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[10] This was already suggested by the results of the ESMS studies.
[11] The monochromator of a fluorescence spectrophotometer (1-mm slit) was employed (65-W Xenon lamp).

Synthesis and Stability of Phosphatidylcholines Bearing Polyenoic Acid Hydroperoxides at the *sn*-2 Position**

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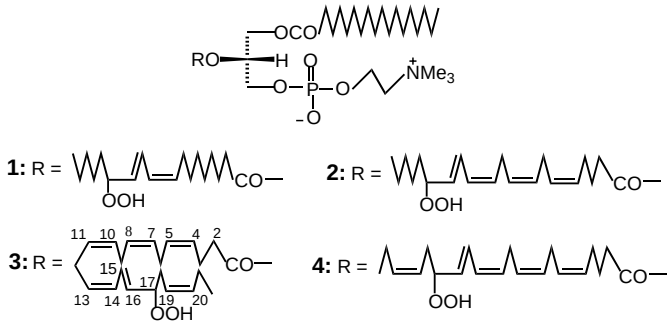
Lipid peroxides, including phospholipid hydroperoxides, have been known to exist in mammalian blood in very low concentrations, which increase during some physiological dysfunctions and aging. Although they are generally considered to have detrimental effects, some reports have described possible beneficial actions of them both in vivo and in vitro.[1] Gonzalez et al. found that administration of a fish oil (menhaden) diet to mice with transplanted human breast cancer significantly reduced the size of the tumors, and showed the involvement of lipid peroxidation products.[1a] Such a diet also significantly protected mice against a strain of the malaria parasite that is resistant to the antimalarial drug chloroquin.[1b] Unfortunately, the nature of the lipid peroxidation products that are responsible for such beneficial effects remains largely unknown. Pure, well-defined lipid peroxides for the necessary biological studies can best be obtained by synthesis. We previously synthesized two phospholipids bearing hydroperoxides of linoleic and arachidonic acids at the *sn*-2 position (PC-LA-OOH (**1**) and PC-AA-OOH (**2**) in Scheme 1, respectively),[2] where PC refers to phosphatidylcholine, and LA-OOH and AA-OOH denote linoleic and arachidonic acid hydroperoxides, and studied their inducement of cell death in normal and cancer cells.



Scheme 1. Phosphatidylcholine hydroperoxides.

The major polyunsaturated fatty acid components in fish oil are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). However, there has been no report on the synthesis of phospholipids bearing DHA or EPA hydroperoxides. These $\omega-3$ fatty acids are known to be very unstable due to their high degree of unsaturation with all-*cis* unconjugated double bonds, and it was considered that the synthesis of their corresponding phospholipid hydroperoxides PC-DHA-OOH (**3**) and PC-EPA-OOH (**4**) by the method we established for **1** might be very difficult. However, with only slight modification, we have now found that it is possible. Typically, the synthesis of **3** (Scheme 2) was achieved by dicyclohexylcarbodiimide(DCC)-mediated esterification of lysophosphatidylcholine **8** with peracetal acid **7**, followed by deprotection of the hydroperoxy group. Compound **8** with *R* configuration was prepared as described previously,[2a] and **7** was synthesized according to Scheme 2.

Docosahexaenoic acid was converted by soybean lipoxygenase to an optically active hydroperoxide, which for easier purification by column chromatography was converted to methyl ester **6** ($[\alpha]_D^{20} = -3$, $c = 1.67$ in $CHCl_3$)[3] followed by protection of the labile hydroperoxy group to give a peracetal methyl ester, which was hydrolyzed by lipase PS (Amano) to give the desired peracetal acid **7**. In the new experimental procedure, omitting the azeotropic removal of residual water from **7** was important for maintaining its structural integrity, while the use of excess DCC facilitated the esterification to give the desired phospholipid **9**. Hydrolytic removal of the

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[**] We are grateful to the API MASS and SC-NMR Laboratories of Okayama University.

