1347. *An Isosteric Non-hydrolysable Phosphatidic Acid Analogue*

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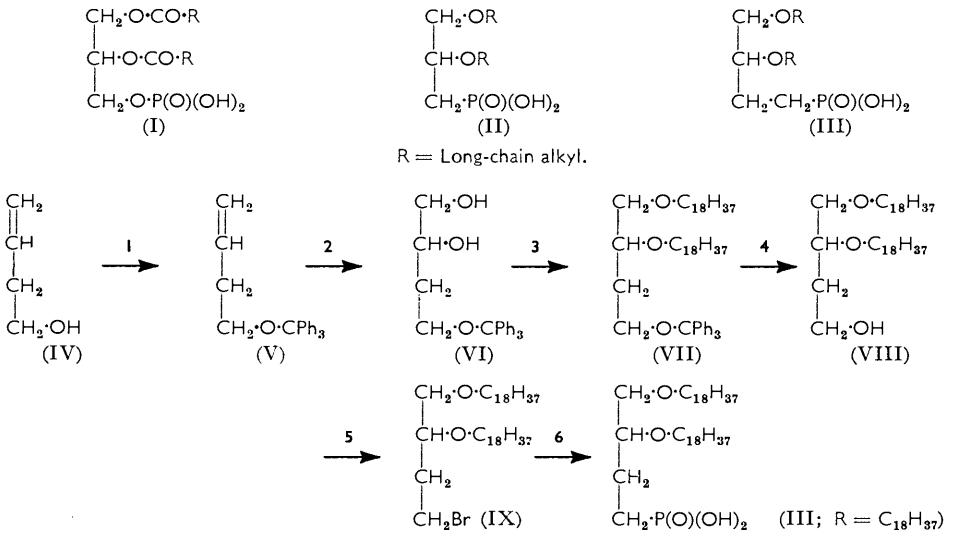
An isosteric diether–phosphonate phosphatidic acid analogue, 3,4-dioctadecyloxybutylphosphonic acid, has been synthesised by the following sequence: but-3-en-1-yltrityl ether → 3,4-dihydroxybutyltrityl ether → 3,4-dioctadecyloxy-1-trityloxybutane → 3,4-dioctadecyloxybutanol → 1-bromo-3,4-dioctadecyloxybutane → diethyl 3,4-dioctadecyloxybutylphosphonate → 3,4-dioctadecyloxybutylphosphonic acid.

The synthesis of analogues of phosphatidic acids (I) has been undertaken with a view to obtaining a specific inhibitor of phosphatidic acid phosphatase, a key enzyme in fat and phospholipid metabolism.[1,2] Only two criteria were possible *a priori* in choosing the types of compounds to be synthesised: a general structural resemblance to the natural substrates, and structures as biologically and chemically stable as possible. The simplest substances satisfying these criteria are obtained by substitution of ether for ester groups and phosphonic acid for phosphoric acid-ester moieties. Accordingly, a series of compounds of structure (II) were prepared,[3] long-chain members of which have been found to be inhibitory toward phosphatidic acid hydrolysis by phosphatidic acid phosphatase.[4]

Compounds of type (II) differ not only in their functional moieties from the natural enzymic substrates, but also sterically, having one less side-chain atom (–O–). In studying the structural specificity of the inhibition, the steric factor could be isolated were there available substances similar to (II) but isosteric with natural phosphatidic acids.



Reagents: 1, Ph_3CBr. 2, $KMnO_4$ 3, $C_{18}H_{37}Br$, KOH, CaH_2. 4, HCl. 5, $(PhO)_3PBr_2$. 6, $NaP(O)(OEt)_2$, $HCl–H_2O$.

