Phosphonolipids. XXVI. Mixed-Acid Phosphonocephalins: Synthesis of α′-Stearoyl-β-oleoyl-L-α-glyceryl-(2-aminoethyl)phosphonate[1]

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The synthesis of α′-stearoyl-β-oleoyl-L-α-glyceryl-(2-aminoethyl)phosphonate is reported. It is a member of a new class of phosphonolipids containing two dissimilar fatty acid substituents, one of which is unsaturated and occupies the 2 or β position of the glycerol moiety. The mixed-acid phosphonolipid, a phosphonic acid analogue of naturally occurring L-(α-stearoyl-β-oleoyl)cephalin, was obtained by phosphonylating D-α-stearoyl-β-oleoylglycerol with (2-phthalimidoethyl)phosphonic acid monochloride and triethylamine, and freeing the resulting α-stearoyl-β-oleoyl-L-α-glyceryl-(2-phthalimidoethyl)phosphonate of its protective phthaloyl group by hydrazinolysis.

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La synthèse de l'α′-stéaroyl-β-oléoyl-L-α-glycéryl-(2-aminoéthyl)phosphonate est décrite. Il s'agit d'un composé d'une nouvelle classe de phosphonolipides contenant deux acides gras substituants différents; l'un est insaturé et il occupe la position 2 ou β de la molécule de glycérol. L'acide phosphonolipidique mixte, analogue d'un acide phosphonolipidique naturel, la L-(α-stéaroyl-β-oléoyl)céphaline, est obtenu par phosphonylation du D-α-stéaroyl-β-oléoylglycérol avec l'acide (2-phthalimidoéthyl)phosphonique monochlorure et la triéthylamine. L'α-stéaroyl-β-oléoyl-L-α-glycéryl-(2-phthalimidoéthyl)phosphonate est ensuite libéré de son groupement protecteur, le phthaloyle, par hydrazinolyse.

[Traduit par le journal]

Introduction

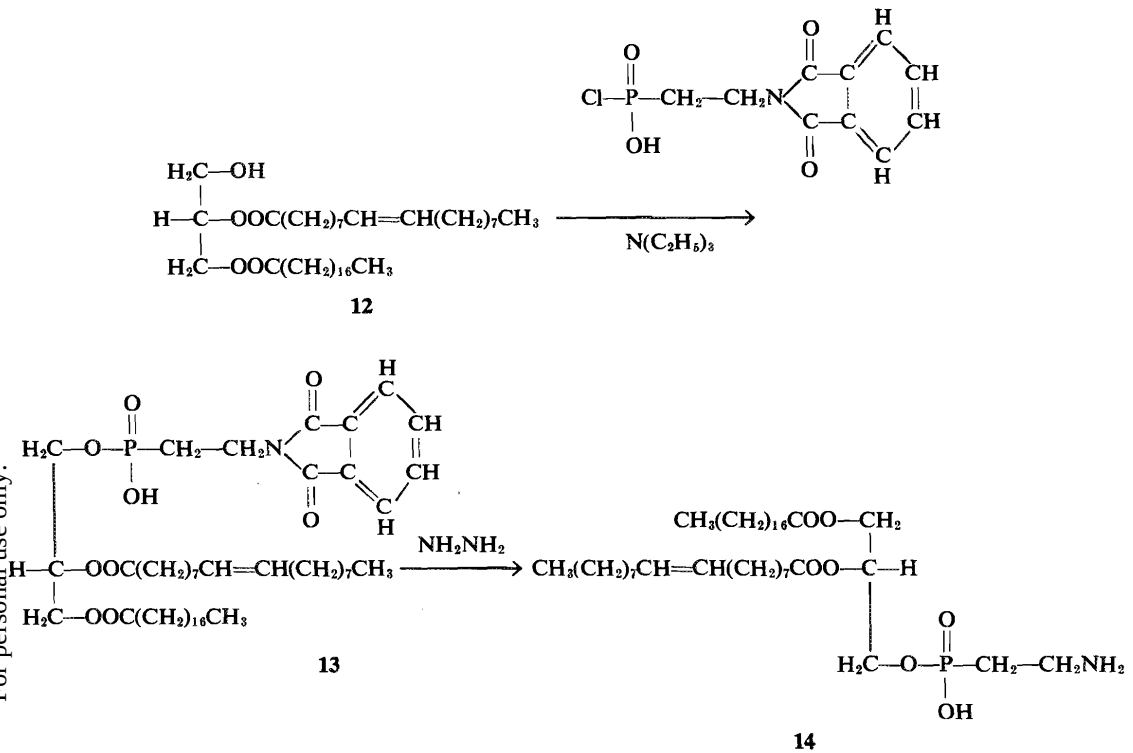
The first chemical synthesis of a phosphonolipid, *i.e.*, the phosphonic acid analogue of L-α-(dipalmitoyl)cephalin was reported by Baer and Stanacev (1) in 1964. It was followed by the synthesis of *phosphonic acid analogues* of several other cephalins (2–6), α-monoalkyl cephalins (7, 8), dialkyl cephalins (9), (*N*-methyl)-cephalins (10), dialkyl-(*N*,*N*-dimethyl)cephalins (11), lecithins (8, 12, 13), dialkyl lecithins (14), ceramide-(2-aminoethyl)phosphates (15, 16), and the fatty acid free moieties of cephalins (17) and lecithins (18). All of the glycerolphosphonolipids thus far synthesized have in common that both their fatty acid substituents, whether saturated or unsaturated, are identical. Naturally occurring glycerolphospholipids in general contain, however, two different fatty acids, of which one usually is unsaturated and occupies the β or 2 position of the glycerol moiety. An interesting exception to the 1-saturated-2-unsaturated pattern was reported recently by Dyatlovitskaya *et al.* (19) for lecithins of rat liver hepatoma-27, in which the 1 position was found to be higher in unsaturated fatty acids. As far as the author is aware neither the isolation from natural sources nor the synthesis of *phosphonic acid analogues* of mixed-acid phospholipids containing an unsaturated fatty acid in the β position have been reported. In view of a possible occurrence of these compounds in nature, their synthesis is being undertaken to provide pure, individual mixed-acid phosphonolipids as substrates for biochemical investigations and as reference compounds for the elucidation of the structure and configuration of naturally occurring phosphonolipids.