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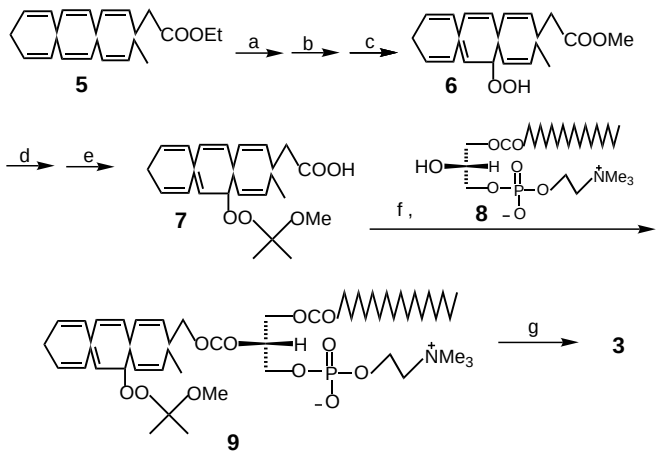
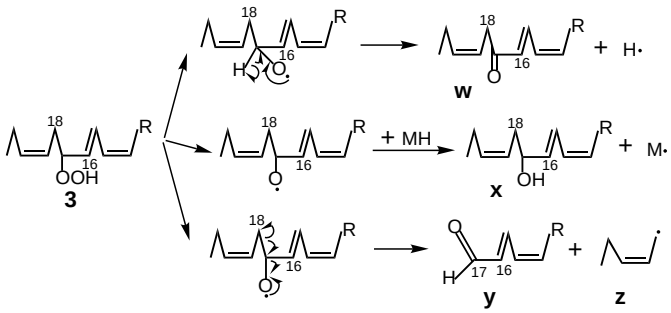


Scheme 2. Synthesis of **3**. a) 1.5 N LiOH, RT; b) O_2, lipoxygenase, borate buffer (pH 9), 0–4 °C, 6 h; c) diazomethane; d) 2-methoxypropene, PPTS, THF, RT; e) lipase, RT; f) DCC, DMAP, $CHCl_3$, RT; g) THF/AcOH/$H_2$0 (4:2:1). DMAP = dimethylaminopyridine, PPTS = pyridinium *p*-toluene sulfonate.



Scheme 3. Homolytic decomposition of **3**. MH refers to an organic compound with readily abstractable hydrogen atoms (such as those of the methylene groups between the remaining unconjugated double bonds in **3**).

peracetal group afforded the target compound **3**, which was purified by reversed-phase HPLC. Its structural integrity was confirmed by its molecular ion peak at *m*/*z* 866.6 $[M+H]^+$ in the electrospray mass spectrum (ES-MS) and by ^{1}H NMR spectroscopy (see Experimental Section). Coloration with potassium iodide further confirmed the presence of the hydroperoxy group, whose assignment at C17 of DHA was based on the known regiospecificity of soybean lipoxygenase I, which oxidizes fatty acids at the $\omega-6$ position,[4] and confirmed by the predominant loss of a C_5 fragment during the decomposition of the compound (vide infra). Chemical shifts and coupling constants ($^3J = 15$ Hz for *trans*, and 11 Hz for *cis*) of the protons of the conjugated diene region confirmed the olefin structure depicted for **3**. Compound **4** was prepared in the same way and confirmed by its molecular ion peak at *m*/*z* 840.6 $[M+H]^+$ and a ^{1}H NMR spectrum very similar to that of **3**.

The stability of these compounds is critical for their convenient use in biological studies. We examined the stability of the more highly unsaturated **3** by ^{1}H NMR spectroscopy and ES-MS, which not only gave simple spectra but could also give information on the major decomposition products. In the structure of **3**, the chemically most labile part is the allylic hydroperoxy group, the weak O–O bond of which is prone to homolytic cleavage, which is expected to be followed by other chemical transformations to produce a ketone **w**, an alcohol **x**, or an aldehyde **y** (Scheme 3).