Attention has been drawn to the $-CH_2·P(O)(OH)_2$ group as an isosteric analogue of $-O·P(O)(OH)_2$;[5-9] this substitution has the obvious advantages of chemical inertness and

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[3] A. F. Rosenthal, G. M. Kosolapoff, and R. P. Geyer, *Rec. Trav. chim.*, 1964, **83**, 1273.
[4] A. F. Rosenthal and M. Pousada, unpublished results.
[5] B. J. Magerlein and F. Kagan, *J. Amer. Chem. Soc.*, 1960, **82**, 593.
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reasonably close isostericity. Thus, the synthesis of a representative of structure (III), 3,4-dioctadecyloxybutylphosphonic acid (R = $C_{18}H_{37}$), is reported herein.

The synthetic scheme is shown above. The trityl ether of but-3-en-1-ol (V) was prepared readily from the alcohol (IV) and trityl bromide. The acid-lability of the trityl group precluded use of peracid hydroxylation, and reaction with hydrogen peroxide in t-butyl alcohol catalysed by either osmium tetroxide or vanadium pentoxide proved unsuccessful. However, permanganate hydroxylation gave the glycol (VI) in excellent yield, provided that the reaction was performed very slowly at 5° in highly dilute solution.

A number of methods for etherification of the glycol were explored, *e.g.*, 1-bromo-octadecane with potassium t-butoxide in t-butyl alcohol, or with silver oxide in toluene; the most satisfactory reagents found were those of Kates *et al.*,[10] *i.e.*, halide and powdered potassium hydroxide. However, these authors' use of refluxing benzene through a Dean–Stark separator to remove the water formed was in our work attended by excessive foaming. Dioxan just under reflux temperature proved a much more satisfactory solvent. A disadvantage of potassium hydroxide with 1-bromo-octadecane in either procedure is that it leads to extensive formation of dioctadecyl ether. The octadecanol presumably formed intermediately apparently arises by direct S_{N2} attack of OH^- on the bromide rather than by any solvolytic reaction; hardly less dioctadecyl ether is formed when calcium hydride is added to the reaction mixture to remove water, although the yield of the desired triether is slightly improved thereby. Further, both etherifications are apparently much more rapid than formation of octadecanol from the bromide since no appreciable hydroxy-ether or octadecanol is found at the end of the reaction.

The great bulk of the dioctadecyl ether could easily be removed from the triether (VII) by virtue of the much greater solubility of the latter in many solvents; but the last trace of this by-product proved difficult to remove. Fortunately its inertness allowed it to be carried through without difficulty to the final product, from which it was readily removed.

Complete hydrolysis of the trityl protecting group required surprisingly vigorous conditions, and was only accomplished by concentrated hydrochloric acid under several hours' reflux. For analytical purposes 3,4-dioctadecyloxybutanol was freed from a trace of dioctadecyl ether by column chromatography, but this was unnecessary for its utilisation in the succeeding synthetic steps.

Phosphorus tribromide, with or without pyridine, yielded no more than a trace of 1-bromo-3,4-dioctadecyloxybutane (IX) from the diether-alcohol; the main products were always a mixture of phosphites. Excellent results, however, were obtained with dibromotriphenoxyphosphorane,[11] the bromide being formed rapidly and almost quantitatively with no observable by-product formation.

Reaction of the bromide with sodium diethyl phosphite proceeded smoothly. The diethyl phosphonate was not purified but was hydrolysed directly to the desired 3,4-dioctadecyloxybutylphosphonic acid. Overall yield for the seven-step synthesis from but-3-en-1-ol was about 25%. Reaction of the bromide with triethyl phosphite followed by hydrolysis gave an identical product, but in considerably poorer yield.

In initial preliminary work it was found that the reaction sequence could be performed satisfactorily using a benzyl rather than a trityl protecting group. The latter is superior, however, for the following reasons: (1) the trityl intermediates are more easily isolated; (2) the necessity for hydrogenolysis of the benzyl group limits the potential utility of the method to saturated alkyl groups; (3) the trityl intermediates in reaction mixtures are readily observed on thin-layer chromatograms by their intense yellow colour when sprayed with sulphuric acid.

