lecithin dipolar ion may not be an essential structural element, but only tightly bound water of crystallization.

The infrared spectra of the two lecithin and two cephalin analogues are shown in Fig. 2; their lack of ester bonds allows interesting comparisons to be made with the spectra of the corresponding natural phosphatides. In their recent study Abramson, Norton, and Katzman (17) have suggested certain modifications in the band assignments usually made for phosphatides, particularly their polar moieties. The view of these authors that the band at 1175–1160 $cm^{-1}$ found in natural phosphatides should be assigned to ester C–O–C and not to P–O–C is borne out in the present work; only the isosteric lecithin (Fig. 2, part A) of the four analogues has any absorption at all in this region, and it is quite weak. The strong P–O–C absorption assigned by the above authors as 1070–1055 $cm^{-1}$ appears at 1060 $cm^{-1}$ in the isosteric lecithin analogue (Fig. 2, part A), at 1070 $cm^{-1}$

in the isosteric cephalin (Fig. 2, part B), at 1061 $cm^{-1}$ in the "hydrocarbon" lecithin (Fig. 2, part C) and at 1071 $cm^{-1}$ in the "hydrocarbon" cephalin (Fig. 2, part D). The strong P→O band, assigned at 1250–1220 $cm^{-1}$ by Abramson et al., appears in a lower frequency region for all the present analogues except for the hydrocarbon lecithin. This suggests an appreciably greater hydrogen bonding of P→O in these substances than in natural phosphate phosphatides.

More intriguing is the assignment of the ionic P–O⁻ bands and with it the question of the ionization state of solid cephalins. A strong peak is seen for the two lecithin analogues in the region assigned (1110–1090 $cm^{-1}$) by Abramson et al. to ionic P–O⁻. A weaker peak at 1090 $cm^{-1}$ is seen for the isosteric cephalin, but none at all in the spectrum of the hydrocarbon cephalin. The two cephalins, but not the lecithins, show strong bands at 1000 $cm^{-1}$, a region assigned by the above authors to a P–O–H absorption. Likewise the cephalins, but not the lecithins, show the characteristic broad P–OH absorption at 2720–2545 $cm^{-1}$. It may thus be concluded that the isosteric cephalin exists as a mixture of ionized and unionized forms, whereas the hydrocarbon cephalin exists primarily in an unionized form. Support for this view arises from the recent work of Wren and Merryfield (18), who provide evidence that the absorption at about 1550 $cm^{-1}$ is due to N⁺H₃. This band is much weaker in the spectrum of the hydrocarbon cephalin than in that of the isosteric cephalin.

A question may also be raised as to the origin of the moderately weak but very distinct band found at 1610–1655 $cm^{-1}$ for all of the phosphatide analogues herein reported. As discussed by Wren and Merryfield, a peak is found in this region in most cephalins, attributable to an unionized –NH₂; but this could not explain its presence in the lecithin analogues. Careful examination of the spectrum of a natural egg lecithin, in fact, shows an absorption shoulder in this region almost masked by nearby stronger absorptions. In our experience, a band in this region is seen in the spectrum of a variety of organic phosphorus compounds, whether or not they contain phosphoric ester, ionic, nitrogen functions, carbon–phosphorus, or phosphorus–hydrogen bonds.

In varying degrees, the lecithin and cephalin analogues whose syntheses are reported in this paper show activity as inhibitors of phospholipases A and C. These effects are under investigation at present.

