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Lp-PLA$_2$ inhibitory activities of fatty acid glycerols isolated from *Saururus chinensis* roots

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Abstract—(*R*)-Glycerol-monolinoleate **4** and (*R*)-glycerol-monostearate **5** were isolated from the ethyl acetate extracts of *Saururus chinensis* roots and (*R*)- or (*S*)-fatty acid glycerols **4** and **5** were synthesized for confirming their structures and evaluating their inhibitory activities against Lp-PLA$_2$. The (*R*)-**4** and (*S*)-**4** exhibited Lp-PLA$_2$ inhibitory activities with IC$_{50}$ values of 45.0 and 52.0 μM, respectively.

Lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$), platelet-activating factor acetylhydrolase (PAF-AH, EC 3.1.1.47), is a secreted calcium-independent member of the phospholipase A$_2$ superfamily produced mainly by cells of the monocyte–macrophage series, T-lymphocytes, and mast cells.[1] The plasma isoform of Lp-PLA$_2$ is about 80% bound to LDL, with the remaining fraction bound to high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL).[2] Especially, accumulation of oxidized LDL in artery wall is known as early stage of atherosclerosis.[3] Lp-PLA$_2$ hydrolyzes the *sn*-2 fatty acid of oxidatively modified LDL to release two lipid products, oxidized free fatty acid (oxFFA) and lysophosphatidylcholine (lyso-PC).[4] These two products are highly effective inflammatory mediators capable of attracting monocytes and driving the atherogenic process.[4] Leach et al.[5] reported that Lp-PLA$_2$ inhibitor reduced atherosclerotic plaque development in the Watanabe heritable hyperlipidemic (WHHL) rabbit study. Therefore, a number of studies have focused on the development of Lp-PLA$_2$ inhibitors to treat atherosclerosis.

Although various synthetic compounds have been developed as potent Lp-PLA$_2$ inhibitors,[6] thus far, however, naturally occurring Lp-PLA$_2$ inhibitors have not been described, except for SB-253514 and analogues isolated from *Pseudomonas fluorescens* DMS 11579.[7] In our search for naturally occurring Lp-PLA$_2$ inhibitors,[8] ethyl acetate extracts of *S. chinensis* roots exhibited Lp-PLA$_2$ inhibitory activities. Two known (*R*)-glycerol-monolinoleate **4** and -monostearate **5** were isolated by bioassay-guided fractionation[9] and identified by their spectroscopic analysis, synthesis, and comparison with the reported data (Fig. 1).[10,11] Additionally, (*S*)-glycerol-monolinoleate **4** and -monostearate **5** were synthesized for evaluating their Lp-PLA$_2$ inhibitory activities (Scheme 1).

Compound **4**, colorless oil, exhibited a value of $[\alpha]_D^{25}$ +5.0° (*c* 0.2, MeOH) (lit. $[\alpha]_D$ +3.0° (*c* 0.36, MeOH)).[10] The

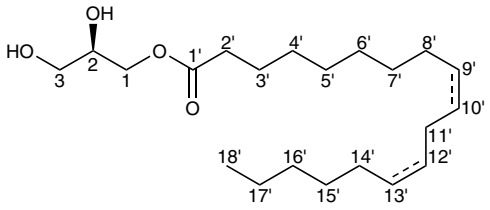

Figure 1. Chemical structures (*R*)-**4** and -**5** of isolated from *S. chinensis*.

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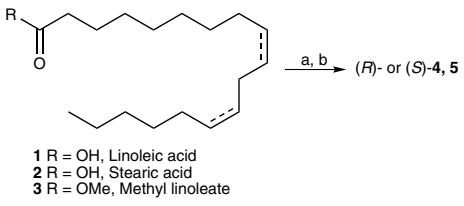
mass spectrum showed molecular ions of *m*/*z* 354 $[M]^+$ and characteristic fragment ions of *m*/*z* 262 [M−2], followed by loss of glycerol in the EI MS data. The ^1H NMR ($CDCl_3$, 500 MHz) showed four olefinic methines at δ_H 5.37 (4H, m, H-9, -10, -12, and -13), α-methylene protons of linoleate at δ_H 2.36 (2H, t, J = 7.5 Hz, H-2′), methylene protons between two olefinic bonds at δ_H 2.77 (2H, t like, J = 5.9 Hz, H-11′), and methyl protons at δ_H 0.89 (3H, t, J = 6.6 Hz, H-18′), respectively. The ^{13}C NMR data indicated ester carbonyl carbon at δ_C 174.3 (C-1′), glycerol group at δ_C 63.3 (C-3), 65.2 (C-1), and 70.3 (C-2), and four olefinic carbons at δ_C 127.9 (C-10′ or 12′), 128.1 (C-12′ or 10′), 130.0 (C-9′ or 13′), and 130.3 (C-13′ or 9′), respectively. Additionally, the stereochemistry of the (2*R*)-glycerol moiety in **4** was determined by comparison of its optical activity to the reported data of (*R*)-glycerol monolinoleate.[10]

