Isolation and Characterization of Eight 1-*O*-Alkyl-*sn*-glycero-3-phosphocholines from the Crude Drug "Jiryu," the Earthworm, *Pheretima asiatica*[1)]

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Eight 1-*O*-Alkyl-*sn*-glycero-3-phosphocholines (lyso platelet-activating factor; lyso PAF) were isolated from the Chinese crude drug "Jiryu," dried body walls of the earthworm, *Pheretima asiatica* MICHAELSEN (Megascolecidae). They were identified by ^{1}H-NMR and mass spectrometry as 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine (1), 1-*O*-pentadecyl-*sn*-glycero-3-phosphocholine (2), 1-*O*-tetradecyl-*sn*-glycero-3-phosphocholine (3), 1-*O*-13-methyltetradecyl-*sn*-glycero-3-phosphocholine (4), 1-*O*-14-methylpentadecyl-*sn*-glycero-3-phosphocholine (5), 1-*O*-15-methylhexadecyl-*sn*-glycero-3-phosphocholine (6), 1-*O*-heptadecyl-*sn*-glycero-3-phosphocholine (7) and 1-*O*-octadecyl-*sn*-glycero-3-phosphocholine (8).

Keywords 1-*O*-alkyl-*sn*-glycero-3-phosphocholine; lyso platelet-activating factor; Jiryu; earthworm; *Pheretima asiatica*; Megascolecidae

The dried body walls of Lumbricus species, such as *Pheretima asiatica* MICHAELSEN,[2)] *P. aspergillum E.* PERRIER[3)] (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.[4)] Chemical investigations on the ingredients of the earthworms have been carried out by many research groups[5)] and so far the presence of fatty acids, phospholipids,[6)] sterols and their esters as well as of various amino acids has been reported. With regard to 1-*O*-alkyl-*sn*-glycero-3-phosphocholine (lyso platelet-activating factor; lyso PAF), two compounds, 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine and 1-*O*-tetradecyl-*sn*-glycero-3-phosphocholine together with 1-*O*-(1-hexadecenyl)-*sn*-glycero-3-phosphoethanolamine were isolated from the hydroid *Solanderia secunda*, by N. Fusetani and his co-workers.[7)] The first compound has also been isolated quite recently from the starfish *Culcita novaeguineae* by M. Iorizzi *et al.*[8)]

In the course of our search for compounds having antiepileptic activity in natural sources, the commercial crude drug, "Jiryu," was found to contain large quantities of phosphorylglycerylethers. As previously reported,[1)] two 1-*O*-alkyl-*sn*-glycero-3-phosphocholines (lyso PAF) were isolated in the pure state from this crude drug and were characterized as 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine (**1**) and 1-*O*-pentadecyl-*sn*-glycero-3-phosphocholine (**2**).

On further examination of the mixture of phosphorylglycerylethers, six new lyso PAFs together with compounds **1** and **2** were obtained. In this paper we describe the isolation and characterization of these compounds.

The mixture previously obtained as a crystal[1)] was subjected to preparative high performance liquid chromatography (HPLC) to give six pure phosphorylglycerylethers, **3**—**8** [positive to Dittmer reagent[9)]], in addition to compounds **1** and **2**.

Compound **3**, colorless needles, mp 122—144 °C (dec.), $[\alpha]_D$ −4.9° exhibited a $[M+H]^+$ ion peak at *m/z* 454 in fast atom bombardment mass spectrometry (FAB-MS) and a $[M+\text{choline}(104)+H]^+$ ion peak[10)] at *m/z* 558 in field desorption mass spectrometry (FD-MS), both of which were 28 mass units less than those of **1**. The proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra of **3** were respectively the same as those of **1** except for the integral value of the proton signals due to the methylene groups. From these findings, it was considered to be 1-*O*-tetradecyl-*sn*-glycero-3-phosphocholine. The configuration at C-2 of the glycerol moiety was regarded to be *R* by comparison of its specific rotation with that of the compound reported by N. Fusetani *et al.*[7)]

