Short Communication

Synthesis of Triacylglyceride Hydroperoxides Derived from Linoleic Acid

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Four triacylglyceride hydroperoxides were synthesized by DCC-mediated esterification of a dimethylperketal of 13-hydroperoxyoctadecadienoic acid with glycerides, in which one or two linoleoyl groups were linked, and by final removal of the protective group with a mixture of THF, acetic acid and water.

Triacylglycerides bearing an unsaturated fatty acyl group are an important source of lipid nutrients which are present in a variety of foods. A serious problem is that these unsaturated lipids are very susceptible to autoxidation by exposure to oxygen, particularly in the presence of a radical initiator, giving lipid hydroperoxides and their degradation products which inevitably cause serious deterioration of such foods. To promote studies on lipid hydroperoxides regarding food deterioration and biological effects on living systems, supply of chemically well-defined lipid hydroperoxides of high purity is essential.

The present study describes a first synthesis of triacylglycerides 6, 8, 12, and 17, in which the three acyl groups in one glyceride molecule are consist of a linoleoyl group(s) and its hydroperoxide(s) alone, with no saturated acyl group. Hydroperoxides like those formed by the autoxidation of a glyceride carrying three linoleoyl groups in the molecule, and such an unsaturated glyceride is an important source of linoleic acid as an essential fatty acid nutrient. With the present synthetic route, the key step is to use perxoxyoctadecadienoic acid (I), from which the hydroperoxide group was protected as a dimethylketel. This acid was prepared from linoleic acid via hydroperoxidation with soybean lipoxygenase, esterification with diazomethane, protection of the hydroperoxide group by 2-methoxypropene, and hydrolysis of the ester with lipase according to our previous report.1

The preparation of a typical triacylglyceride hydroperoxide (6) is given here. A solution of dihydroxycetone (2; 0.5 g, 5.5 mmol) in 1 N-HCl (1 ml) was left to stand for 3 h, and dimethylformamide (20 ml), linoleic acid (3.0 g, 11 mmol), dicyclohexylcarbodiimide (DCC; 2.4 g, 11.5 mmol), and dimethylaminopyridine (DMAP; 0.80 g, 6.5 mmol) were then added. The solution was stirred at room temp. for 2 days in the dark. The products were extracted with hexane and purified in a silica gel column (hexane/ EtOAc: 9:1) to afford 3 (0.90 g, 40%). To a solution of ketone 3 (0.8 g, 1.3 mmol) in methanol (100 ml) was added sodium borohydride (0.5 g, 13.5 mmol) at 0°C, the mixture being stirred for 30 min at this temperature, and then for an additional 30 min at room temperature. After adjusting the acidity of the solution to pH 4, the solvent was evaporated. The residue was extracted three times with ether, and the combined organic phase was washed with water and dried over anhydrous sodium sulfate. After being concentrated, the residue was chromatographed on silica gel to afford glyceride 4 (0.55 g, 69%). A solution of 4 (0.55 g, 0.89 mmol), peracid 1 (0.50 g, 1.3 mmol), DCC (0.80 g, 3.9 mmol), and DMAP (0.50 g, 4.1 mmol) in ethanol-free dry CHCl₃ (20 ml) was stirred at room temperature overnight in the dark, and after evaporating the solvent, the residue was chromatographed on silica gel (hexane/EtOAc: 9:1) to afford triglyceride 5 (0.84 g, 96%). A solution of 5 (0.20 g, 0.20 mmol) in a mixture of THF/AcOH/H₂O (4:2:1, 49 ml) was stirred at room temp. for 4 days in the dark in the presence of a trace of butylated hydroxytoluene, and after evaporating the solvent, the

Scheme. Synthesis of Triglyceride Hydroperoxides.

Reagents: i) R'OHT or R'OH, DCC, dimethylaminopyridine; ii) NaBH₄; iii) THF-CH₂COOH/H₂O (4:2:1); iv) lipase P from Pseudomonas fluorescens; v) 1 N HCl/THF (3:5).
residue was chromatographed on silica gel to afford the triglyceride hydroperoxide 6 (0.11 g, 59%). This hydroperoxide thus obtained was a colorless liquid, and its structural integrity and purity were confirmed by TLC and $^1$H-NMR. The hydroperoxy group was confirmed by KI-coloration on the TLC plate.

The other three hydroperoxides (8, 12, and 17) were similarly prepared according to Scheme, and their TLC data and $^1$H-NMR spectra confirmed their purity and structural integrity. Each of these hydroperoxides as a CHCl$_3$ solution was found to be so stable that it showed a single spot by TLC and no change in $^1$H-NMR spectra after two weeks of storage at $-20^\circ$C.

The triacylglyceride hydroperoxides thus obtained can be utilized in many research fields, particularly for studies on food deterioration by oxidation. The present study also demonstrates that the use of a key intermediate, peroxylipoyl acid I, lipase, and a general synthetic reaction is potentially useful for synthesizing many other triacylglyceride hydroperoxides derived from other unsaturated fatty acids.

