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 ^{1}H-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) -> $CHCl_3$-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° (*c*=1.0, $CHCl_3$-MeOH, 1:1), – 3.5° (*c*=1.0, MeOH); FD-MS *m/z* (%): 586 $[M+choline(104)+H]^+$ (33.6), 104 (100); FAB-MS *m/z* (%): 482 $(M+H)^+$ (45), 184 (100); ^{1}H-NMR (CD_3OD, 600MHz) δ: 0.89 (3H, t, *J*=7Hz, $C\underline{H}_3$), 1.20~1.37 (24H, br s), 1.56 (2H, m, -O-$CH_2C\underline{H}_2$-), 3.22 (9H, s, N-$C\underline{H}_3$), 3.46 (2H, t, *J*=10.0 Hz), 3.48 (2H, dd, *J*=5.0, 10.0Hz), 3.63 (2H, ddd, *J*=1.5, 2.5, 6.4Hz), 3.83~3.96 (3H, m), 4.28 (2H, m, P-O-$C\underline{H}_2$-); ^{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-CH_3), 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=5-8Hz) because of the coupling with ^{31}P].

Compound (**2**), colorless needles, mp 185°C (dec.), $[\alpha]_D^{20}$ – 4.0° (c=1.0, MeOH); FD-MS m/z (%): 572 $(M+choline+H)^+$ (39), 104 (100); FAB-MS m/z: 468 $(M+H)^+$ (75), 184 (100); ^{1}H-NMR (CD_3OD) δ: 0.89 (3H, t, J=7Hz, $C\underline{H}_3$), 1.20~1.47 (24H, br s), 1.57 (2H, m, -O-$CH_2C\underline{H}_2$-), 3.22 (9H, s, N-$C\underline{H}_3$), 3.46 (2H, t, J=10.0 Hz) 3.48 (2H, dd, J=5.0, 10.0Hz), 3.64 (2H, ddd, J=1.5, 2.5, 6.4Hz), 3.83~3.96 (3H, m), 4.28 (2H, m, P-O-$C\underline{H}_2$-); ^{13}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-CH_3), 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 $(M+H)^+$ and [M+choline (104) +H]$^+$ ions,[4] respectively. Its ^{1}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=7Hz) 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}H-NMR spectrum to that of **1**. Its FAB- and FD-MS showed the peaks at m/z 468 $(M+H)^+$ and 572 $(M+choline+H)^+$, 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]

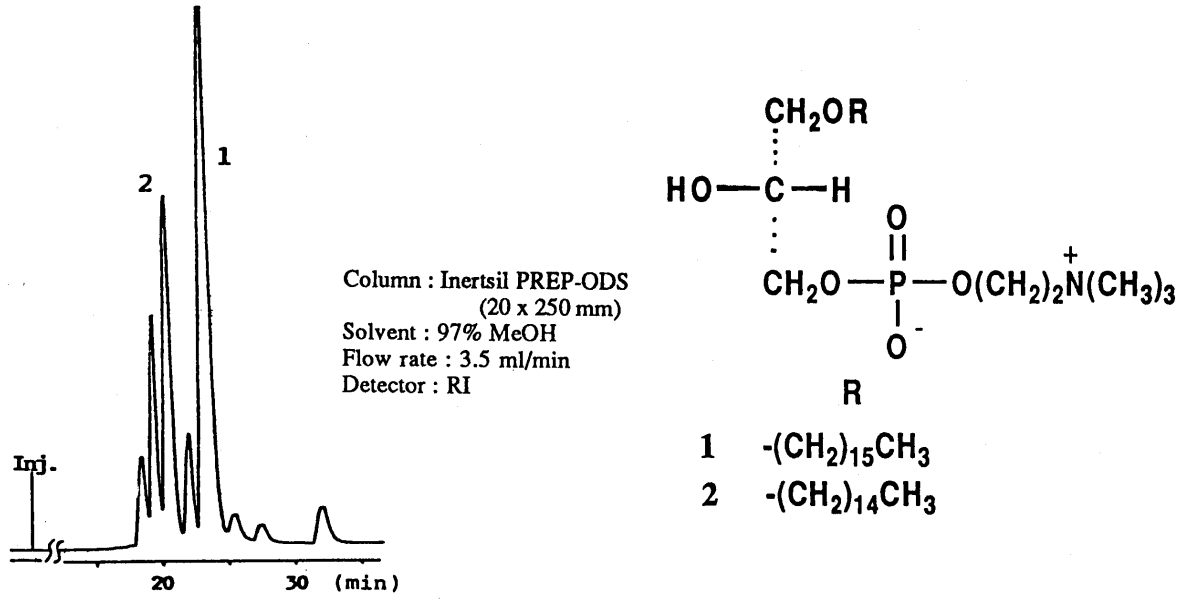

Fig. 1. HPLC of the Crystal

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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REFERENCES

1) Y. E. Kim, W. K. Lee, C. S. Lee and H. J. Yoon, *Yakhak Hoeji*, **25**, 137 (1981).

2) Y. Naka and M. Kotake, *Bull. Chem. Soc. Jpn.*, **40**, 880 (1967). R. P. Hansen and Z. Czochanska, *J. Sci. Fd Agric.*, **26**, 961 (1975). K. Okamoto and M. Kametaka, *Agric. Biol. Chem.*, **52**, 2379 (1988).

3) N. Okamura, M. Stoskopf, H. Yamaguchi and Y. Kishimoto, *J. Neurochem.*, **45**, 1875 (1985).

4) A. Tokumura, K. Kamiyasu, K. Takauchi and H. Tsukatani, *Biochem. Biophys. Res. Commun.*, **145**, 415 (1987).

5) M. Ohno, K. Fujita, H. Nakai, S. Kobayashi, K. Inoue and S. Nojima, *Chem. Pharm. Bull.*, **33**, 572 (1985).

6) N. Fusetani, K. Yasukawa, S. Matsunaga and K. Hashimoto, *Comp. Biochem. Physiol.*, **83B**, 511 (1986).

7) M. Iorizzi, L. Minale, R. Riccio, T. Higa and J. Tanaka, *J. Nat. Prod.*, **54**, 1254 (1991).

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