Methods

The frequent and simultaneous occurrence of stearic acid and oleic acid in phospholipids suggested the synthesis of a phosphonolipid containing both these acids, *e.g.*, α′-stearoyl-β-oleoyl-L-α-glyceryl-(2-aminoethyl)-

[1]Synonym: 1-stearoyl-2-oleoyl-*sn*-glyceryl-(2-aminoethyl)phosphonate.

D-Mannitol → 1,2-5,6-diacetone-D-mannitol (**1**) → acetone D-glyceraldehyde (**2**) → D-acetone glycerol (**3**) → D-acetone glyceryl benzyl ether (**4**) → L-glyceryl benzyl ether (**5**) → D-α-tritylglyceryl-α-benzyl ether (**6**) → D-α-trityl-β-stearoylglyceryl-α-benzyl ether (**7**) → D-α-stearoylglyceryl-α-benzyl ether (**8**) → D-α-stearoyl-β-oleoylglyceryl-α-benzyl ether (**9**) → D-α-stearoyl-β-9,10-dibromostearoylglyceryl-α-benzyl ether (**10**) → D-α-stearoyl-β-9,10-dibromostearoylglycerol (**11**) →



REACTION SCHEME 1. Synthesis of a mixed-acid L-α-phosphonocephalin

phosphonate, the phosphonic acid analogue of naturally occurring L-(α-stearoyl-β-oleoyl)cephalin. Its synthesis (Reaction Scheme 1, compounds **1–14**) involved the preparation of 13 intermediates, most of which were required for the preparation of the mixed-acid diglyceride (**12**). The final steps of the synthesis of the phosphonolipid, *i.e.*, the phosphonylation of D-α-stearoyl-β-oleoylglycerol (**12**) with (2-phthalimidoethyl)phosphonic acid monochloride and triethylamine, and the removal of the protective phthaloyl group of α′-stearoyl-β-oleoyl-L-α-glyceryl-(2-phthalimidoethyl)-phosphonate (**13**) by hydrazinolysis are shown in detail by Reaction Scheme 1. For the preparation of D-α-stearoyl-β-oleoylglycerol (**12**), two procedures were available: one reported by Buchnea and Baer (20), in 1960, starting with commercially available D-mannitol and requiring the preparation of 11 intermediates, and a more recent procedure by Buchnea (21) starting with L-mannitol and requiring the preparation of 7 intermediates, not taking into consideration 4 intermediates required for the preparation of L-mannitol from quebracitol. Since L-mannitol was available in this laboratory, it seemed desirable to investigate the feasibility of obtaining the mixed-acid diglyceride (**12**) by the method of Buchnea. No difficulties were encountered until the very end of the procedure, when it was found that the detritylation of the trityl ether of D-α-stearoyl-β-oleoylglycerol by silicic acid – boric acid is accompanied by a partial racemization of the diglyceride. Since no description for the preparation of the silicic acid – boric acid mixture was given by Buchnea, a series of experiments was undertaken, although in vain, to establish conditions that would yield a silicic acid – boric acid mixture capable of removing the trityl group without partial racemization of the diglyceride. It included keeping the mixture at 125° for 24 h. In 22 attempts at removing the trityl group with silicic acid – boric acid mixtures prepared under a variety of experimental conditions, the diglyceride (**12**) was obtained with specific rotations ranging from −0.54 to −2.07°, Reported (20): $[\alpha]_D$ −2.8°.