## EXPERIMENTAL DETAILS

### Materials and Methods

3,4-Dioctadecoxybutylphosphonic acid was prepared as previously described (13). 2-Octadecyleicosanoic acid was prepared by a two-step alkylation of diethyl mal-
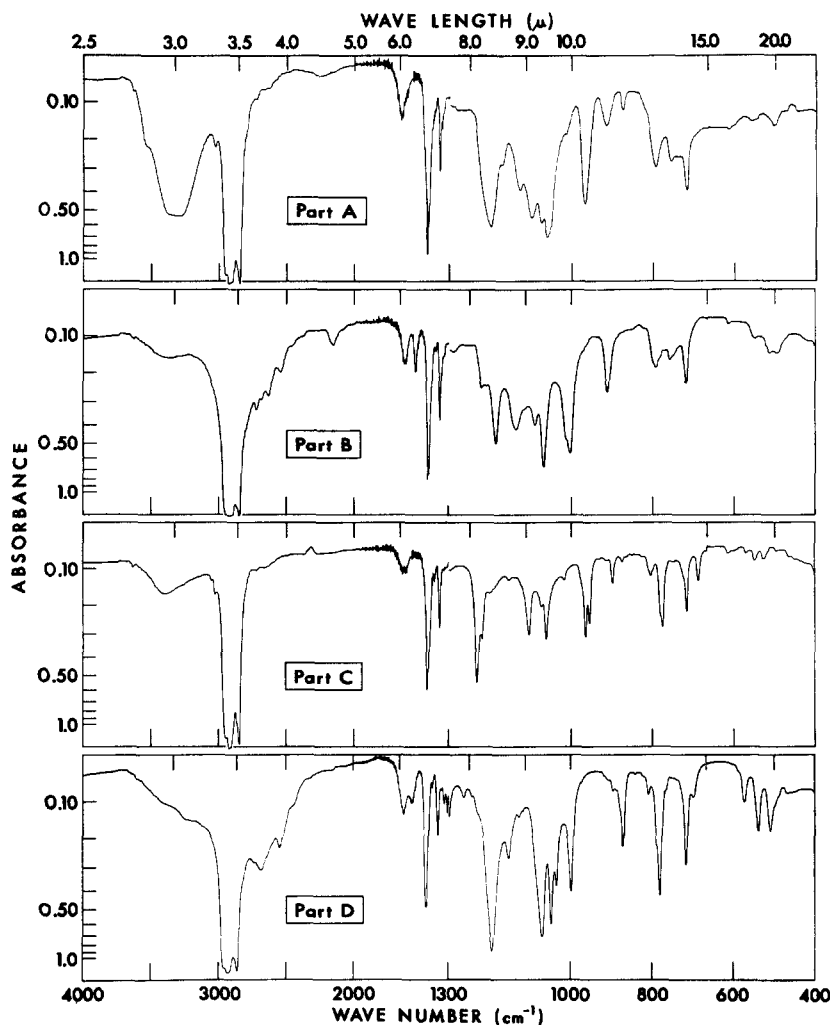
FIG. 2. Infrared spectra of four phosphonate-containing phosphatide analogues. Part A: isosteric lecithin analogue, 3,4-dioctadecoxybutylphosphonyl choline; part B: isosteric cephalin analogue, 2-aminoethyl 3',4'-dioctadecoxybutylphosphonate; part C: hydrocarbon lecithin, 2-octadecyleicosylphosphonyl choline; part D: hydrocarbon cephalin, 2-aminoethyl 2'-octadecyleicosylphosphonate. All spectra were taken as Nujol mulls.

onate followed by hydrolysis and decarboxylation as described by Adam and Dyer (15), except that anhydrous n-propanol was used as a solvent for the alkylations rather than a mixture of ethanol and ether. Choline p-toluenesulfonate was prepared by treatment of 2-dimethylaminoethanol in either with methyl p-toluenesulfonate overnight, and crystallization of the precipitated product from acetone; the hygroscopic salt was stored over CaSO₄. Other materials were commercial products.

Elemental microanalyses were performed by Schwartzkopf Microanalytical Laboratories, Inc., Woodside, N. Y.

Compounds containing phosphonate phosphorus were made visible on thin-layer chromatograms as previously described (12).

Infrared spectra were obtained in KBr discs or as Nujol mulls on a Perkin-Elmer model 337 infrared spectrometer.

### 3,4-Dioctadecoxybutylphosphonyl Choline

3,4-Dioctadecoxylbutylphosphonic acid (674 mg, 1.00 mmole) and choline p-toluenesulfonate (2.0 g) were dissolved in a mixture of pyridine (15 ml) and trichloroacetonitrile (4 ml) at 50°C., and the solution was kept at this temperature for 48 hr. About two-thirds of the volatile material was carefully removed in vacuo and the product was precipitated by addition of acetonitrile (50 ml). Filtration and washing with acetonitrile yielded a tan product, which was dissolved in tetrahydrofuran–water 7:3 at 30°C (100 ml) and passed through a short

column of Amberlite MB-3 previously equilibrated with the same solvent (flow rate, about 5 ml/min). The resin was washed with a further 500 ml of aqueous tetrahydrofuran and the combined eluates were taken to dryness in vacuo.

