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Synthesis of Phospholipids Bearing a Conjugated Oxo-polyunsaturated Fatty Acid Residue†

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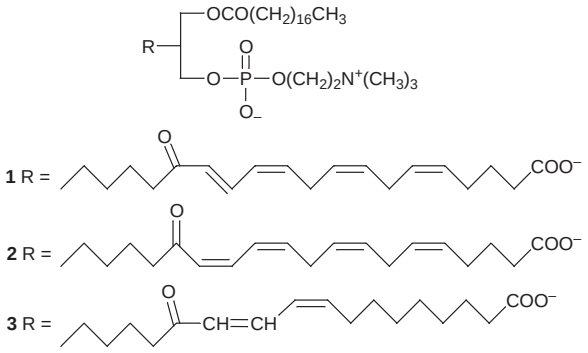
2-(15′-Oxo-5′,8′,11′,13′-eicosatetraenoyl)-1-stearoyl- *sn*-glycerol(3)phosphocholine (APC-CO) **1** and **2** and 2-(13′-oxo-9′,11′-octadecadienoyl)-1-stearoyl-*sn*-glycero(3)phosphocholine (LPC-CO) **3** are synthesized and an analytical system established for the determination of geometrical isomers at the 13′ position of APC-CO.

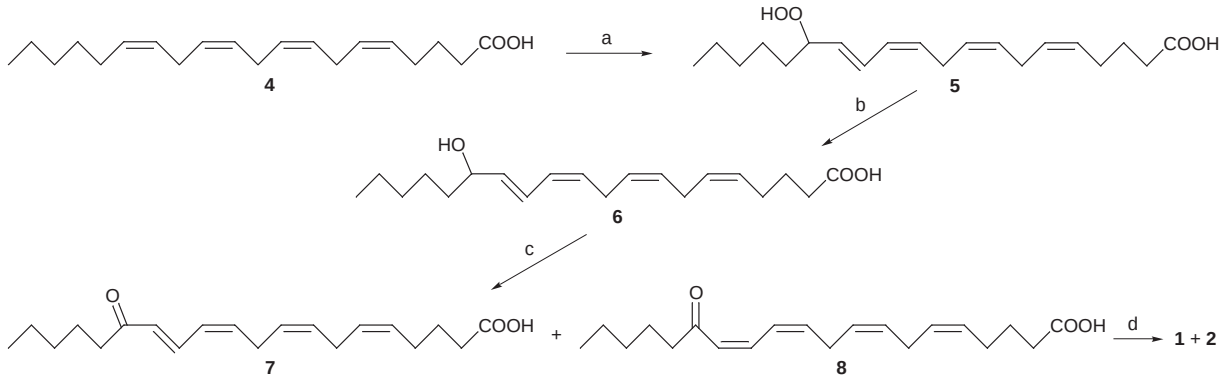
An increase in lipid peroxides (LOOHs) is often observed in diseased or stressed tissue. These LOOHs are known to generate oxidative stress which triggers free radical-mediated chain reactions allowing deteriorative reactions with biological targets through radical damage and cytotoxic smaller compounds such as aldehydes formed from the decomposition of LOOHs. However the details of the mechanism of the LOOHs action are still poorly understood.

We have previously synthesized two phospholipid hydroperoxides (LPC-OOH and APC-OOH)[1,2] and investigated their effect on cultured human cell lines. Neither compound was cytotoxic on human umbilical vein endothelial cell.[3] However LPC-OOH was cytotoxic on a human promyelocyte line U937. We have also observed that the synthesized LPC-OOH is liable spontaneously to decompose[4] even at ambient temperature and its major product shows a molecular peak at *m/z* 800.6 ($[M + H]^+$ in the atmospheric pressure ionization (API) mass spectrum after isolation. Considering our observation in relation to other studies on the formation, identification, reaction, and toxicity of free oxo-fatty acids[5–10] we believe that this decomposition product may bear a 13-oxo group in the linoleic acid residue of the phospholipid, and that this may be an important intermediate in biochemical processes.



Therefore, in this present study we have synthesized the oxo-phospholipids APC-CO **1** and **2** from arachidonic acid and LPC-CO **3** from linoleic acid. Here we focus on the description of the APC-CO synthesis and the identification of its geometrical isomers **1** and **2**.