The NMR signals of the olefinic protons in **6** and in **3** are well characterized for the conjugated diene system at the 14-, 15- and 16-positions, and for the unconjugated systems at the positions 4, 5, 7, 8, 10, 11, 19, and 20 ($\delta = 5.42$, broad), with the signal for H13 overlapping with those of the latter group. However, upon formation of **w** or **y**, the resulting conjugated olefin–carbonyl π-bond systems introduce new and distinct signals in between those for H14, H15, and H16, whose intensities decrease thereby. Thus, decomposition could be easily detected and its extent estimated by the intensity of the signals for the conjugated olefinic protons relative to those of the stable glycerol or choline moieties. New molecular ion peaks in the ES-MS spectrum indicate the kind of decomposition products being formed. When a solution of **3** in chloroform or deuteriochloroform (15 mM) without a radical-scavenging antioxidant was stored at −20 °C, the ^{1}H NMR and ES-MS data remained surprisingly unchanged, even after three weeks. Moreover, in the presence of a trace of the antioxidant butylated hydroxytoluene (BHT), which can be readily removed when necessary,[5] it was even more stable, and the NMR and ES-MS data were unchanged even after three months. However, when kept at room temperature (ca. 30 °C) without BHT, it degraded much faster, and after 3 d there were considerable changes in the NMR spectrum, with an estimated loss of about 40% of **3**. After one week, decomposition was almost complete, and the signals for H14, H15, and H16 disappeared from the NMR spectrum. The main peak in the ES-MS spectrum of this decomposition product was at *m*/*z* 780.6 (100%), which is 86 amu less than that for **3** at *m*/*z* 866.6 (40%). Since its daughter ion at *m*/*z* 184 confirmed the presence of the glycerophosphocholine moiety, it must have been formed by the loss of a $C_5H_{10}O$ fragment from the DHA-OOH moeity, which is in agreement with the hydroperoxy group being at C17 (vide supra) and cleavage between C17 and C18 to produce aldehyde **y**. A peak at *m*/*z* 850.6 (65%), for alcohol **x**, was the second most prominent, while a peak for ketone **w** could not be identified. This differs from our previous report on the decomposition of **1** and similar observations for **2**, for which the major decomposition product corresponded to ketone **w**.[6] A posssible explanation for this is that bond cleavage between C17 and C18 of the DHA-OOH moiety in **3** is favored because the presence of a double bond at C19 leads to formation of a stable allylic radical **z** (Scheme 3), as opposed to the unstable alkyl radical that would form from **1** or **2**. At the present stage, where the true chemical species involved in the beneficial effects of fish oils and their oxidation products are not yet identified, information on the in vitro decomposition products from such phospholipid hydroperoxides as **3** may play an important role in mechanistic studies of their activities.

In summary, two new phospholipid hydroperoxides were synthesized from DHA and EPA, and the stability of the former is sufficient for storage and for use in biological studies.