References and Notes


2) TLC (SiO$_2$) and $^1$H-NMR (CDCl$_3$, 500 MHz) data for 6, 8, 12, and 17: TLC $R_f$ = 0.33 (hexane:EtOAc=85:15); $^1$H-NMR $\delta$ = 0.89 (9H, t, 3 $\times$ CH$_3$), 1.30 (44H, m, CH$_2$), 1.59 (6H, m, 3 $\times$ CH$_2$CH$_2$COO), 2.04 (8H, m, 2 $\times$ CH$_2$-CH = CHCH$_2$CH = CHCH$_2$), 2.18 (2H, m, CH = CHCH = CHCH$_2$), 2.31 (6H, m, 3 $\times$ -CH$_2$CH$_2$COO), 2.76 (4H, m, 2 $\times$ CH = CHCH$_2$CH = CH), 4.14, 4.29 (4H, m, OCH$_2$-CH(OR)-CH$_2$O), 4.37 (1H, m, -CH$_2$(OCH)), 5.26 (1H, m, OCH$_2$-CH(OR)-CH$_2$O), 5.35 (8H, m, 2 $\times$ CH = CH-CH = CH), 5.48 (1H, m, CH(OOH)-CH = CH-CH = C(=CH$_2$)CH$_2$), 5.57 (1H, dd, CH(OOH)-CH = CH-CH = C(=CH$_2$)CH$_2$, J = 8.1, 15.1 Hz), 6.00 (1H, dd, CH(OOH)-CH = CH-CH = C(=CH$_2$)CH$_2$, J = 10.9, 11.2 Hz), 6.56 (1H, dd, CH(OOH)-CH = CH-CH = C(=CH$_2$)CH$_2$, J = 11.2, 15.1 Hz), 7.30 (1H, s, OCOO), 8: TLC $R_f$ = 0.36 (hexane:EtOAc=80:20); $^1$H-NMR $\delta$ = 0.88 (9H, t, CH$_3$), 1.29 (48H, m, 6 $\times$ CH$_2$), 1.60 (6H, m, 3 $\times$ CH$_2$CH$_2$COO), 2.18 (6H, m, 3 $\times$ CH = CHCH$_2$), 2.31 (6H, m, 3 $\times$ CH = CHCH$_2$COO), 4.14, 4.28 (4H, m, OCH$_2$-CH(OR)-CH$_2$O), 4.37 (3H, m, 3 $\times$ CH(OOH)), 5.26 (1H, m, OCH$_2$-CH(OR)-CH$_2$O), 5.57 (3H, dd, 3 $\times$ CH(OOH)-CH = CH = CHCH$_2$), 5.67 (3H, dd, 3 $\times$ CH(OOH)-CH = CH = CHCH$_2$), J = 10.7, 11.2 Hz). 6.56 (3H, dd, 3 $\times$ CH(OOH)-CH = CH = CHCH$_2$), J = 11.2, 15.1 Hz), 7.95 (3H, s, 3 $\times$ OCOO); 12: TLC $R_f$ = 0.18 (hexane:EtOAc=9:1); $^1$H-NMR $\delta$ = 0.89 (9H, t, 3 $\times$ CH$_3$), 1.30 (44H, m, -CH$_2$CH$_2$), 1.59 (6H, m, 3 $\times$ CH$_2$CH$_2$COO), 2.04 (8H, m, 2 $\times$ CH = CHCH = CHCH$_2$), 2.18 (2H, m, CH = CHCH = CHCH$_2$), 4.14, 4.29 (4H, m, CH$_2$O-CH(OR)-CH$_2$O), 4.37 (1H, m, CH(OOH)), 5.26 (1H, m, OCH$_2$-CH(OR)-CH$_2$O), 5.35 (8H, m, 2 $\times$ CH = CH-CH$_2$-CH = CH), 5.49 (1H, m, CH(OOH)-CH = CH-CH = C(=CH$_2$)CH$_2$, J = 7.2, 15.1 Hz), 6.00 (1H, dd, CH(OOH)-CH = CH-CH = C(=CH$_2$)CH$_2$, J = 10.8, 11.2 Hz), 6.56 (1H, dd, CH(OOH)-CH = CH, J = 11.2, 15.1 Hz), 7.89 (1H, s, OCOO). [x]$_D$ = 3.3° (c = 1.415, CHCl$_3$), 17: TLC $R_f$ = 0.25 (hexane:EtOAc=85:15); $^1$H-NMR (CDCl$_3$), $\delta$ = 0.88 (9H, t, 3 $\times$ CH$_3$), 1.30 (46H, m, -CH$_2$CH$_2$), 1.60 (6H, m, 3 $\times$ CH$_2$CH$_2$COO), 2.04 (4H, m, OCH$_2$-CH=CHCH$_2$-CH=CHCH$_2$), 2.19 (4H, m, 2 $\times$ CH = CHCH = CHCH$_2$), 2.31 (6H, m, 3 $\times$ CH$_2$CH$_2$COO), 2.76 (2H, m, CH = CHCH = CHCH$_2$), 4.14, 4.28 (4H, m, OCH$_2$-CH(OR)-CH$_2$O), 4.37 (2H, m, 2 $\times$ CH(OOH)), 5.26 (1H, m, OCH$_2$-CH(OR)-CH$_2$O), 5.35 (4H, m, CH = CH-CH = CHCH$_2$), 5.49 (2H, m, 2 $\times$ CH(OOH)-CH = CH-CH = CHCH$_2$), 5.57 (2H, dd, CH(OOH)-CH = CH, J = 8.1, 15.1 Hz), 6.00 (2H, dd, 2 $\times$ CH(OOH)-CH = CH-CH = CHCH$_2$), J = 10.9, 11.2 Hz), 6.56 (2H, dd, 2 $\times$ CH(OOH)-CH = CH, J = 11.2, 15.1 Hz), 7.90, 7.91 (2H, s, 2 $\times$ OCOO).