A Regioselective, Stereoselective Synthesis of a Diacylglycerophosphocholine Hydroperoxide by use of Lipoxygenase and Lipase

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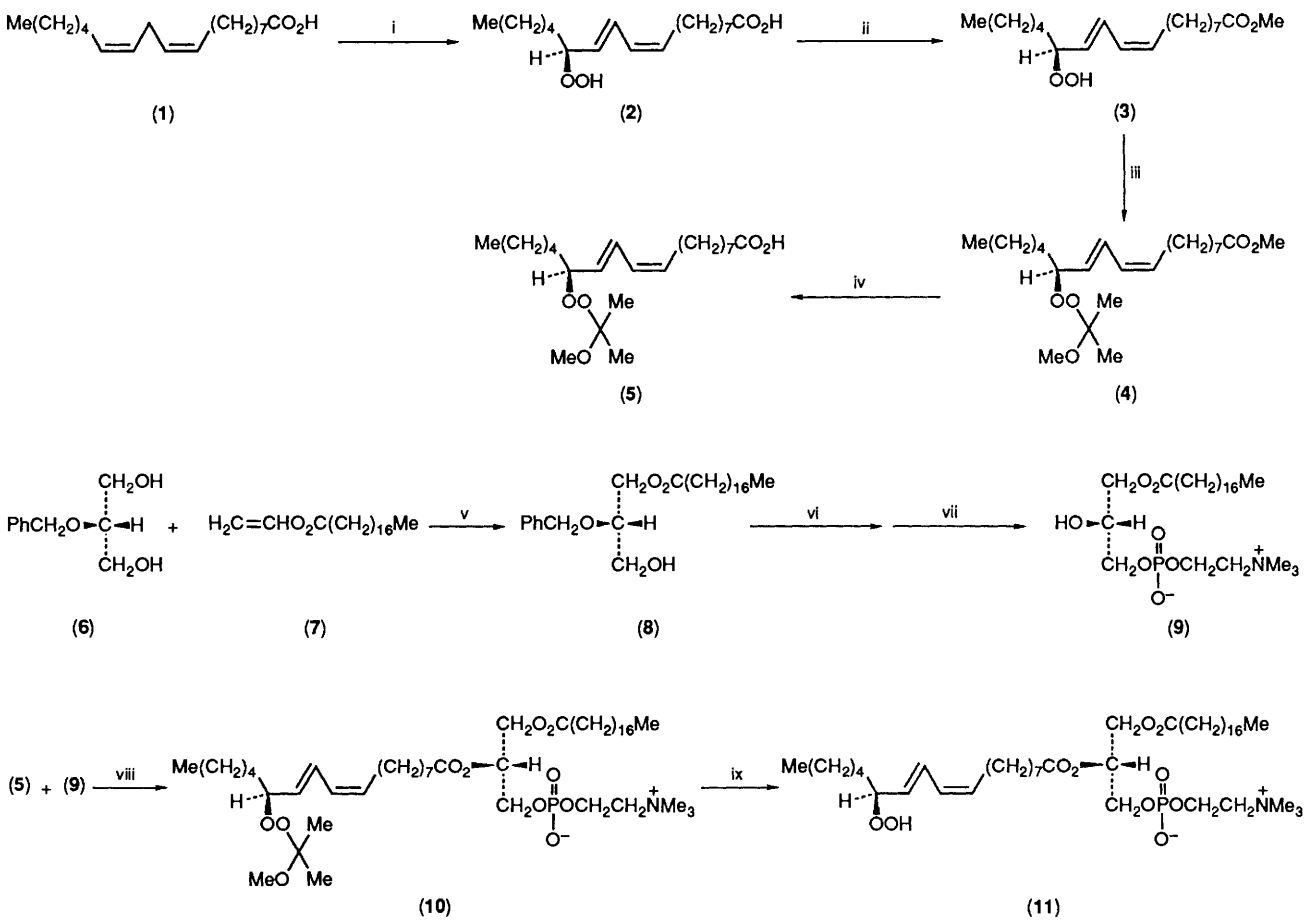
1-Stearoyl-2-[13′-(*S*)-hydroperoxy-(9′*Z*,11′*E*)-octadecadienoyl]-*sn*-glycero-3-phosphocholine (**11**) was synthesized without contamination by any regio- and stereo-chemical isomers by a combination of lipoxygenase-catalysed peroxidation, lipase-catalysed stearoylation, and dicyclohexyl carbodiimide-mediated (DCC) esterification.

The formation of lipid peroxides *in vivo* is considered to be involved in some serious diseases and dysfunctions including arteriosclerosis, cancer, and ageing.[1] In order to investigate the deteriorative effects of lipid peroxides, we need to obtain the peroxides of biologically functional lipids, such as phospholipid hydroperoxides, in a high state of purity and in a large amount. Regio- and stereo-chemically pure phospholipid hydroperoxides, however, have been unavailable, as they are difficult to isolate from a mixture of conventionally prepared peroxidized phospholipids. There has been no report of a synthesis of pure phospholipid hydroperoxides.

We report here the first synthesis of highly pure 1-stearoyl-2-[13′-(*S*)-hydroperoxy-(9′*Z*,11′*E*)-octadecadienoyl]-*sn*-glycero-3-phosphocholine (**11**) through nine steps.