As shown in Scheme 1, hydroperoxidation of arachidonic acid **4** with soybean lipoxygenase in borate buffer at pH 9.0 and 0–5 °C under oxygen yielded 15*S*-hydroperoxyeicosatetraenoic acid **5** in 57% yield. This compound gave a proton signal at δ 4.28 (15-H) in the ^{1}H NMR spectrum,



Scheme 1 Synthetic reactions for APC-CO. a, Soybean lipoxygenase, borate buffer, pH 9, O_2, 0–5 °C, 6 h; b, $NaBH_4$, MeOH, rt, 1 h; c, Dess–Martin reagent, CH_2Cl_2, $NaHCO_3$, pyridine, 0–5 °C, 1 h; d, lyso PC, DCC, DMAP, $CHCl_3$, rt, 20 h

which showed the presence of a hydroperoxy group. Reduction of **5** with $NaBH_4$[11] in alcohol at 0–5 °C under nitrogen gave 15*S*-hydroxyeicosatetraenoic acid **6** in 35%, yield. The shift of the proton signal to δ 4.13 (dd, 1 H, 15-H) indicated the formation of a hydroxy group.

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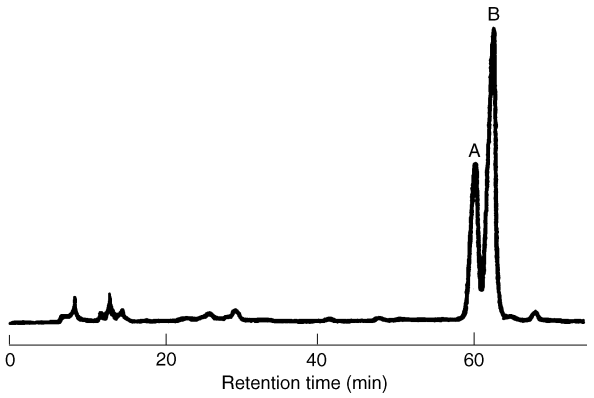

Fig. 1 HPLC profile (ODS) for derivatives of APC-CO. Peak A was identified as **2**, and B as **1**. Mobile phase, MeOH–$CHCl_3$–H_2O (100:4.5:5)

Oxidation of **6** with 2 equiv. of Dess–Martin reagent[12] in the presence of $NaHCO_3$ and pyridine at 0–5 °C yielded a mixture of two geometrical isomers **7** and **8** of 15-oxo-5,8,11,13-eicosatetraenoic acid in 84% yield. The proton signal at δ 4.13 disappeared indicating the formation of a carbonyl group. This isomeric mixture was subjected to the next reaction without further separation.

Reaction of the mixture of compounds **7** and **8** with lysophosphatidylcholine (lyso PC), prepared as described previously,[1] in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP)[1,2] resulted in the production of the desired compounds, APC-CO (**1** and **2**), in 19% yield. Spectral analysis of the product by ^{1}H NMR and API mass spectroscopy combined with HPLC analysis indicated that this product consisted of two geometrical isomers. Proton signals at δ 6.0–7.5 showed the α,β (14′,13′ position) unsaturated structure of the oxo-form, for which a proton signal at δ 7.13 and another at δ 7.49 suggested the presence of two β (13′) protons and thus the presence of 13′ positional isomers. Comparison of these ^{1}H NMR data and calculated chemical shifts for α,β-protons also supported this suggestion and allowed assignments of a 13′-*trans* form showing 14′- and 13′-proton signals at δ 6.21–6.10 and 7.49, and 13′-*cis* form showing 14′- and 13′-proton signals at δ 6.08 and 7.13, respectively. Correlation between their proton signal intensities in the ^{1}H NMR spectrum and their HPLC peak intensities revealed that peak A in HPLC was the 13′-*cis* form **2** and B the 13′-*trans* form **1** with a ratio of 1 : 1.9 as shown in Fig. 1. Furthermore, separation of the two peaks by HPLC showed both have a molecular ion at m/z 824.6 ($[M+H]^+$) in the API mass spectrum. This analytical system can thus be applied to the determination of the geometrical isomers **1** and **2** derived from APC-OOH in biochemical and chemical processes.

We also synthesized LPC CO **3** in 10% overall yield from linoleic acid in the same way. It was shown that **3** was also a mixture of two geometrical isomers at the 11′ position, from ^{1}H NMR spectrum, but these isomers could not be separated under the conditions for the geometrical isomers of APC-CO **1** and **2**.

Thus, phospholipids bearing a conjugated oxo-poly-unsaturated fatty acid residue were synthesized for the first time. These results will contribute to further studies involving the detection and the role of oxo-phospholipids in biological systems.

Experimental

^{1}H NMR spectra were recorded on a Varian VXR 500 spectrophotometer, mass spectra on an API III triple quadruple mass spectrometer (PE-Sciex) HPLC was conducted on Innertsil ODS-2 (5 μm, 4.6 × 250 mm). Since LPC-CO **3** was synthesized by the same procedure as for **1** and **2**, only characterizing data for it are given.