When it was finally realized that the procedure of Buchnea was unlikely to yield a stereochemically pure enantiomer of the mixed-acid diglyceride (compound

12), it was abandoned and the diglyceride was prepared by the method of Buchnea and Baer (20) as outlined by Reaction Scheme 1 (compounds **1**–**12**). The procedure was carried out as reported, except for some changes which are described in the experimental section of this paper. Thus, both D-α-stearoyl-β-9,10-dibromostearoylglyceryl benzyl ether (**10**) and D-α-stearoyl-β-9,10-dibromostearoylglycerol (**11**) were purified by column chromatography on silicic acid. Furthermore, the activation of zinc dust by hydrochloric acid for the debromination of compound **11** was omitted, as it was found that an equally active zinc preparation can be obtained by washing the commercial product several times with alcohol and ether.

Experimental

Materials

Silicic acid (Mallinckrodt, 100 mesh powder, analytical reagent) for chromatographic purposes was freed from finer particles by sifting the commercial product, using a sieve of 200 mesh per linear inch. The D-α-stearoyl-β-oleoylglycerol was prepared as described by Buchnea and Baer (20), using stearic acid and oleic acid with a purity of 99.6% or better. Commercial zinc dust (100 g) was activated by suspending it for 5 min with vigorous stirring in 99% ethanol (100 ml), separating the mixture by centrifugation, and repeating the procedure with two 100-ml portions of fresh alcohol, followed by three 100-ml portions of ether. The zinc dust was dried *in vacuo*.

Improvements in the Synthesis of D-α-*Stearoyl-β-oleoylglycerol*

Purification of D-α-*Stearoyl-β-9,10-dibromostearoylglyceryl Benzyl Ether (10)*

A solution of 6.76 g of the crude title compound (20) in 15 ml of petroleum ether (b.p. 30–60°) was added to a column of silicic acid (3 × 36 cm), and the column was eluted by passing in succession 300 ml of petroleum ether, 180 ml of a mixture of petroleum ether and diethyl ether (95:5, v/v), 390 ml of petroleum ether and ether (90:10, v/v), and 300 ml of petroleum ether and ether (70:30, v/v), and collecting the eluates in 30-ml fractions at a flow rate of 5 ml/min. The separation was monitored by thin-layer chromatography (T.L.C.) on silicic acid microslides using a solvent mixture of low boiling petroleum ether, diethyl ether, and acetic acid (70:20:4, v/v/v). Evaporation of the combined fractions 21–29 inclusive under reduced pressure (8–10 mm Hg) at a bath temperature of 25–30°, and drying the remaining colorless oil to constant weight at room temperature and a pressure of 0.02 mm Hg gave 4.91 g (72.6% recovery) of chromatographically homogenous D-α-stearoyl-β-9,10-dibromostearoylglyceryl benzyl ether. $[\alpha]_D$ +5.2° in ethanol-free chloroform (*c*, 10). Reported (20): $[\alpha]_D$ + 5.0° in chloroform (*c*, 10).

