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Synthesis of Carbon Analogues of Phosphatidylcholines Having a Polyunsaturated Fatty Acid at the 2-Position

Chiyo Seki[a], Yoshihiro Mori[b], Shuhei Nakajima[a], Sakayu Shimizu and Naomichi Baba[a]

(Department of Bioresources Chemistry)

Carbon analogues of phosphatidylcholines having linoleic or arachidonic acid at the 2-position were synthesized. The synthetic route involves conversion of the polyunsaturated fatty acid esters to their malonic acid diethyl ester derivatives via reduction, tosylation and iodination. The derivatives were converted to diols by $LiAlH_4$ reduction and submitted to lipase-catalyzed monostearoylation in isopropylether. The mono-ester was converted to phosphatidylcholines by the usual phosphodiester synthesis.

Key words : phospholipid, arachidonic acid, synthesis, phospholipase A_2

Introduction

Glycerophospholipids like phosphatidylcholines, phosphatidylinositols and phosphatidylserines have been recognized in recent years to be important not only as constituents of cell membranes but also as biological signal transducers. A number of enzymes which catalyze conversion of glycerophospholipids also plays important roles in biological signal transductions. Phospholipase A_2 widely distribute in many biological systems and has various physiological functions. It has been claimed that one of the important roles of the enzyme is to cleave out oxidized unsaturated fatty acid from damaged phospholipid in cell membrane.[1] This enzyme is also known to be involved in arachidonic acid cascade and when stimulated by some triggers, it catalyzes cleavage of arachidonic ester linkage at *sn*-2 position of glycerophosphocholine.[2] The arachidonic acid thus liberated goes into arachidonic acid cascade in which the acid is converted to eicosanoids like leukotrienes, which cause a variety of undesirable pathological symptoms. Therefore, regulation of enzyme activity upstream stage of the cascade is very important.[3] With this in mind, a lot of studies have been done to find inhibitors for those enzymes aiming at developing new drugs. Carbon analogues (**8**) of glycerophosphocholine have very similar structure, being different only in linkage between polyunsaturated carbon chain and glycerol moiety. In the present study, we developed a new method for synthesis of these carbon analogs which are novel phospholipids and might be utilized for biological studies.

Result and Discussion

Synthesis. The synthetic route is shown in the Scheme. Polyunsaturated fatty acyl groups having all *cis* non-conjugated polyolefinic structure like ethyl arachidonate (**1**) are generally known

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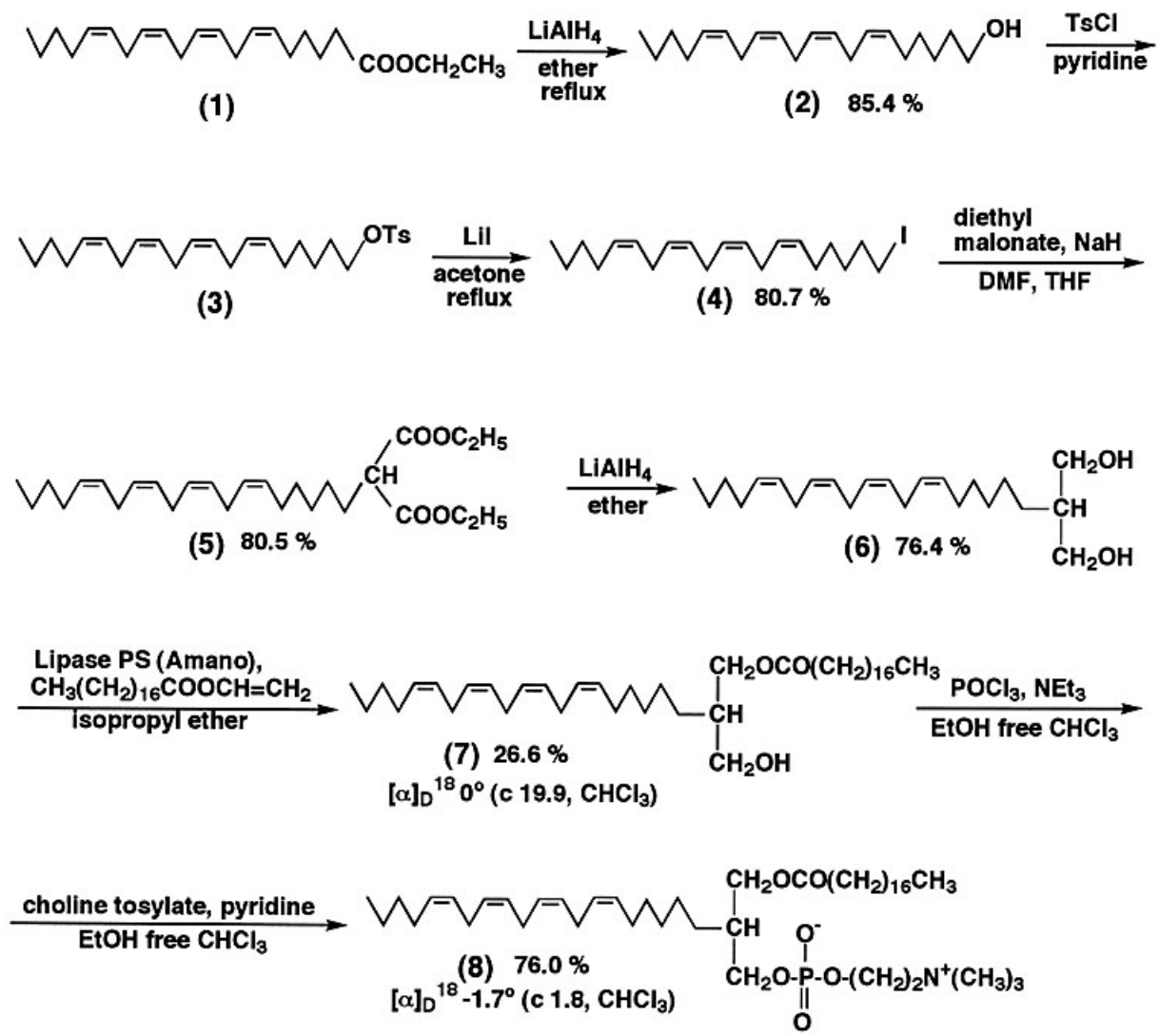
a) *Department of Bioresources Chemistry, Faculty of Agriculture, Okayama University, Tsushimanaka, Okayama 700-8530*
b) *Graduate School of Natural Science and Technology, Okayama University, Tsushimanaka, Okayama 700-8530*
c) *Division of Applied Bioscience, Graduate School of Agriculture, Kyoto University*

