ISOLATION OF TWO 1-*O*-ALKYL-*sn*-GLYCERO-3-PHOSPHOCHOLINES FROM THE EARTHWORM, *PHERETIMA ASIATICA*

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Two 1-*O*-alkyl-*sn*-glycero-3-phosphocholines were isolated from the dried body walls of earthworms, *Pheretima asiatica* MICHAELSEN (Megascolecidae), in excellent yield. They were identified by ^1H -NMR and mass spectrometry as 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine (1) and 1-*O*-pentadecyl-*sn*-glycero-3-phosphocholine (2).

KEYWORDS 1-*O*-alkyl-*sn*-glycero-3-phosphocholine; earthworm; *Pheretima asiatica*; Megascolecidae

The dried body walls of Lumbricus species, such as *Pheretima asiatica* MICHAELSEN (Megascolecidae) and *Allolobophora caliginosa trapezoides* ANT. DRUGES (Lumbricidae), have been known as the Chinese crude drug "Jiryu" and used as a depressant, an anticonvulsant and an antifebrile in Southeast Asia.¹⁾ Chemical investigations on the ingredients of the Lumbricus species have been carried out by many investigators,²⁾ and so far the presence of fatty acids, sterols and their esters as well as various amino acids has been reported. With regard to the phospholipids, Okamura *et al.*³⁾ examined the lipid composition of the ventral nervous systems of earthworms, *Lumbricus terrestris*, and identified phosphatidylethanolamine, phosphatidylserine and phosphatidylcholine with a number of fatty acids.

During our study of phospholipids, we found that the commercial crude drug "Jiryu" (dried body walls of *P. asiatica*) contains large quantities of phosphorylglycerylether. This communication describes the isolation and identification of two 1-*O*-alkyl-*sn*-glycero-3-phosphocholines.

The MeOH extract (130g) of the crude drug (1 kg), purchased from Tochimoto Tenkaido, was treated with CHCl_3 -MeOH- H_2O (1:2:1), and the lower phase was collected and concentrated to give a brown extract (75 g). It showed a main blue spot [R_f 0.26, CHCl_3 -MeOH- H_2O (5:5:1), silica-gel HPLC, (Merck, Art 5556), Dittmer reagent] together with two minor spots. This was separated on silica-gel column [CHCl_3 -MeOH (6:4) $\rightarrow$ CHCl_3 -MeOH-28% NH_4OH (6:4:1)] and ODS (MeOH) to give a yellow powder (3.0 g, R_f 0.26). It was crystallized from AcOEt-MeOH (15:1) to give colorless needles (2.0 g, 1.7% yield based on the extract). HPLC analysis revealed that the crystal is a mixture consisting of at least eight compounds (Fig. 1). Separation of the mixture by preparative HPLC using ODS column (97% MeOH) followed by recrystallization from AcOEt-MeOH (15:1) gave compounds 1 (1.2 g) and 2 (0.45 g).

Compound (1), colorless needles, mp 190°C (dec.), $[\alpha]_D^{20} - 5.8^\circ$ ($c=1.0$, CHCl_3 -MeOH, 1:1), -3.5° ($c=1.0$, MeOH); FD-MS m/z (%): 586 [$\text{M}+\text{choline}(104)+\text{H}$] $^+$ (33.6), 104 (100); FAB-MS m/z (%): 482 ($\text{M}+\text{H}$) $^+$ (45), 184 (100); ^1H -NMR (CD_3OD , 600MHz) δ : 0.89 (3H, t, $J=7\text{Hz}$, CH_3), 1.20~1.37 (24H, br s), 1.56 (2H, m, $-\text{O}-\text{CH}_2\text{CH}_2-$), 3.22 (9H, s, $\text{N}-\text{CH}_3$), 3.46 (2H, t, $J=10.0\text{ Hz}$), 3.48 (2H, dd, $J=5.0, 10.0\text{Hz}$), 3.63 (2H, ddd, $J=1.5, 2.5, 6.4\text{Hz}$), 3.83~3.96 (3H, m), 4.28 (2H, m, $\text{P}-\text{O}-\text{CH}_2-$); ^{13}C -NMR (CD_3OD , 150MHz) δ : 14.5 (C-16), 23.7 (C-15), 27.2 (C-3),