D-α-Stearoyl-β-9,10-dibromostearoylglycerol (11)

The removal of the protective benzyl group of compound **10** (8.09 g) by hydrogenolysis was carried out as described (20) using, however, a dry palladium black catalyst. The acetic acid was distilled off under reduced pressure (8–10 mm Hg) at a bath temperature of 20°. The remaining traces of acetic acid were removed, not as reported by treating the ethereal solution of the residue with a saturated solution of sodium bicarbonate, but by keeping the residue at a pressure of 0.02 mm Hg until its weight was constant. The remaining material, weighing 6.9 g, was dissolved in 16 ml of dry acetone, the solution was cooled in ice-water, and the precipitate was removed by centrifugation at 0 °C. The decanted supernatant solution on evaporation under reduced pressure at 20° gave 5.6 g of compound **11**, which was further purified by column chromatography on silicic acid. Its solution in 20 ml of petroleum ether (b.p. 30–60°) was added to a column of sifted silicic acid (3 × 30 cm) and the column was eluted successively with 700 ml of petroleum ether, 500 ml of a mixture of petroleum ether and ether (9:1, v/v), and 550 ml of petroleum ether and ether (7:3, v/v), collecting the eluate in 50-ml fractions at a flow rate of 5 ml/min. Evaporating the combined fractions of the petroleum–ether (7:3) eluate under reduced pressure at 20° and drying the remaining material at a pressure of 0.02 mm Hg gave 4.70 g (82% recovery) of chromatographically homogenous D-α-stearoyl-β-9,10-dibromostearoylglycerol (**11**). $[\alpha]_D$ −1.9° in ethanol-free chloroform (*c*, 14).

D-α-Stearoyl-β-oleoylglycerol (12)

A solution of 5.70 g of D-α-stearoyl-β-9,10-dibromostearoylglycerol in 125 ml of moist ether[2] to which 65 g of alcohol–ether-treated

[2]Prepared by stirring 100 ml of ether with 2 ml of water and removing the excess of water in a separatory funnel.

zinc dust had been added was stirred vigorously (magnetic stirrer) at room temperature (25°) for 1 h. The zinc dust was removed by centrifugation and extracted with four 100-ml portions of moist ether. The combined ether solution and extracts were washed with four 100-ml portions of distilled water[3] and dried with 100 g of anhydrous sodium sulfate. Evaporation of the ether solution under reduced pressure at a bath temperature of 20–25° and drying of the remaining material to constant weight at a pressure of 0.02 mm Hg gave 4.60 g (90% of theory) of D-α-stearoyl-β-oleoylglycerol. $[\alpha]_D$ −2.8° in ethanol-free chloroform (*c*, 9.20). Reported (20): $[\alpha]_D$ −2.8° in chloroform (*c*, 10).

α′-Stearoyl-β-oleoyl-L-α-glyceryl-(2-phthalimidoethyl)phosphonate (13)

Phthalimidoethylphosphonic acid monochloride, prepared from 3.57 g (14 mmol) of phthalimidoethylphosphonic acid (1) as described by Baer and Sarma (2), was dissolved in 85 ml of anhydrous and ethanol-free chloroform and to the solution, kept at 20°, was added over a period of 30 min a solution of 4.36 g (7.0 mmol) of D-α-stearoyl-β-oleoylglycerol and 7 ml (33 mmol) of anhydrous triethylamine in 165 ml of chloroform. The reaction mixture was allowed to stand at room temperature for 18 h. At the end of this time, 3.5 ml of triethylamine and 7 ml of water were added, and the mixture was stirred vigorously for 2 h. The chloroform and excess of triethylamine were distilled off under reduced pressure (8–10 mm Hg) at a bath temperature of 30–35°. To the residue were added 150 ml of benzene, and the benzene was distilled off under reduced pressure at 30–35°. This process was repeated twice more, using each time 150 ml of benzene. The remaining material, after drying at 30° and a pressure of 0.02 mm Hg, was extracted with three 150-ml portions of anhydrous diethyl ether, and the combined extracts were cleared by centrifugation. The ethereal solution was evaporated to dryness under reduced pressure at a bath temperature of 15–20°. The residue, weighing 7.0 g, was dissolved in 400 ml of a mixture of chloroform, methanol, and water (5:4:1, v/v/v), and the solution was treated with 40 ml of Amberlite ®IR-120 (H^+ form) for 20 min. The ion-exchange resin was filtered off, washed with two 80-ml portions of chloroform, and the combined filtrates were evaporated to dryness under reduced pressure at 30–35°. The residue was triturated with three 50-ml portions of water, the mixtures were separated by centrifugation, and the solid material was dried over anhydrous calcium chloride at a pressure of 0.2 mm Hg. It was dissolved in 100 ml of benzene, the solution was decolorized with 2 g of charcoal (Norit, acid-washed), and the benzene was removed by distillation under reduced pressure. The material remaining, weighing 4.37 g, was purified by column chromatography on silicic acid. Its solution in 30 ml of a mixture of chloroform and benzene (1:1, v/v) was passed through a column (2.4 cm width) containing 65 g of silicic acid, and the column was eluted successively with a 1:1 mixture of chloroform and benzene (fractions 1–18), ether (fractions 19–29), and methanol (fractions 30–37), collecting the eluates in 30-ml fractions. Evaporation of the combined fractions 22–24 under reduced pressure at 35–40°, and drying of the residue at room temperature and a pressure of 0.02 mm Hg gave 2.0 g (33% of theory) of chromatographically homogenous α′-stearoyl-β-oleoyl-L-α-glyceryl-(2-phthalimidoethyl) phosphonate. $[\alpha]_D$ +4.4° in ethanol-free chloroform (*c*, 7).