to be very unstable in that they are easily oxidized being accompanied by conjugation and *cis*/*trans* isomerization especially in the presence of radical initiators like metal ions, heat and light. Therefore, all the reactions in the present study were done in the presence of butylated hydroxytoluene (BHT) as an antioxidant under nitrogen atmosphere in the dark. Ethyl arachidonate was converted to an alcohol (**2**) by lithium aluminum hydride reduction. The hydroxy group was converted to iodide (**4**) with lithium iode in acetone through the tosylate (**3**) which was obtained using tosyl chloride in pyridine. The iodide (**4**) in a mixture of dimethylformamide (DMF) and THF was treated with diethyl malonate and sodium hydride. Usual work-up and silica gel column purification afforded a diethyl malonate derivative (**5**).[4] This diester was reduced with lithium alminum hydride in ether and, after usual work-up, silica gel column purification afforded the diol (**6**). This diol is considered as a mimic of glycerol structure and may serve as a good substrate for any lipases in the presence of acylation reagent to afford a monoacyl form of the diol in an appropriate organic solvent. Thus, we conducted a lipase-catalyzed acylation of **6** using lipase PS (gift of Amano Pharmaceutical Co. Ltd., Nagoya) and vinyl stearate as an acylation reagent in isopropyl ether. The vinyl stearate may react with



some functional hydroxy group provided by amino acid residue like serine in the enzyme active site, to afford an active ester and vinyl alcohol. Vinyl alcohol liberated to the outside of the active site is unstable and is promptly converted to acetaldehyde. This becomes an important driving force for shifting the equilibrium to give the active ester in the enzyme active site. The activated ester thus formed is attacked by one of the hydroxy groups in the diol (**6**) as a next step to yield the monoacyl form (**7**) in good quantity. The irreversibility renders the enzyme-catalyzed acylation much faster and more effective. By this procedure, monoacyl glycerol was successfully obtained and the structure was confirmed by electrospray MASS and ^{1}H NMR analyses. The stereochemistry of the asymmetric center and its optical purity are however, as yet unknown since chiral unnatural glycerol mimic (**7**) can not be converted to a compound with known-stereochemistry. As a final step, the mono-ester (**7**) was submitted to usual phosphodiester synthesis using phosphorus oxychloride and choline tosylate in the presence of triethylamine and pyridine in ethanol-free chloroform. The reaction was conducted at room temperature in a nitrogen atmosphere in the dark for 48 h. The product was purified by silica gel column eluted with chloroform/methanol/28% NH_3 (aq.) (40: 60: 2) to afford the desired phospholipid (**8**).

Biochemical Studies. There are a number of phospholipases whose sources are snake venom, pancreas, bee venom and other biological systems. Arachidonic acid cascade is an important metabolic pathway of arachidonic acid in which the acid is converted to compounds like leukotriene which operates as a trigger of allergy symptoms. The cascade commences by the liberation of arachidonic acid from *sn*-2 position of phosphatidylcholine by phospholipase A_2 through activation of this enzyme by some stimulation. Therefore, from the medicinal aspect, inhibiting this enzyme with inhibitors is an important subject. Inhibitors of enzymes very often have similar chemical structure to their substrates. From this view, the phosphatidylcholine mimic (**8**) was considered to be a possible inhibitor of phospholipase A_2 since this enzyme catalyses hydrolysis of ester function between fatty acyl group and *sn*-2 position of the glycerol moiety. Thus, we conducted an experiment to measure the inhibitory activity of **8** using pig pancreatic phospholipase A_2 and 2-linoleoyl-1-stearoyl-*sn*-phosphatidylcholine as a substrate in the presence of **8** in varying amounts. This experiment showed that **8** has no inhibitory effect.