15-*Hydroperoxyeicosatetraenoic Acid* **5**.—The peroxide **5** (774 mg, 2.3 mmol, 57%) was produced from arachidonic acid (1228 mg, 4.04 mmol) as previously described.[1]

15-*Hydroxyeicosatetraenoic Acid* **6**.—Alcohol **6** (100 mg, 0.31 mmol, 35%) was formed from **5** by reduction with $NaBH_4$.

15-*Oxoeicosatetraenoic Acid* **7** *and* **8**.—To a solution of 15-hydroxyeicosatetraenoic acid (33 mg, 0.1 mmol) in CH_2Cl_2 (2.6 ml) was added $NaHCO_3$ (49 mg, 0.58 mmol), pyridine (0.05 ml, 0.06 mmol), and Dess–Martin reagent (78 mg, 0.20 mmol, 2 equiv.) in CH_2Cl_2 (2.6 ml) at 0–5 °C. The reaction mixture was stirred for 1 h at the same temperature and then quenched by consecutive addition of saturated $NaHCO_3$ (5.8 ml) and $Na_2S_2O_3\cdot5H_2O$ (338 mg, 1.36 mmol). The resulting solution was stirred for 30 min and extracted with ether (3 × 20 ml). The combined organic extracts were purified by flash chromatography (silica gel, $CHCl_3$–MeOH, 10 : 0.3) to give a mixture of compounds **7** and **8** (27 mg, 0.09 mmol, 84%): R_f = 0.49 (silica gel, $CHCl_3$–MeOH, 10:0.5).

15′-*Oxo*-(cis-5′,8′,11′,trans-*13′-eicosatetraenoyl*)-1-*stearoyl*-sn-*glycero*(3)*phosphocholine* (*APC-CO*) **1** *and* **2**.—A mixture of **1** and **2** (13 mg, 0.02 mmol, 19%) were produced from **7** and **8** by the method previously described.[1] Compound **1**: R_f = 0.47 [silica gel, 60 : 30 : 5, $CHCl_3$–MeOH–NH_3 (aq)]; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (dd, J = 14, 11, 1 H, 13′-H), 6.21–6.10 (m, 2H, 14′- and 12′-H), 5.5–5.3 (m, 11′-, 9′-, 8′-, 6′-, and 5′-H), 5.20 (m, 1 H, 2-H), 4.38 (dd, J = 11, 2.2, 1 H, OCH_2CHCH_2O), 4.29 (m, 2 H, $OPOCH_2$), 4.12 (dd, J = 11, 7, 1 H, OCH_2CHCH_2O), 3.94 (m, 2 H, OCH_2CHCH_2O), 3.79 (m, 2 H, CH_2N), 3.38 (s, 9 H, $N(CH_3)_3$), 2.97 (m, 2 H, 10′-H), 2.79 (m, 2 H, 7′-H), 2.55 (dd, J = 14, 7, 1 H, 4′-H), 2.28 (dd, J = 7, 7, 2 H, 2′-H), 2.25 (dd, J = 7, 7, 2 H, $OCOCH_2CH_2(CH_2)_{14}CH_3$), 2.07 (m, 2 H, 16′-H), 2.03 (dd, J = 7, 7 Hz, 1 H, 4′-H), 1.59 (m, 6 H, $OCO(CH_2)_2CH_2(CH_2)_{13}CH_3$, 3′- and 17′-H), 1.25 (m, 26 H, $OCO(CH_2)_2(CH_2)_{14}CH_3$, 4′-, 5′-, 18′-, and 19′-H), 0.87 (m, 6 H, $OCO(CH_2)_{16}CH_3$ and 20′-H) compound **2**: δ_H 7.13 (dd, J = 14, 11, 1 H, 13′-H), 6.08 (d, J = 14, 1 H, 14′-H), 5.83 (dd, J = 8.3, 7 Hz, 1 H, 12′-h), other data for **1**.

2-(13′-*Oxo*-9′,11′-*octadecadienoyl*)-1-*stearoyl*-sn-*glycerophosphocholine* (*LPC-CO*) **3**.—R_f = 0.22 (CH_3Cl–MeOH–NH_4 (aq), 60 : 30 : 3; ^{1}H NMR (500 MHz, $CDCl_3$): 11′-*trans* form, δ 7.43 (dd, J = 15, 11 Hz, 1 H), 6.18–6.07 (m, 2 H), 5.35 (m, 1 H), 5.21 (m, 1 H), 4.38 (m, 3 H), 4.12 (m, 1 H), 3.99 (m, 2 H), 3.84 (m, 2 H), 3.36 (s, 9H), 2.52 (m, 2 H), 2.28 (m, 4 H), 2.01 (m, 2 H), 1.59 (m, 6 H), 1.24 (m, 42 H) and 0.88 (m, 6 H); 11′-*cis* form, δ 7.14 (dd, J = 15, 11 Hz, 1 H), 5.19 (m, 1 H), other data as for 11′-*trans*.

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