Synthesis of all-*cis*-1,3-diacylcyclopentane-1,2,3-triol-2-phosphate via acyl group migration in a cyclic diglyceride analog

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Abstract The acid-catalyzed isomerization of the diglyceride analog (1,2,3/0)-1,2-dipalmitoylcyclopentane-1,2,3-triol has been used to generate *syn-syn*-1,3-diacyl-cyclopentane-1,2,3-triol, a required intermediate in the synthesis of a symmetrical all-*cis*-1,2,3/0-2P cyclopentanoid phosphatidic acid analog. The all-*cis* cyclo-phosphatidic acid analog has therefore been obtained in the free acid form and as the diphenyl ester, dimethyl ester, and dipotassium salt derivatives. The compounds have been characterized by microanalysis and spectroscopic methods. The 1,2,3/0-2P analog is now available for comparative studies with the corresponding all-*trans* cyclophosphatidic acid (1,3/2-2P). —**Hancock, A. J., and M. D. Lister.** Synthesis of all-*cis*-1,3-diacylcyclopentane-1,2,3-triol-2-phosphate via acyl group migration in a cyclic diglyceride analog. *J. Lipid Res.* 1979. **20:** 271–274.

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The synthesis of cyclopentanoid analogs of phosphatidic acid has recently been reported (1). The compounds are derivatives of one or another of the three diastereoisomeric cyclopentane-1,2,3-triols. Since each of the triols closely mimics a particular rotameric state of the flexible glycerol moiety of many natural lipids, the synthetic lipid derivatives promise to be useful probes of the conformational states of glycerol-derived lipids in their interaction with proteins. A more detailed account of this rationale has been presented in an earlier communication (2).

The consequence of varying the geometry of the ring substituents in diacylphosphoryl cyclopentanetriols (1) has been revealed in biological studies involving proteins. Morrisett et al. (3) showed that the extent of binding of cyclopentanoid PA analogs to apolipoproteins C-III from human serum HDL differed with the stereochemistry of the cyclo-PA analogs, suggesting that the geometry of the glycerol backbone may significantly influence interaction between protein and lipid components. The symmetrical all-*trans* isomer (1,3/2-2-P) caused the greatest effect on the protein.

In view of the striking ability of the all-*trans* derivative to bind, it is of importance to evaluate the hitherto unavailable all-*cis* positional isomer, *syn-syn*-1,3-dipalmitoyl-2-*O*-phosphoryl-1,2,3-triol. This communication therefore describes the synthesis and characterization of this isomer.

MATERIALS AND METHODS

Melting points were measured on a Fisher Unimelt apparatus and are uncorrected. Infrared spectra were measured for KBr dispersions with a Perkin-Elmer 621 spectrometer (Perkin-Elmer Corp., Norwalk, CT) and were calibrated with polystyrene. NMR spectra were recorded on Varian A-60 or HA-100 spectrometers (Varian Associates, Palo Alto, CA) for dilute solutions in [^{2}H]chloroform using TMS as internal standard. Chemical shifts are reported as ppm downfield from TMS (δ-scale). Microanalyses were performed by Galbraith Laboratories, Knoxville, TN.

Analysis of diacyltriol isomerization products was carried out by TLC using 250-μm silica layers (EM Laboratories, Inc., Elmsford, NY). Preparative TLC was done using 1500-μm layers of silica gel G (Analtech, Inc., Newark, DE). Phosphates were detected on analytical plates by the spray method of Dittmer and Lester (4). For preparative purposes, lipids were visualized by exposing edge portions of the plate to iodine vapor.

Abbreviations and nomenclature: DPPC, diphenylphosphoryl chloride; GLC, gas–liquid chromatography; NMR, nuclear magnetic resonance; PA, phosphatidic acid; Pd–C, palladium–charcoal (10%); ppm, parts per million; TLC, thin-layer chromatography; TMS, trimethylsilane. Cyclic compounds described in this report are named according to the tentative Rules for Nomenclature of Cyclitols. The names are derived from those of the parent cyclanes of which they are formal derivatives. A summary of these rules has been presented in an earlier communication (2). The 1-phosphate and 2-phosphate positional isomers are designated by -1P or -2P, respectively, immediately following the cyclane nomenclature.

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(1,2,3/0)-1,2-Dipalmitoyl-3-*O*-benzylcyclopentane-1,2,3-triol *8* was synthesized from cyclopentadiene according to the methods of Sable et al. (5) and Hancock, Stokes, and Sable (1). The reaction sequences and the structures are shown in **Fig. 1.** Debenzylation of *8* to give dipalmitoyltriol *9* was achieved by hydrogenolysis over Pd–C as previously described (1) except that $NaHCO_3$ was suspended with the catalyst.