The present study established a synthetic route to obtain a novel carbon analog of phosphatidylcholine, and this compound may be used as a mimic of glycerophospholipids. This route can be applied to synthesis of others that have a different polyunsaturated carbon chain at the same position. These mimics may be utilized for experiments on the inhibitory properties in other enzymes relating to glycerophospholipids like phospholipase C and D.

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Spectral data

Ethyl 2-Ethoxycarbonyl-7, 10, 13, 16-docosatetraene-1-ol (**5**) —— ^{1}H NMR (200MHz, $CDCl_3$): δ 0.88 (3H, t, CH_3), 1.25-1.43 (16H, m, $CH_2 \times 5$, $COOCH_2CH_3$), 1.88 (2H, dt, β-CH_2), 2.06 (4H, m, C6, C18-CH_2), 2.08 (6H, m, C9, C12, C15-CH_2), 3.30 (1H, m, a-CH_2), 4.18 (4H, q, $COOCH_2$ $CH_3 \times 2$), 5.34 (8H, m, C7, C8, C14, C11, C13, C14, C16, C17-CH olefine protons).

2-Hydroxymethyl-7, 10, 13, 16-docosatetraene-1-ol (**6**) —— ^{1}H NMR (500MHz, CDC13): δ 0.88 (3H, t, CH_3), 1.25-1.38 (10H, $CH_2 \times 5$), 1.78 (2H, dt, C2'-CH_2 in stearoyl group), 2.06 (4H, m, C6, C18-CH_2), 2.81 (6H, m, C9, C12, C15-CH_2), 3.64-3.84 (4H, m, $CH_2O \times 2$), 5.39 (8H, m, C7, C8, C14, C11, C13, C14, C16, C17-CH olefine protons).

2-Hydroxymethyl-7, 10, 13, 16-docosatetraene-1-ol (**7**) —— ^{1}H NMR (500MHz, $CDCl_3$): δ 0.88 (3H, t, CH_3), 1.25-1.38 (40H, CH_2 x 20), 1.61 (2H, dt, C3'-CH_2 in stearoyl group), 1.78 (2H, dt, C2'-CH_2 in stearoyl group), 2.05 (4H, m, C6, C18-CH_2), 2.30 (2H, m, -CH_2OCO-), 2.80 (6H, m, C9, C12, C15-CH_2), 3.45-3.60 (2H, m, C1-CH), 4.05-4.23 (1H, m, C1-CH), 5.35 (8H, m, C7, C8, C14, C11, C13, C14, C16, C17-CH olefine protons).

2-Hydroxymethyl-7, 10, 13, 16-docosatetraene-1-ol (**8**) —— ^{1}H NMR (500MHz, $CDCl_3$): δ 0.88 (3H, t, CH_3), 1.21-1.36 (40H, $CH_2 \times 20$), 1.57 (2H, m, C3'-CH_2 in stearoyl group), 1.90 (2H, dt, b-CH_2 in stearoyl group), 2.05 (4H, m, C6, C18-CH_2), 2.28 (2H, t, C2'-CH_2), 2.81 (6H, m, C9,C12, C15-CH_2), 3.35 (9H, s, $N(CH_3)3$), 3.64 (1H, m, C1-CH), 3.81 (2H, m, -CH_2-N), 3.84 (1H, m, C1-CH_2), 4.27 (2H, m, O-CH_2CH2N), 5.35 (8H, m, C7, C8, C14, C11, C13, C14, C16, C17-CH olefine protons).

多価不飽和脂肪酸を2位に結合するホスファチジルコリンの炭素アナローグの合成

勢喜 千代・森 義裕[a)]・中島 修平・清水 昌[b)]
馬場 直道
(生物資源化学講座)

自然界に広く存在するホスフォリパーゼA_2はグリセロリン脂質の2位のエステル結合を選択的に切断する酵素であり, 消化, アラキドン酸カスケードの起動, リン脂質過酸化物の代謝等,生理作用に広く関わっている。本研究ではホスフォリパーゼA_2の基質ミメテイックとしてホスファチジルコリンの2位エステル結合が炭素-炭素結合に置き換わった化合物をアラキドン酸とステアリン酸を出発原料としてリパーゼ触媒によるアシル化反応及び有機化学反応によって合成した。

a) 岡山大学大学院自然科学研究科生命分子科学専攻
b) 京都大学大学院応用生命科学専攻