As shown in Scheme 1, linoleic acid (**1**) is converted to hydroperoxide (**2**) with soybean lipoxygenase 1 and then to methyl ester (**3**) with diazomethane in 36% yield. The significant step is the introduction of a protecting group for the hydroperoxy group, which is labile on exposure to an acid, a base, a radical, or an acylation reagent. The protecting group must be stable throughout iv—viii shown in Scheme 1, and be easily removed in the final mild acidic hydrolysis, ix. Dussault and Porter[2] reported that perketal was suitable as a protecting group for a hydroperoxy function during the HPLC analysis.

Ester (**3**) is converted in 78% yield to perketal (**4**), which after purification by silica gel column chromatography using a mixture of hexane and ethyl acetate as eluent, is hydrolysed with lipase from *Pseudomonas fluorescens* or lithium hydroxide to give perketal acid (**5**) in 90 and 99% yield, respectively. The perketal acid is stable in a concentrated state at −18 °C for



Scheme 1. *Reagents:* i, O_2, lipoxygenase; ii, diazomethane; iii, 2-methoxypropene, PPTS; iv, lipase or LiOH; v, lipase; vi, $POCl_3$, choline tosylate; vii, H_2, $Pd(OH)_2$; viii, DCC, DMAP; ix, THF/AcOH/H_2O.

more than 10 days. This indicates that its perketal group is not hydrolysed by the possible catalytic action of a carboxylic group in the same molecule.

Recently, the stereoselective acylation of alcohols with lipase in organic media has been developed,[3] in which a vinyl or isopropenyl ester of carboxylic acids was used as an activated acylation reagent. This approach is promising for the preparation of products having high optical purity because of the irreversible nature of the enzymatic reaction. Thus, the counterpart 1-stearoyl-*sn*-glycero-3-phosphocholine (**9**) was prepared *via* the lipase-catalysed enantiotopic group-specific stearoylation of 2-*O*-benzylglycerol (**6**) with vinyl stearate (**7**). In di-isopropyl ether in the presence of the lipase, the direct acylation of 2-*O*-benzylglycerol with vinyl stearate afforded 1-stearoyl-2-*O*-benzyl-*sn*-glycerol (**8**) in 50% yield and 92% e.e.; the ratio of *S* to *R* forms was 96:4. The stearoylbenzylglycerol (**8**) thus obtained, was converted to 1-stearoyl-*sn*-glycero-3-phosphocholine (**9**) by treatment with phosphorus oxychloride and choline *p*-toluenesulphonate,[4] followed by removal of the benzyl group. Its yield was 49%. By virtue of high yield and availability of starting materials, this synthetic method has a great advantage over the conventional method for the preparation of chiral lysophosphatidylcholines by use of phospholipase A_2.

In chloroform containing DCC and dimethylaminopyridine, the phosphocholine (**9**) was esterified with the perketal (**5**) under an argon atmosphere for 48 h to give phospholipid perketal (**10**), which was purified by silica gel column chromatography using a mixture of chloroform, methanol, and 28% ammonium hydroxide as an eluent.[5] Deprotection of the phospholipid perketal (**10**) obtained was conducted at room temperature, overnight in a mixture of tetrahydrofuran, acetic acid, and water (4:2:1) in the presence of a trace amount of butylated hydroxy toluene to give the hydroperoxide (**11**).[2] The hydroperoxide was purified by reverse-phase column chromatography (RP-8 Rober column) using a mixture of methanol, chloroform, and water (10:1:1) as the eluent[6] and was isolated in 90% yield as a resinous solid. Its structure was confirmed by NMR spectroscopy and mass spectrometry, as well as colouration with potassium iodide. Its purity was proved by silica gel TLC using a mixture of methanol, chloroform, and 28% ammonium hydroxide (15:30:2; $R_F = 0.6$) and by reverse-phase HPLC (ODS) using a mixture of methanol, chloroform, and water (20:2:1; R_F = 26 min). The yield of hydroperoxide (**11**) based on the starting material (**1**) was 10%.†

Here, the regio- and stereo-chemically well-defined diacyl-*sn*-glycerophosphocholine hydroperoxide (**11**) was successfully synthesized. The toxicity of the hydroperoxide toward human endothelial cells is being examined. The present study shows that the enzymes are useful in the synthesis of a phospholipid hydroperoxide, and that the synthetic strategy might be applied to the preparation of other medically and biologically important phosholipids with an acyl moiety bearing a hydroperoxy group.

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† Spectroscopic data for compound (**11**). 1-Stearoyl-2-[13′-(*S*)-hydroperoxy-(9′*Z*,11′*E*)-octadecadienoyl]-*sn*-glycero-3-phosphocholine (**11**). $[\alpha]_D^{25}$ + 2.00 (*c* 1.92 in MeOH/$CHCl_3$, 1:1 v/v); ^{1}H NMR (500 MHz; $CDCl_3$) δ 0.87 (6 H, m, ω-Me × 2), 1.28 (44 H, m, CH_2 × 22), 1.58 (4 H, m, $OCOCH_2CH_2$ in each chain), 2.15 (2 H, m, 8′-H), 2.28 (4 H, m, $OCOCH_2$ in each chain), 3.30 (9 H, s, NMe_3), 3.75 (2 H, m, CH_2N), 3.98 (2 H, m, $-OCH_2$-CH-CH_2O-), 4.14 (1 H, m, one proton of -CH_2O-CH-CH_2O-), 4.34 (4 H, m, one proton of -OCH_2-CH-CH_2O-, $OPOCH_2$, 13′-H), 5.22 (1 H, m, CO-CH-CO), 5.44 (1 H, m, 9′-H), 5.58 (1 H, m, 12′-H), 5.98 (1 H, m, 10′-H), 6.52 (1 H, m, 11′-H); fast atom bombardment mass spectrum, *m/z* 818(MH^+).