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A Phospholipase A_2 Inhibitor from *Arisaema amurense* Max. var. *serratum* Nakai

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Phospholipase A_2 [EC 3.1.1.4] (PLA_2) catalyzes the hydrolysis of the sn-2 fatty acyl ester bond of glycerophospholipids (Dennis, 1983) and exists in both extracellular and intracellular forms (Bosch, 1980). PLA_2 has recently received much attention because the release of arachidonic acid by PLA_2 from cellular membrane seems to be the rate-limiting step in the biosynthesis of pro-inflammatory eicosanoids, such as prostaglandins, leukotrienes, thromboxanes (Johnson, *et al.*, 1983). Inhibition of phospholipase A_2 may therefore be therapeutically beneficial to the control of inflammation.

For the development of new antiinflammatory agents from natural sources, we screened many extracts of medicinal plants for the inhibitory activity against PLA_2. As a result, we found that the extract of *Arisaema amurense* Max. var. *serratum* Nakai (Araceae) showed strong PLA_2 inhibitory activity. Several *Arisaema* spp. are used as folk medicines for the treatment of rheumatism, ulcer of digestive tract and cancer. Investigation of the PLA_2 inhibitory principles of the extract led us to the isolation of 2,3-dihydroxypropyl 9Z,12Z-octadecadienoate. We wish to report here the isolation of 2,3-dihydroxypropyl 9Z,12Z-octadecadienoate as a PLA_2 inhibitory principle in *Arisaema amurense* Max. var. *serratum* Nakai.

The dried plant of *A. amurense* Max. var. *serratum* Nakai (6 kg) purchased from a folk medicine market in Seoul was extracted by soaking in $CHCl_3$-MeOH (1 : 1) for a day at room temperature. The extract was evaporated under reduced pressure and the residue (44.3 g) was chromatographed over silica gel columns to give a PLA_2 inhibitory compound (0.32 g).

The compound shows bands for hydroxyl groups at 3400 cm^{-1} and an ester carbonyl group at 1750 cm^{-1} in the IR spectrum. Also, the compound shows a molecular ion at m/z 354 [M^+] and characteristic fragmentation ions at m/z 323 [M^+-CH_2OH], 305 [323-H_2O]$^+$, 293 [M^+-CH_2OH-CHOH] and 279 [$C_{18}H_{30}O_2$, octadecadienoyl]$^+$ in the EI-MS spectrum and the fragmented ions suggested that the isolated compound is a glycerol derivative in which a octadecadienoyl group is attached to the C-1 position. The presence of 2,3-dihydroxypropyl group has been further confirmed from the ^{1}H-NMR spectrum ($CDCl_3$, 500 MHz) of the compound, which shows signals of two pairs of geminally coupled protons which should be assigned from two methylene groups attached to a chiral carbon atom at 3.60 (dd, J=5.8, 11.5 Hz, H-3a), 3.70 (dd, J=4.0, 11.5 Hz, H-3b), 4.15 (dd, J=6.1, 11.7 Hz, H-1a), 4.20 (dd, J=4.7, 11.7 Hz, H-1b) ppm. The signal of the proton (H-2) of the chiral carbon atom is observed as a multiplet at 3.93 ppm. The couplings H-2 with H-1 and H-3 are confirmed in its the ^{1}H-^{1}H COSY spectrum. Furthermore, the ^{13}C-NMR and DEPT spectra ($CDCl_3$, 125 MHz) establish the signals of one oxygenated methine carbon atom at 70.8 ppm (C-2) and two oxygenated methylene carbon atoms at 63.4 (C-3) and 65.2 ppm (C-1). The ester carbonyl carbon signal is observed at 174.3 ppm in its ^{13}C-NMR spectrum. Additionally, the signals for protons of the aliphatic chain are observed at 0.88 (t, J=6.8 Hz, 3H, CH_3), 1.25 (m, 8H, H-5', 6', 16', 17'), 1.31 (m, 6H, H-4',7',15'), 1.63 (m, 2H, H-3'), 2.04 (m, 4H, H-8',14'), 2.35 (t, J=7.6 Hz, 2H, H-2'), 2.77 (m, 2H, H-11') ppm and for 4 olefinic protons at 5.34 (m, 4H, H-9', 10', 12', 13'). The ^{13}C-NMR spectrum shows signals of four olefinic carbons at 127.9, 128.1, 129.7 and 130.0 ppm. The DEPT spectrum of the compound establishes twelve methylene carbon signals at 22.6 (C-17'), 24.9 (C-16'), 25.6 (C-11'), 27.2 (C-8', 14'), 29.2 (C-3'), 29.5 (C-4'), 29.7 (C-5'), 29.8 (C-6'), 31.5 (C-15'), 31.9 (C-7'), 34.2 (C-2') ppm and one methyl carbon signal at 14.1 (C-18') ppm. These spec-