The decolorized product was recrystallized twice from hexane (50°–18°C) and then from trichloroethylene–acetone (ca. 1:2). The final yield of white crystalline material was 490 mg (63%).

The isosteric lecithin softens slightly above 120°C, becomes partially transparent above 150°C, browns slightly at 190–195°C, and finally melts sharply at 200.5–201.5°C. In chloroform–methanol–water 65:25:4 and in 10% trifluoroacetic acid in anhydrous, alcohol-free chloroform on Silica Gel G plates it ran as a single spot; $R_f$ values 0.43 and 0.53 respectively. The substance is very soluble in chloroform, warm trichloroethylene, and hexane–isopropanol, moderately soluble in warm hexane, poorly soluble in methanol, and insoluble in acetone and acetonitrile. The substance gave a correct analysis for a monohydrate:

Analysis: $C_{45}H_{96}NO_6P$;
  calculated: C, 69.45; H, 12.43; N, 1.80; P, 3.98
  found: C, 69.40; H, 12.69; N, 2.00; P, 3.84

The infrared spectrum of the isosteric lecithin analogue is given in Fig. 2, part A.

### 2-Phthalimidoethyl 3′,4′-dioctadecoxybutylphosphonate

3,4-Dioctadecoxybutylphosphonic acid (337 mg, 0.500 mmole) and 2-hydroxyethylphthalimide (500 mg) were dissolved in dry pyridine (5 ml) and trichloroacetonitrile (2 ml) and the solution was kept at 50°C for 48 hr. The product obtained by addition of acetonitrile (30 ml) was filtered off and washed well with acetonitrile.

The free acid was obtained by dissolving the pyridinium salt in chloroform (2 ml), adding trifluoroacetic acid (0.5 ml), and reprecipitating the product with acetonitrile; yield, 400 mg (94%). The filtered and washed product was recrystallized from boiling acetone and from acetone–hexane. The melting point of the phthalyl cephalin analogue was 70–71°C; it was chromatographically homogeneous in chloroform–trifluoroacetic acid 9:1 on Silica Gel G ($R_f$ 0.64).

Analysis: $C_{50}H_{90}NO_7P$;
  calculated: C, 70.80; H, 10.69; N, 1.65; P, 3.65
  found: C, 70.89; H, 10.58; N, 1.70; P, 3.70
Neut. equiv.: calcd, 848; found, 849

Pertinent features of the infrared spectrum are: strong sharp bands at 1760 and 1710 cm$^{-1}$ (imide carbonyl); moderate, broad absorption at 2750–2600 cm$^{-1}$ (P–OH), and a strong and rather complex ether and phosphorus pattern at 1140–990 cm$^{-1}$.

### 2-Aminoethyl 3′,4′-dioctadecoxybutylphosphonate

2-Phthalimidoethyl 3′,4′-dioctadecoxybutylphosphonate (380 mg, 0.45 mmole) was dissolved in isopropanol (15 ml) at 60°C; hydrazine hydrate (0.5 ml; 99%) was added and the solution was kept at 60°C for 12 hr.

The copious precipitate was filtered off and washed well with isopropanol. The solid was then stirred with chloroform–acetic acid 9:1 and filtered at 45°C through a Celite mat, which was then washed well with warm chloroform. The residue obtained by evaporation of the filtrate and washings was crystallized from chloroform–acetone; yield, 240 mg (75%). The cephalin analogue was further crystallized from trichloroethylene–acetonitrile. The substance softens slightly above 110°C, browns slowly above 140°C, and melts at 162.5–163.5°C. It is soluble in warm chloroform and trichloroethylene, in chloroform–methanol, and in isopropanol–trichloroethylene at room temperature. It is poorly soluble in ethanol and methanol and insoluble in acetone and acetonitrile.