Isomerization of DL-(1,2,3/0)-1,2-dipalmitoylcyclopentane-1,2,3-triol

Diacyltriol *9* (500 mg) was isomerized to *10* when treated with 10 ml of 0.1 N methanolic-HCl containing 5% H_2O (15 min, 45°C). Treatment of *9* with anhydrous 2.5% methanolic-HCl, however, rapidly transesterified the lipid. TLC and GLC analysis revealed the presence of methyl palmitate and almost no desired product *10*.

The reaction mixture was cooled, treated with excess $NaHCO_3$ and dried over anhydrous Na_2SO_4. Evaporation of solvent from the filtrate gave an approximately equivalently proportioned mixture of isomers *9* and *10* (mp range 49–58°C) as estimated by TLC.

Phosphorylation of isomeric mixture of diacyltriols *9* and *10*

The mixture of isomeric diglyceride analogs *9* and *10* was phosphorylated with DPPC under anhydrous conditions as described previously (1). TLC analysis showed two phosphate-positive spots, R_f 0.45 and 0.55 ($CHCl_3$–EtOAc, 10:1). The faster moving isomer (R_f 0.55) was shown to be the new compound *6a* by TLC comparison of the mixture with authentic *3a*. Each of the diphenyl esters was isolated by preparative TLC [developing solvent hexane–diethyl ether 60:40 and eluting solvent $CHCl_3$–MeOH–Et_2O 1:1:1], and recrystallized from MeOH (mp *3a*, 50–51°C, lit. (1) 52–53°C; mp *6a*, 61–62°C).

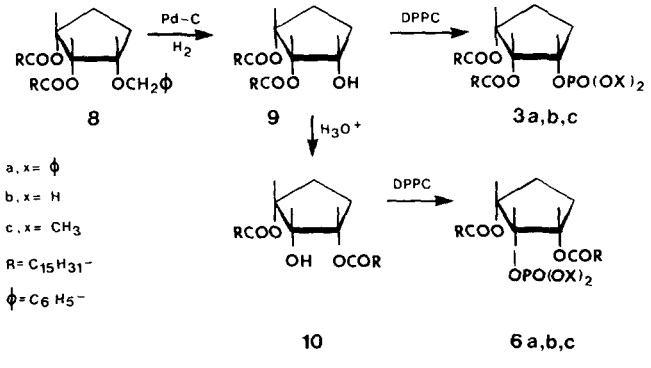

Fig. 1. Reaction sequences and compound structures.

Analysis. Calc. for *6a*: $C_{49}H_{79}O_8P$ (827.1), C, 71.15; H, 9.63; P, 3.75. Found: C, 71.09; H, 9.72; P, 3.70.

Cleavage of PA-diphenyl esters *3a* and *6a*

The PA analog was obtained by hydrogenolytic cleavage of the protecting phenyl groups over Pt in glacial acetic acid as previously described (1). Isomers *6b* and *3b* were quantitatively obtained as sharply melting solids (80–81°C and 75–76°C, respectively). (R_f values in $CHCl_3$–MeOH–H_2O 65:25:4 were 0.32 and 0.35, respectively.)

Analysis. Calc. for *6b*: $C_{37}H_{71}O_8P$ (675.9), C, 65.84; H, 10.60; P, 4.59. Found: C, 65.88; H, 10.79; P, 4.60.

Conversion of free acid *6b* to potassium salt

The 1,2,3/0-2P isomer *6b* was freed of metal ions introduced by the catalyst by a modification (6) of the procedure of Bligh and Dyer (7). The cation-free lipid (50 mg in 2 ml of $CHCl_3$–MeOH 2:1) was converted to its potassium salt by titration to the phenolphthalein end-point with KOH (0.2 N in MeOH–H_2O 98:2) and purified by acetone precipitation as described by Kates et al. (6); the potassium salt had the same mobility on TLC as the free acid in neutral and alkaline solvent systems.

Analysis. Calc. for $C_{37}H_{69}O_3PK_2 \cdot 3H_2O$ (805.2): C, 55.19; H, 9.39; P, 3.85; K, 9.71, K/P, 2.00. Found: C, 55.47; H, 9.20; P, 3.98; K, 9.50; K/P, 1.90.