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Experimental Section

3: All reactions were carried out in the presence of a small amount of BHT and, except for the lipoxygenase-catalyzed hydroperoxidation, under a nitrogen atmosphere. Methyl peracetal acid **7** was prepared from DHA as previously described for linoleic acid,[2a] and isolated by flash column chromatography, followed by drying over $MgSO_4$. After filtration, the solvent was evaporated at below 30 °C, and the resulting **7** (ca. 0.15 g, 0.4 mmol) dissolved in ethanol-free chloroform, followed by the addition of DCC (0.2 g, 0.9 mmol), dimethylaminopyridine (0.012 g, 0.09 mmol) and lyso-PC **8** (0.15 g, 0.3 mmol). The reaction mixture was stirred at room temperature for 48 h, and the product purified on a silica gel column ($CHCl_3$/MeOH/NH_3 (aq.) 60:30:1 to 50:30:3) to give phospholipid **9** (0.09 g, 30 % from **8**), which was then dissolved in THF/AcOH/H_2O (4:2:1) and stirred for 24 h, which achieved complete deprotection of the hydroperoxy group. The product thus obtained was purified by reversed-phase chromatography (ODS, $CHCl_3$/MeOH/H_2O, 4.5:100:5) to give high-purity **3** as a resinous solid (0.065 g, 7 % from **5**). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.88$ (t, 3H; ω-CH_3), 0.95 (t, 3H; ω'-CH_3), 1.26 (m, 32H; $OCO(CH_2)_{16}CH_3$), 2.03 (m, 2H; H21′), 2.28 (m, 1H; one proton of C18′), 2.39 (m, 4H; H2′ and H3′), 2.46 (m, 1H; one proton of C18′), 2.87 (m, 4H; H6′ and H9′), 2.98 (m, 2H; H12′), 3.28 (s, 9H; $N(CH_3)_3$), 3.72 (m, 2H; OCH_2CH_2N), 3.97 (m, 2H; CH_2OP), 4.18 (m, 1H; one proton of $OCH_2CH(OR)CH_2OP$), 4.30 (m, 2H; OCH_2CH_2N), 4.38 (m, 2H; H17′, one proton of $OCH_2CH(OR)CH_2OP$), 5.24 (br s, 1H; $OCH_2CH(OR)CH_2$-OP), 5.42 (m, 9H; H4′, H5′, H7′, H8′, H10′, H11′, H13′, H19′, H20′), 5.64 (dd, $J = 15$, 8 Hz, 1H; H16′), 6.10 (t, $J = 11$ Hz, 1H; H14′), 6.58 (dd, $J = 11$, 15 Hz, 1H; H15′); ES-MS: found: m/z: 866.6; calcd for $[C_{48}H_{84}NO_{10}P+H^+]$: m/z: 866.6.

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Gel-Phase MAS NMR Spectroscopy of a Polymer-Supported Pseudorotaxane and Rotaxane: Receptor Binding to an "Inert" Polyethylene Glycol Spacer**

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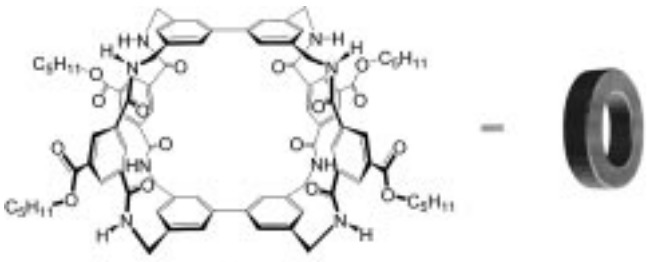
The combination of magic-angle spinning (MAS) and high-resolution probe technology has provided chemists with a powerful technique for obtaining solutionlike ^{1}H NMR spectra of resin-bound samples.[1] As previously described, we have been successful in applying gel-phase MAS spectroscopy to the study of metal – ligand interactions at the solid – liquid interface.[2] Following this success, we set out to extend our understanding of other interactions, such as those between the tricyclic polyamide receptor **1** and carbohydrates. However, during the course of this study, we discovered an unexpected phenomenon which led to the synthesis of a resin-bound rotaxane as described below.



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Macrocycle **1** is a good receptor for octyl pyranosides.[3] Therefore, a galactose residue was attached onto ArgoGel resin through the primary hydroxide group, so that **1** would bind to the sugar unit and allow us to study the binding between the solid and liquid interfaces. ArgoGel resins contain highly flexible polyethylene glycol (PEG) chains (30 – 40 units) appended to a 1 % cross-linked polystyrene bead; the flexibility of these PEG chains allows us to obtain solutionlike NMR spectra using MAS techniques. An NMR titration was performed to investigate for possible binding between **1** and the solid-supported galactoside. However, this titration unexpectedly showed a splitting of the PEG reso-

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