30.5 (C-14), 30.7~30.8 (10 C), 33.1 (C-2), 54.7 (N-CH₃), 60.4* (C-1"), 67.6* (C-3'), 68.5* (C-2"), 71.1* (C-2'), 72.7 (C-1'), 73.0 (C-1). [the signals marked with the asterisk appear as doubles ($J=5-8\text{Hz}$) because of the coupling with ^{31}P].

Compound (2), colorless needles, mp 185°C (dec.), $[\alpha]_{\text{D}}^{20} - 4.0^\circ$ ($c=1.0$, MeOH); FD-MS m/z (%): 572 ($\text{M}+\text{choline}+\text{H}$)⁺ (39), 104 (100); FAB-MS m/z : 468 ($\text{M}+\text{H}$)⁺ (75), 184 (100); $^1\text{H-NMR}$ (CD_3OD) δ : 0.89 (3H, t, $J=7\text{Hz}$, CH₃), 1.20~1.47 (24H, br s), 1.57 (2H, m, -O-CH₂CH₂-), 3.22 (9H, s, N-CH₃), 3.46 (2H, t, $J=10.0\text{ Hz}$) 3.48 (2H, dd, $J=5.0, 10.0\text{Hz}$), 3.64 (2H, ddd, $J=1.5, 2.5, 6.4\text{Hz}$), 3.83~3.96 (3H, m), 4.28 (2H, m, P-O-CH₂-); $^{13}\text{C-NMR}$ (CD_3OD , 150MHz) δ : 14.4 (C-15), 23.7 (C-14), 27.2 (C-3), 30.4 (C-13), 30.6~30.8 (9 C), 33.0 (C-2), 54.7 (N-CH₃), 60.4* (C-1"), 67.5* (C-3'), 68.5* (C-2"), 71.1* (C-2'), 72.8 (C-1'), 72.9 (C-1).

The FAB- and FD-MS of 1 showed peaks at m/z 482 and 586, which were assignable to ($\text{M}+\text{H}$)⁺ and [$\text{M}+\text{choline}$ (104) +H]⁺ ions,⁴⁾ respectively. Its $^1\text{H-NMR}$ spectrum showed the signal at δ 3.22 (9H, s) due to the choline group, and the signals at δ 3.43~4.28 (11H), δ 1.56 (2H, m) and 0.89 (3H, t, $J=7\text{Hz}$) along with those at δ 1.20~1.37 assignable to the methylene groups. Its spectrum was superimposable on that of an authentic sample, 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine (Sigma). The specific rotation of 1 was almost the same as that reported by M. Ohno *et al.*⁵⁾ Thus, 1 was identified as 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine.

Compound (2) gave quite similar $^1\text{H-NMR}$ spectrum to that of 1. Its FAB- and FD-MS showed the peaks at m/z 468 ($\text{M}+\text{H}$)⁺ and 572 ($\text{M}+\text{choline}+\text{H}$)⁺, respectively, which were 14 mass units less than those of 1. These data showed that 2 differs only in the alkyl group consisting of the C_{15:0} alkyl chain. The configuration at C2 position of 2 is considered to be the same as that of 1 from the specific rotation. Accordingly, compound 2 is 1-*O*-pentadecyl-*sn*-glycero-3-phosphocholine. Compounds 1 and 2 are precursors of platelet activating factors (PAF), and the former was recently isolated from the hydroid *Solanderia secunda*⁶⁾ and the starfish *Culcita novaeguineae*.⁷⁾

Fig. 1. HPLC of the Crystal

We now found that the gram order quantities of C_{16:0} lyso PAF together with odd-numbered (C_{15:0}) lyso PAF can be obtained in the pure state from 1 kg of the crude drug.

Earthworms belong to the annelids, so studies of the phosphorylglycerylethers of some annelids are in progress.

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