Analysis: $C_{42}H_{88}NO_5P$;
  calculated: C, 70.24; H, 12.35; N, 1.95; P, 4.31
  found: C, 69.98; H, 12.34; N, 2.05; P, 4.33

On Silica Gel G plates 2-aminoethyl 3′,4′-dioctadecoxybutylphosphonate ran as in single spot in chloroform–methanol–water 65:25:4 ($R_f$ 0.67) and in chloroform–trifluoroacetic acid 9:1 ($R_f$ 0.54). The infrared spectrum of the isosteric cephalin analogue is shown in Fig. 2, part B.

### 2-Octadecyleicosanol

2-Octadecyleicosanoic acid (15), mp 80–82°C (17.0 g, 0.030 mole), in tetrahydrofuran (200 ml) was treated at room temperature with a stream of diborane prepared from sodium borohydride (3.6 g, 0.09 mole) and boron fluoride etherate (13.5 ml) in diglyme (75 ml). When all the diborane had been added the mixture was allowed to stand at room temperature for 15 hr. Excess borane was destroyed by careful addition of 95% ethanol followed by a few milliliters of 3 N HCl. The solvent was removed in vacuo, the residue shaken with ether and N HCl, the ethereal solution dried over $MgSO_4$ and filtered, the ether removed in vacuo, and the final residue crystallized from acetone. The yield was 14.9 g (90%), mp 59–59.5°C. If the HCl treatment of the diborane reduction mixture is omitted, a substance, mp 54–56°C, apparently a borate ester, is obtained as the main product.

2-Octadecyleicosanol has $R_f$ 0.60 in chloroform and 0.40 in toluene on Silica Gel G. The substance gives a relatively simple infrared spectrum containing strong alcoholic OH (3320 cm$^{-1}$) and the usual C–C and C–H absorptions.

Analysis: $C_{38}H_{78}O$;

calculated: C, 82.83; H, 14.27; O, 2.90
found: C, 82.63; H, 14.11; O, 3.00

### 2-Octadecyleicosyl Tosylate

2-Octadecyleicosanol (1.10 g, 0.0020 mole) was dissolved in pyridine (5 ml) and p-toluenesulfonyl chloride (1.0 g) was added. The mixture was kept at 17°C for 12 hr, then poured into a mixture of 3 N HCl and ice. The solid was filtered and air-dried. The product was dissolved in warm acetone and the solution allowed to stand at 18°C for a few hours. The small precipitate was filtered off isothermally, and the filtrate was evaporated in vacuo. The residue was crystallized from acetone–acetonitrile at 18°C; yield, 1.2 g (85%) of product of mp 57.5–58.5°C. For analysis the product was decolorized with Norit A in hexane solution, and the residue obtained by filtration and evaporation was crystallized several more times from acetone–acetonitrile. The final product had mp 59–60°C; $R_f$ 0.88 in chloroform and 0.80 in toluene on Silica Gel G.

Analysis: $C_{45}H_{84}SO_3$;

calculated: C, 76.64; H, 12.01; S, 4.54
found: C, 76.60; H, 12.00; S, 4.85

The infrared spectrum of 2-octadecyleicosyl tosylate shows the many strong sharp bands characteristic of tosylate esters, e.g., 1190, 1378, and 661 cm$^{-1}$.

### 2-Octadecyleicosylphosphonic acid

Sodium hydride dispersion (50%; 3.0 g; 0.06 mole) was deoiled with hexane. The hydride was suspended in anhydrous tetrahydrofuran (80 ml) and dissolved by portionwise addition of redistilled diethyl hydrogen phosphite (12 ml) with stirring under reflux. To the solution was added 2-octadecyleicosyl tosylate (7.05 g, 0.010 mole) and the mixture was heated under reflux for 12 hr.

The solvent was evaporated in vacuo and the residue was extracted with ether and N HCl. The ethereal solution was washed with water and dried over $MgSO_4$ and the solvent was removed in vacuo.