Compound **4**, colorless needles, mp 200—205 °C (dec.), $[\alpha]_D$ −2.3° gave the same molecular ion peaks at *m/z* 468 $[M+H]^+$ and 572 $[M+\text{choline}+H]^+$ in the FAB- and FD-MS spectra, respectively, as those of **2**. Its ^{1}H-NMR spectrum was similar to that of **2** except for one doublet (6H) assignable to two secondary methyl groups instead of a triplet (3H) due to the primary methyl in **2**. Moreover, the ^{13}C-NMR spectrum of **4** was also similar to that of **2**, but a signal at δ 14.4 arising from the primary methyl carbon was absent, instead, a signal at δ 22.9 ascribable to the secondary methyl carbon appeared. Therefore, compound **4** is different from **2** only in the alkyl moiety and it has a C_{15} monomethyl-branched alkyl chain attached.

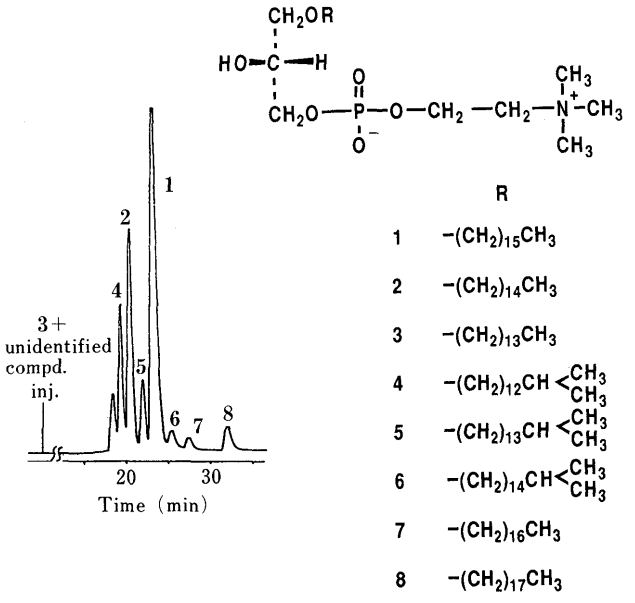

Fig. 1. HPLC Separation of Phosphorylglycerylethers

Column, (Inertsil Prep-ODS) 20.0×250 mm; solvent, 97% MeOH; flow rate, 3.5 ml/min; chart speed, 12 cm/h; detector, RI.

Accordingly, the structure of **4** was assigned the structure 1-*O*-13-methyltetradecyl-*sn*-glycero-3-phosphocholine.

Compound **5**, colorless needles, mp 188—202 °C, $[\alpha]_D$ −4.0°, showed, in its FAB- and FD-MS, the same ion peaks as those of **1** at *m/z* 482 and 586, respectively. The ^{1}H-NMR spectrum of **5** was almost identical to that of **1**, except for the signal (6H, d) due to two secondary methyl groups instead of the primary methyl signal (3H, t). Its ^{13}C-NMR spectrum showed that no signal at δ 14.5 (primary methyl carbon) in **1** was observed, but a signal at δ 23.1 (secondary methyl carbon) appeared. These observations indicate that compound **5** has a C_{16} monomethyl-branched alkyl chain, and hence it was assigned the structure 1-*O*-14-methylpentadecyl-*sn*-glycero-3-phosphocholine.

Compound **6**, colorless needles, mp 245—260 °C, $[\alpha]_D$ −3.3° and **7**, powder, mp 235—250 °C, $[\alpha]_D$ −3.0° gave the same ion peaks at *m/z* 496 and 600 in their FAB- and FD-MS, respectively, which were 14 mass units more than those of **1**. The ^{1}H-NMR spectra of **6** and **7** were identical to those of **5** and **1**, respectively. Therefore, compounds **6** and **7** are to be isomeric to each other, and the former was regarded as 1-*O*-15-methylpentadecyl-*sn*-glycero-3-phosphocholine, while the latter as 1-*O*-heptadecyl-*sn*-glycero-3-phosphocholine.