Preliminary studies show that 3,4-dioctadecyloxybutylphosphonic acid is an inhibitor of phosphatidic acid phosphatase; comparison of its inhibitory power with that of 2-hexadecyloxy-3-octadecyloxypropylphosphonic acid [3] will be reported later.

[10] M. Kates, T. H. Chan, and N. Z. Stanacev, *Biochemistry*, 1963, **2**, 394.
[11] D. G. Coe, S. R. Landauer, and H. N. Rydon, *J.*, 1954, 2281.

Experimental

Thin-layer chromatography was performed on silica-gel G-coated plates; phosphorus compounds were visualised as described previously.[3] Trityl compounds are seen as brilliant yellow spots when plates are sprayed with 40% sulphuric acid in the cold, and other organic compounds are observed as char-spots when such plates are heated >200°. Infrared spectra were obtained on a Perkin-Elmer 337 spectrometer.

Trityl But-3-en-1-yl Ether (V).—But-3-en-1-ol (K and K Laboratories, Plainview, N.Y.) (5·0 g., 0·069 mole) in pyridine (25 ml.) was added to a suspension of trityl bromide (22·3 g., 0·069 mole) in anhydrous ethyl acetate (175 ml.). After stirring overnight at 40° the solvents were evaporated and the yellow residual oil taken up in ether and washed several times with water; the ether layer was dried over magnesium sulphate and evaporated. Hexane (100 ml.) was added and the solution was kept at 2° and filtered from a small precipitate of tritanol. The oil obtained by evaporation of the filtrate crystallised from aqueous acetonitrile at 2°, to yield the *product* (16·3 g.), m. p. 53—54°; a further 2·3 g. were obtained by recrystallising the residue from the evaporated filtrate (total yield, 85·5%) (Found: C, 88·1; H, 7·2; O, 4·9. $C_{23}H_{22}O$ requires C, 87·9; H, 7·1; O, 5·1%). The substance was chromatographically homogeneous (R_F 0·90 in 19 : 1 hexane–ethyl acetate).

3,4-*Dihydroxybutyl Trityl Ether* (VI).—Potassium permanganate (15·8 g., 0·100 mole) in water (500 ml.) was added to a solution of but-3-en-1-yltrityl ether (31·6 g., 0·100 mole) in acetone (3·2 l.) dropwise during 9·5 hr. at 5° ± 1°. The product crystallised at 3° after filtration and evaporation of the filtrate to about 1 l. Recrystallisation from aqueous acetonitrile yielded the *product* (31·2 g., 89·5%), m. p. 124·5—126°. Further recrystallisation raised the m. p. to 129—130° (Found: C, 79·3; H, 6·8. $C_{23}H_{24}O_3$ requires C, 79·3; H, 6·9%); periodate consumption, 100·0% of theory. The product was chromatographically homogeneous (R_F 0·70 in ethyl acetate).

3,4-*Dioctadecyloxybutanol* (VIII).—A mixture of 3,4-dihydroxybutyltrityl ether (1·05 g., 0·0030 mole), powdered dry potassium hydroxide (6·0 g.), 1-bromo-octadecane (7·0 g., 0·021 mole), calcium hydride (2·0 g.), and dioxan (35 ml.) was stirred just below reflux for 16 hr. The mixture was filtered through Celite and the filtrate evaporated. The residue was dissolved in warm 3 : 1 acetone–ethyl acetate (50 ml.), kept at 21° overnight, and filtered. Evaporation of the filtrate gave a white powder (1·58 g., 62%) consisting of almost pure triether (VII) but containing a small amount of dioctadecyl ether (R_F 0·44 and 0·63, respectively, in 19 : 1 hexane–ethyl acetate). The acetone–ethyl acetate precipitate was shown to be dioctadecyl ether by infrared and chromatographic comparison with an authentic specimen.