To the residue was added propionic acid (80 ml) and 47% hydrobromic acid (15 ml). The mixture was heated under reflux for 16 hr. As much solvent as possible was evaporated in vacuo, the residue was precipitated by addition of water, and the preciitate was filtered and air-dried. The crude material in hexane solution was decolorized with Norit A, the filtered solution evaporated, and the residue recrystallized three times from hexane–acetone (ca. 1:2) at 18°C. The yield of product, mp 64.5–65.0°C, was 2.45 g (40%). 2-Octadecyleicosylphosphonic acid moved as a single spot on Silica Gel G in chloroform–trifluoroacetic acid 9:1 ($R_f$ 0.67).

Analysis: $C_{38}H_{79}PO_3$;

calculated: C, 74.21; H, 12.95; P, 5.04
found: C, 74.32; H, 12.68; P, 4.80

Neut. equiv.: calcd, 307.5; found, 307

The acid gives a relatively simple infrared spectrum which includes the P–OH bands at 2750–2650 and 995 cm$^{-1}$ (?) and a strong hydrogen-bonded P→O at 1170 cm$^{-1}$. Bands at 1141 and 939 cm$^{-1}$ could not be assigned with certainty.

### 2-Octadecyleicosylphosphonyl Choline

2-Octadecyleicosylphosphonic acid (615 mg, 1 mmole) and choline tosylate (1.0 g) were dissolved in pyridine at 50°C and trichloroacetonitrile (4 ml) was added. The solution was kept at 50°C for 48 hr. About half the pyridine was removed in vacuo and the product was precipitated by addition of acetonitrile (30 ml). The filtered, washed, and dried product weighed 600 mg (87%). It was dissolved in tetrahydrofuran–water 7:3 and passed through a short column of Amberlite MB-3 previously equilibrated with the same solvent. The resin was washed thoroughly with aqueous tetrahydrofuran and the eluates were combined and evaporated in vacuo; water was removed by repeated evaporation with isopropanol. The decolorized product was crystallized from hexane–acetone and from trichloroethylene–hexane.

The hydrocarbon lecithin analogue softens at 170°C and melts at 177–179°C. It is chromatographically homogeneous in chloroform–methanol–water 65:25:4 ($R_f$ 0.51) and in chloroform–trifluoroacetic acid 9:1 ($R_f$ 0.54). It is soluble in ethanol, isopropanol, and chloroform, fairly soluble in warm trichloroethylene, and very insoluble in acetone and acetonitrile.

On drying at 20 mm Hg at room temperature over desiccant silica gel, the hydrocarbon lecithin gave an analysis corresponding to the expected monohydrate.

Analysis: $C_{43}H_{90}NO_3P \cdot H_2O$;

calculated: C, 71.91; H, 12.91
found: C, 71.66; H, 13.19

On drying at 1 mm Hg over phosphorus pentoxide at 40°C for 48 hr, the lecithin analogue then yielded the analysis corresponding to the anhydrous compound:

Analysis: $C_{43}H_{90}NO_3P$;

calculated: C, 73.76; H, 12.95; N, 2.00; P, 4.42
found: C, 73.51; H, 12.83; N, 2.08; P, 4.31

The infrared spectrum in Nujol of the well-dried material (Fig. 2, part C) shows very little water OH (around 3450 cm$^{-1}$) compared to other lecithins and their analogues.

## 2-Phthalimidoethyl 2'-Octadecyleicosylphosphonate

2-Octadecyleicosylphosphonic acid (615 mg, 1 mmole), 2-hydroxyethylphthalimide (1.0 g), pyridine (8 ml), and trichloroacetonitrile (3 ml) were kept at 50°C for 48 hr. The pyridine salt of the phthalyl cephalin was precipitated with acetonitrile (30 ml), filtered, washed, and dried in vacuo. The material was dissolved in a mixture of chloroform (3 ml) and trifluoroacetic acid (0.5 ml); the free acid was precipitated by acetonitrile (20 ml). The filtered and dried product was recrystallized from hexane–acetone at 5°C; yield, 580 mg (74%) of product melting at 56–56.5°C. Several more crystallizations yielded an analytically pure product, homogeneous ($R_f$ 0.77) in chloroform–trifluoroacetic acid 9:1 on Silica Gel G.