Anal. Calcd. for $C_{49}H_{82}O_9NP$ (860.1): C, 68.42; H, 9.61; N, 1.63; P, 3.60. Found: C, 68.40; H, 9.90; N, 1.54; P, 3.59.

α′-Stearoyl-β-oleoyl-L-α-glyceryl-(2-aminoethyl)phosphonate (14)

To a solution of 1.720 g (2.0 mmol) of compound (**13**) in 50 ml of 90% ethanol, heated to gentle reflux in a nitrogen atmosphere, were added 5.9 ml of a 1% solution of hydrazine in 90% ethanol, followed at hourly intervals by 5.9 ml and 4.1 ml, respectively, of the hydrazine solution. Total reflux time 4 h. After standing overnight at room temperature (25°), the solution was filtered and the filtrate was kept for 2 h at 6°. The precipitate was filtered off in the cold, and the filtrate was concentrated under reduced pressure to approximately one-half of its original volume. On keeping the concentrate overnight at 6°, a further amount of crude compound was obtained. The combined precipitates on drying at room temperature and a pressure of 0.05 mm Hg weighed 0.769 g (52.6% of theory). The

[3]The wash water, on the addition of nitric acid and silver nitrate, gave 2.452 g (89% of theory) of silver bromide.

crude phosphonocephalin was triturated with a small volume of acetone, and the solid material remaining, weighing 0.727 g after drying, was triturated with two 9-ml portions of benzene. Evaporation of the combined benzene extracts under reduced pressure (bath 30°) and drying of the residue for 2 days at room temperature over phosphorus pentoxide at a pressure of 0.02 mm Hg gave 0.540 g of phosphonolipid. For further purification, a solution of 140 mg of the substance in chloroform was added to a column (1.1 cm width) containing 5.0 g of silica gel 60 (E. Merck, catalogue No. 7734). The material was eluted with a solvent mixture of chloroform and methanol (65:25, v/v), collecting the eluate in 10-ml fractions. The separation was monitored by T.L.C. on microslides of silica gel G, using for the development a solvent mixture of chloroform, methanol, and water (65:25:2, v/v/v). On evaporating fractions 5–15 under reduced pressure at 30–35°, triturating the material remaining briefly with acetone and drying it over phosphorus pentoxide at room temperature and a pressure of 0.01 mm Hg for 2 days gave 70.4 mg of chromatographically homogenous α'-stearoyl-β-oleoyl-L-α-glyceryl-(2-aminoethyl)phosphonate. Chromatographic purification of the remaining crude material (400 mg) gave an additional 201.1 mg of phosphonocephalin. Total yield of pure material 271.5 mg (m.p.[4] 171–172° (decomp.)). At room temperature (25°), the compound is readily soluble in 99% ethanol, chloroform, ethyl ether, petroleum ether (b.p. 30–60°), and benzene, but insoluble in acetone. $[\alpha]_D^{23}$ +7.5° in anhydrous chloroform and methanol (4:1, v/v) (*c*, 4.2). Reported for distearoyl L-α-glyceryl-(2-aminoethyl)phosphonate (2): $[\alpha]_D$ +7.4° in chloroform–methanol (4:1, v/v). The n.m.r. spectrum of compound **14** in $CDCl_3$ shows a triplet at δ 5.36 p.p.m. ($J = 4$Hz, 2H) characteristic for vinylic protons (oleic acid).

Anal. Calcd. for $C_{41}H_{80}O_7NP$ (730.04): C, 67.45; H, 11.05; N, 1.92; P, 4.24. Found: C, 67.00; H, 10.96; N, 2.01; P, 3.94.

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[4]The melting point of the substance was determined in a capillary tube using a short-stem thermometer with a range of 50° and an electrically heated bath of diisobutylphthalate.