The triether (1·00 g., 0·0017 mole) in propanol (15 ml.) and 12N-hydrochloric acid (7 ml.) was refluxed for 3 hr. with vigorous magnetic stirring. The residue obtained after thorough evaporation of the mixture was precipitated with acetonitrile and the product filtered off. Reprecipitation with acetonitrile removed traces of trityl compounds and left fairly pure 3,4-dioctadecyloxybutanol (700 mg., 97%) (R_F 0·05 in 19 : 1 hexane–ethyl acetate) containing a small quantity of dioctadecyl ether.

For analysis, the product (250 mg.) was adsorbed from hexane on to a 35-cm. column of 7 : 3 silicic acid–Celite. Successive washings with 5% ethyl acetate in hexane (800 ml.) and 10% ethyl acetate in hexane (200 ml.) removed only dioctadecyl ether and trace impurities. 3,4-Dioctadecyloxybutanol was eluted with 20% ethyl acetate in hexane (800 ml.), dried, and recrystallised from acetone, to give a chromatographically pure product, m. p. 52—52·5° (Found: C, 78·9; H, 13·6; O, 8·0. $C_{40}H_{82}O_3$ requires C, 78·6; H, 13·5; O, 7·9%).

3,4-*Dioctadecyloxybutylphosphonic Acid* (III; R = $C_{18}H_{37}$).—Fairly pure 3,4-dioctadecyloxybutanol (5·00 g., 0·0082 mole) was dissolved in warm 1,2-dimethoxyethane, and added in two portions with shaking to dibromotriphenoxyphosphorane (10 g.).[11] The homogeneous yellow solution was allowed to stand for 45 min. at room temperature and the solvent evaporated. To the residue was added acetonitrile (60 ml.) and the granular product filtered off. The 1-bromo-3,4-dioctadecyloxybutane (5·50 g., 99%) (R_F 0·48 in 19 : 1 hexane–ethyl acetate) was fairly pure but contained a small amount of dioctadecyl ether and traces of 3,4-dioctadecyloxybutanol and phosphorus compounds. The bromide was used directly in the Michaelis–Becker reaction.

A solution of sodium diethyl phosphite was prepared from de-oiled sodium hydride (1·50 g.) and diethyl hydrogen phosphite (30 ml.) in anhydrous peroxide-free 1,2-dimethoxyethane

(200 ml.). 1-Bromo-3,4-dioctadecyloxybutane (3·00 g., 0·0464 mole) was added and the mixture heated under reflux for 4·5 hr., evaporated, and the residue extracted with ether and washed several times with water and dilute hydrochloric acid. The dried ether solution was evaporated and the residue was dissolved in propionic acid (85 ml.), 8N-hydrochloric acid (15 ml.) was added, and the residue heated under reflux for 16 hr. The product was obtained by diluting the mixture with cold water (1 l.), filtering, and recrystallising from acetone–hexane. Yield of product, m. p. 76—78°, was 1·62 g. (53%). Recrystallisation from propan-2-ol–acetonitrile and from chloroform–acetone yielded an analytically pure product, m. p. 77·5—78·5° (R_F 0·42 in 9 : 1 chloroform–trifluoroacetic acid) (Found: C, 71·3; H, 12·5; P, 4·7%; Neut. Equiv., 334. $C_{40}H_{83}O_5P$ requires C, 71·2; H, 12·4; P, 4·6%; Neut. Equiv., 337). Pertinent assignments of infrared adsorptions for the acid are: (a) POH, about 2750—2550br cm.$^{-1}$; (b) P=O, 1290m 1190s cm.$^{-1}$; (c) ether, 1137 and 1062vs cm.$^{-1}$. In addition, a very strong peak is found at 988 cm.$^{-1}$, probably representing an unassigned absorption found in many organic phosphorus compounds.[12] The spectrum is very similar to that of 2-hexadecyloxy-3-octadecyloxypropylphosphonic acid.[3]

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