Analysis: $C_{48}H_{86}NO_5P$;

calculated: C, 73.15; H, 11.00; N, 1.77; P, 3.93
found: C, 73.33; H, 10.94; N, 1.72; P, 3.88

Neut. equiv.: calcd, 788; found, 781

The infrared spectrum of the phthalyl cephalin analogue showed two imide carbonyl absorptions at 1710 and 1768 cm$^{-1}$, and a very broad P→O absorption at about 1190 cm$^{-1}$. The bands at 1072 and 1020 cm$^{-1}$ probably represent P–O–C and P–OH bands respectively.

## 2-Aminoethyl 2'-Octadecyleicosylphosphonate

2-Phthalimidoethyl 2'-octadecyleicosylphosphonate (300 mg; 0.38 mmole) was dissolved in isopropanol (10 ml) at 50°C and 85% hydrazine hydrate (1.0 ml) was added. After 12 hr at 50°C the mixture was filtered and washed successively with isopropanol, acetonitrile, and acetone. The air-dried precipitate was stirred with chloroform–acetic acid 9:1 and filtered slowly at 40°C through Celite. The insoluble material was washed with chloroform, and the filtrate and washings were evaporated to dryness. The residue was dissolved in chloroform (20 ml), and the solution was again filtered and evaporated. The residue was crystallized from chloroform–acetone; yield, 230 mg (92%). The material was recrystallized from trichloroethylene–hexane and again from chloroform–acetone.

The hydrocarbon cephalin analogue softened above 95°C and melted without browning at 152–153°C. It is the least soluble of the phosphatide analogues herein described; no solvent was found in which the substance was very soluble at room temperature, although it is soluble in warm chloroform and trichloroethylene. It was chromatographically homogeneous in chloroform–methanol–water 65:25:4 ($R_f$ 0.69) and in chloroform–trifluoroacetic acid 9:1 ($R_f$ 0.55) on Silica Gel G.

Analysis: $C_{40}H_{84}NO_3P$;

calculated: C, 73.00; H, 12.87; N, 2.13; P, 4.71
found: C, 73.24; H, 12.85; N, 2.15; P, 4.63

The infrared spectrum of the hydrocarbon cephalin analogue is shown in Fig. 2, part D.

### REFERENCES

1. Horiguchi, M., and M. Kandatsu. *Nature* **184:** 901, 1959.
2. Kittredge, J. S., E. Roberts, and D. G. Simonsen. *Biochemistry* **1:** 624, 1962.
3. Rouser, G., G. Kritchevsky, D. Heller, and E. Lieber. *J. Am. Oil Chemists' Soc.* **40:** 425, 1963.
4. Quin, L. D. *Biochemistry* **4:** 324, 1965.
5. Baer, E., and N. Z. Stanacev. *J. Biol. Chem.* **239:** 3209, 1964.
6. Baer, E., and G. R. Sarma. *Can. J. Biochem.* **43:** 1353, 1965.
7. Rosenthal, A. F., and M. Pousada. *Proc. Chem. Soc.* **no vol:** 358, 1964.
8. Rosenthal, A. F., and M. Pousada. *Rec. Trav. Chim.* **84:** 833, 1965.
9. Baer, E., and N. Z. Stanacev. *J. Biol. Chem.* **240:** 44, 1965.
10. Baer, E., and N. Z. Stanacev. *J. Am. Chem. Soc.* **87:** 679, 1965.
11. Baer, E., and N. Z. Stanacev. *J. Biol. Chem.* **240:** 3754, 1965.
12. Rosenthal, A. F., G. M. Kosolapoff, and R. P. Geyer. *Rec. Trav. Chim.* **83:** 1273, 1964.
13. Rosenthal, A. F. *J. Chem. Soc.* **no vol:** 7345, 1965.
14. Cramer, F., and G. Weimann. *Chem. Ber.* **94:** 996, 1961.
15. Adam, N. K., and J. W. W. Dyer. *J. Chem. Soc.* **127:** 70, 1925.
16. Brown, H. C., and B. C. Subba Rao. *J. Am. Chem. Soc.* **82:** 681, 1960.
17. Abramson, M. B., W. T. Norton, and R. Katzman. *J. Biol. Chem.* **240:** 2389, 1965.
18. Wren, J. J., and D. S. Merryfield. *Nature* **209:** 1315, 1966.