Compound **5**, white powder, showed a base peak at *m*/*z* 358 $[M]^+$ and characteristic fragment ions of *m*/*z* 270 [M+2], followed by loss of glycerol in the EI MS data and has mp 71–72 °C (CH_2Cl_2) (lit. mp 70–72 °C).[11] The signals at δ_H 3.60 (1H, dd, J = 5.7, 11.4 Hz, H-3), 3.70 (1H, dd, J = 3.9, 11.4 Hz, H-3), 3.94 (1H, m, H-2), 4.15 (1H, dd, J = 6.6, 11.6 Hz, H-1), and 4.22 (1H, dd, J = 4.7, 11.7 Hz, H-1) indicated the presence of a glycerol group, which was confirmed by its spectroscopic analysis (e.g., EI MS and ^{13}C NMR) and by comparing with reported data.[11] The stereochemistry of the (2*R*)-glycerol moiety in **5** was considered by comparison of its optical activity, $[\alpha]_D^{25}$ −35.0° (*c* 0.25, $CHCl_3$), to the reported data of (*R*)-glycerol stearate, $[\alpha]_D$ −36.36° (*c* 0.055, $CHCl_3$).[11]

Fatty acid derivatives **4** and **5** were prepared to confirm more exact structures and structural specificity against Lp-PLA_2. The results are summarized in Scheme 1. The reaction of linoleic acid **1** (or stearic acid **2**) with (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol and EDC in THF at 0 °C gave the corresponding (*R*)-isopropylidene glycerol-monolinoleate (or -stearate), which was then deprotected with Dowex 50W × 2 to produce (*R*)-glycerol-monolinoleate **4** (or -stearate **5**) in good yield.[11] To obtain their enantiomers **4** and **5**, linoleic acid **1** (or -stearic acid **2**) was treated with (*R*)-(−)-2, 2-dimethyl-1,3-dioxolane-4-methanol under similar reaction conditions to give the corresponding (*S*)-(+)-glycerol-monolinoleate **4** (or -stearate **5**). The synthetic compounds (2*R*)-**4** and -**5** were identical with the spectroscopic data of compounds **4** and **5** that were isolated from *S. chinensis*. Therefore, the structures of compounds **4** and **5** were elucidated to be (*R*)-(+)-glycerol 1-9(*Z*),12(*Z*)-octadecadienate (**4**) and (*R*)-(−)-glycerol 1-octadecanate (**5**). Also, stereochemistry of the glycerol group in isolated compounds **4** and **5** was determined by comparing the optical activities of the synthetic compounds (*R*)-**4** and -**5** with $[\alpha]_D^{25}$ +3.7° (*c* 0.2, MeOH) and $[\alpha]_D^{25}$ −32.0° (*c* 0.25, $CHCl_3$), respectively, to those of isolated compounds **4** and **5** with $[\alpha]_D^{25}$ +5.0° (*c* 0.2, MeOH) and $[\alpha]_D^{25}$ −35.0° (*c* 0.25, $CHCl_3$), respectively.



Scheme 1. Reagents and conditions: (a) (*S*)-(+)- or (*R*)-(−)-2,2-dimethyl-1,3-dioxolane-4-methanol, EDC, THF, 0 °C; (b) Dowex 50W × 2, MeOH, rt.

Although 1-fatty acid glycerols[10] and 2-fatty acid glycerols[12] have been reported to show various biological activities, the potential of compounds **4** and **5** as inhibitors of Lp-PLA_2 (LDL-PLA_2) was evaluated for the first time according to a previous testing method.[13] Compound (2*R*)-**4** exhibited moderate Lp-PLA_2 inhibitory activity with an IC_{50} value of 45.0 μM, whereas (2*R*)-**5** showed a very weak inhibitory activity (20% inhibition at 25 μM). Their enantiomers (2*S*)-**4** and -**5** were assessed for structural specificity against Lp-PLA_2, resulting in a very similar degree of inhibitory activity with an IC_{50} value of 52.0 μM and inhibition of 21% at 25 μM, respectively. These results suggested that the fatty acid group is concerned with biological activity, regardless of the stereochemistry of the glycerol moiety in compounds **4** and **5**. In general, polyunsaturated fatty acid analogues have been known to exhibit anti-inflammatory activity,[14] in vitro or in vivo antioxidant activity,[15] and beneficial effects on the risk factors of coronary heart disease (CHD).[16] Based on these results, linoleic and stearic acids (**1** and **2**) and methyl linoleate **3**, purchased from Sigma–Aldrich Co., were tested tentatively for their Lp-PLA_2 inhibitory activities, resulting in being inactive against Lp-PLA_2.

(*R*)- or (*S*)-Fatty acid glycerol **4**, isolated first from the EtOAc extracts of *S. chinensis* or synthesized from fatty acid **1**, exhibited moderated Lp-PLA_2 inhibitory activities. Furthermore, the in vivo efficacy tests of cholesterol lowering and antiatherogenic activity of **4** are underway.

Acknowledgment

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9. *Procedure for extraction and isolation.* The dried roots of *S. chinensis* (1.0 kg), which was collected in Guchang, Korea, were extracted three times each with EtOAc (4 L × 3) for 10 days. The EtOAc extracts (40 g) were subjected to silica-gel column chromatography (230–400 mesh, Merck) with a gradient elution of *n*-hexane/EtOAc (9:1, 1.0 L; 9:5, 1.0 L; 1:1, 1.0 L; 5:9, 1.0 L; 1:9, 1.0 L) to afford five fractions. The active fraction 3 (3.5 g) was purified by column chromatography on SiO_2 with a gradient elution of $CHCl_3/(CH_3)_2CO$ (95:5, 1 L; 9:1, 1 L; 85:15, 1 L; 80:20, 1 L, 75:25, 1 L) to obtain five fractions. The active fraction 5 (1.5 g) was purified by reverse-phase column chromatography (ODS-A, RP-18; 70–230 mesh; YMC-Gel), eluting successively with an isocratic of aqueous MeOH (90%) to produce compound **4** (3.7 mg) as a colorless oil and compound **5** (6.2 mg) as a white powder.
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