Conversion of 1,2,3/0-2P free acid *6b* to dimethyl ester *6c*

The PA analog *6b* (30 mg in 2 ml of $CHCl_3$) was treated with excess ethereal diazomethane (8) for 10 min. The solvents were removed in a stream of N_2 to give the ester (32 mg) as a sharply melting solid (mp 72–73°C). The NMR spectrum of this compound is discussed later.

Analysis. Calc. for $C_{39}H_{75}O_8P$ (703.0): P, 4.41. Found: P, 4.36.

RESULTS AND DISCUSSION

Synthetic lipids derived from one or another of the three diastereoisomeric cyclopentane-1,2,3-triols are proving invaluable in the investigation of the effect of conformational variation on lipid properties. However, in addition to the planned variation in stereochemistry amongst the analogs themselves, these compounds differ from their natural lipid counterparts both in having an extra ethylene bridge in the backbone moiety and in containing exclusively secondary ester functions. In the present work, advantage is taken of the vicinal secondary alcoholic functions of

the synthetic intermediate. This disposition changes the extent and direction of acyl group migration from that observed for glyceride intermediates (9).

The isomerization of diacyltriol *9* appears to proceed to equilibrium in the presence of 0.1 N HCl in 95% methanol; TLC analysis showed the 2-hydroxy isomerization product *10* was at its maximum level after 15 min. The proton-scavenging effect of water probably lowers the effective concentration of $MeOH_2^+$ and hence transesterification via $MeOH_2^+$ attack is almost eliminated. In an earlier study (10), it was observed that water inhibits the acid-catalyzed desulfation of the phosphatidylglycerosulfate from *Halobacterium cutirubrum*, a reaction that in strictly anhydrous solvents is extremely rapid.

Spectral characteristics of 1,2,3/0-2P phosphatidic acid

The proton magnetic resonance spectrum of *6c*, the dimethyl ester of 1,2,3/0-2P PA analog, shows the expected symmetrical doublet (centered at δ 3.78 ppm, J_{PH} = 11.3 Hz, which is indicative of $^{31}P-^{1}H$ coupling between the methoxyl group protons and the proximal phosphorus atom, but shows no additional multiplicity even at high resolution. The resonance signals of the methoxyl protons in unsymmetrical phospholipids exhibit an additional multiplicity to that expected from $^{31}P-^{1}H$ coupling; this multiplicity has been shown to be due to the nonequivalence of the diastereotopic methoxyl groups on phosphorus, the line separation being frequency dependent (11). The line separation was measurable in three unsymmetrical isomers (1,2/3-3P, 1,2/3-1P, 1,2,3/0-1P) but not observable in the symmetrical all-*trans*-1,3/2-2P isomer. The spectrum of cyclo-PA isomer *6c*, which shows only a simple methoxyl doublet at high resolution, is therefore supportive of the proposed all-*cis*-structure (1,2,3/0-2P).

The infrared spectra of KBr dispersions of 1,2,3/0-2P isomer *6a* and 1,2,3/0-1P isomer *3a* were empirically different. For example, the methylene rock mode (720 cm^{-1}) gave a sharp singlet for the 1-phosphate and a well-resolved doublet for the 2-phosphate, a difference associated with subcell packing in the crystal (12).

Structure proof of diacyltriol *10*

The sensitivity of *10* to acyl migration is sufficiently great that analytical and spectral characteristics were instead obtained for the phosphorylated derivative *6*. Elemental analyses of *6* as its diphenylester *6a* and its dipotassium salt were consistent with the proposed molecular formula. The latter salt analyzed as a trihydrate after precipitation from acetone, a characteristic shared by all the cyclopentano-phosphatidic acid isomers described earlier (1). Since isomerization of DL-1,2-diacyltriol *9* can give only one isomeric product, *meso*-1,3-diacyltriol *10*, only one new phosphorylated derivative is possible. The molecular formula of this derivative is that of a cyclopentano-phosphatidic acid diphenylester, but the melting point, chromatographic mobility, and spectral characteristics differ from those of the 1,2,3/0-1P isomer *3*; it therefore follows that the isomer *6* must have the configuration shown, i.e., 1,2,3/0-2P.

The availability of the 1,2,3/0-2P isomer will allow assessment of the contribution made to physical and biological properties of cyclopentano-lipid analogs by the "abnormal" 2-phosphate substituent in the all-*cis* configuration. This information should aid in the interpretation of data obtained for the other PA analogs, particularly the all-*trans* 2-phosphate (1,3/2-2P). The present work describes the synthesis of the sixth PA isomer from a possible seven derived from cyclopentane-1,2,3-triol.

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