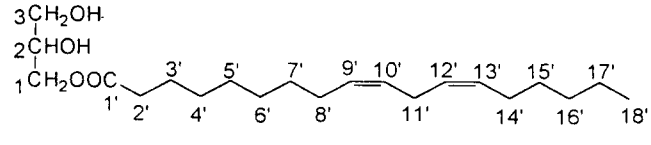

Fig. 1. The structure of phospholipase A_2 inhibitor isolated from *Arisaema amurense* Max. var. *serratum* Nakai (Araceae)

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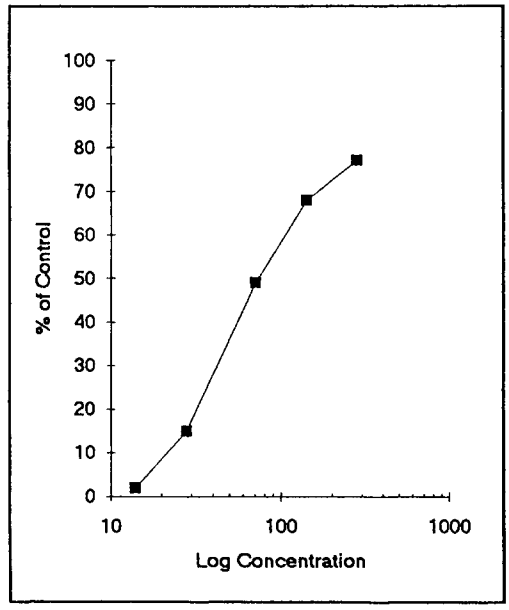

Fig. 2. Inhibition of phospholipase A_2 activity by 2,3-dihydroxypropyl 9Z,12Z-octadecadienoate. PLA_2 activity is measured by using 1-stearoyl-2-[1-^{14}C]arachidonyl-L-3-phosphatidylcholine as a substrate. The reaction mixture containing Tris-HCl buffer (100 mM, pH 7.4), $CaCl_2$ (3 mM), substrate (40 μM) and enzyme is incubated at 37°C for 40 minutes. The reaction is terminated by addition of 1.25 ml of Dole's reagent and the free fatty acid extracted with n-heptane is subjected to liquid scintillation countings.

tral data infer that the fatty acid attached to glycerol is octadecadienoic acid. Comparison of these spectral data with those of the 2,3-dihydroxypropyl 9Z,12Z-octadecadienoate reported by Gunstone (1991) indicates the the isolated PLA_2 inhibitory principle is 2,3-dihydroxypropyl 9Z,12Z-octadecadienoate (Fig. 1).

The compound was isolated from the roots of *Cymbidium*, Orchidaceae (Stoessl, 1980). It was reported that the value for (R)-(Z,Z)-form of this compound is -5.2° (c=1.56 in methanol) and the value for (S)-(Z,Z)-form is +5.4° (c=1.16 in methanol) (Daubert, B.F., *et al.*, 1944). However the isolated compound shows an optical rotational value, -0.51° (c=1.16) in methanol. Currently, we assume that the isolated compound is a racemic mixture. When the inhibitory activity against PLA_2 from *Naja naja* snake venom is measured by employing the method reported by Tanaka, *et al.* (1992), 2.3-dihydroxypropyl 9Z,12Z-octadecadienoate shows an IC_{50} value 75 μM (Fig. 2). 2,3-Dihydroxypropyl 9Z,12Z-octadecadienoate was reported to have antiviral, antibacterial activity (Stoessl, *et al.*, 1980) and antiinflammatory activity. Thus, it should be reasonable to assume that the antiinflammatory activity of *Arisaema amurense* Max. var. *serratum* Nakai may be originated from inhibition of PLA_2 by 2,3-dihydroxypropyl 9Z,12Z-octadecadienoate.

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