Compound **8**, powder, mp 255—260 °C, $[\alpha]_D$ −4.0° was identified as 1-*O*-octadecyl-*sn*-glycero-3-phosphocholine by comparison of its physical and spectral data with those reported by M. Ohno *et al.*[11]

Except for compounds **1**, **3** and **8**, the configurations at C-2 of the glycerol moiety of the other compounds remained undetermined, but they were conventionally regarded as *R* in conjunction with the facts that they show the same sign (minus) as those of **1**, **3** and **8** in the specific rotations, and that 2*R* configuration is commonly found in nature. To our knowledge, compounds **4**, **5**, **6** and **7**, which have a monomethyl-branched or odd numbered alkyl chain, have not yet been isolated from natural sources.

Recently, PAF[12] has attracted a great deal of attention and is now recognized as an important chemical mediator in certain pathophysiological processes, such as acute inflammatory reaction.[13]

The present result, that a large amount of lyso PAF having a monomethyl-branched or odd-numbered alkyl chains isolated from the crude drug "Jiryu," is of interest.

Experimental

Melting points (mp) were determined on a Yanaco MP-S3 apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM GX-400 spectrometer at 30° using TMS as an internal reference. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; sep, septet; m, multiplet, br s, broad singlet. * The signal with asterisks appear as doublets (J=6—7 Hz) because of the coupling with ^{31}P. MS were acquired on a JEOL JMS DX-300 spectrometer in combination with a JMA 3500 data system (FAB-MS: accelerating voltage 3 kV; matrix, glycerin; collision gas, Xe, FD-MS: emitter, carbon; cathode voltage 5 kV; emitter current 17—22 mA). Optical rotations were measured at 20 °C with a JASCO DIP-140 polarimeter. TLC was carried out on silica-gel pre-coated Al sheets (Merck Art. 5554) and HPTLC with Al sheets (Merck Art. 5556). Column chromatography was carried out on Merck Silica-gel 60 (230—400 mesh, Art. 9385), and Cosmosil 75C_{18}-OPN (Nacalai Tesque, Inc.). Preparative HPLC was conducted over Inertsil Prep-ODS (10 μm, 20 × 250 mm, GL Sciences Inc.) on a JASCO 880-PU equipped with JASCO 830-RI. Recycling HPLC was carried out on a JASCO 880-PU equipped with a JASCO preparative recycle valve.

Isolation of Compounds 1—8 The crushed powder (1 kg) of the dried body walls of the commercial crude drug "Jiryu," *P. asiatica* purchased from Tochimoto Tenkaido was extracted with $CHCl_3$–MeOH (1 : 1, 4 l) and MeOH (4 l), successively, at room temperature. The combined extract was concentrated to give a brown syrup (75 g). It was shaken with $CHCl_3$–MeOH–H_2O (1 : 2 : 1, 900 ml) and the lower phase was collected. After removal of the solvent, the residue (27 g) was chromatographied over silica-gel ($CHCl_3$–MeOH, 8 : 2→6 : 4→$CHCl_3$–MeOH–28% NH_4OH, 6 : 4 : 1), successively, to yield three fractions; fr. 1 (13 g), fr. 2 (7 g) and fr. 3 (7 g). Chromatography of fr. 3 on Cosmosil 75C_{18}-OPN with MeOH afforded three fractions; fr. 4 (1 g), fr. 5 (4 g) and fr. 6 (2 g). Fraction 5 was dissolved in a minimum volume of MeOH (*ca.* 2 ml) and AcOEt (*ca.* 30 ml) was added. On standing at room temperature, crystals were formed. The crystals were filtered off, and the filtrate was subjected to HPLC (97% MeOH) to yield fr. 7 (th first peak; Fig. 1) and seven compounds, **1**, **2** and **4**—**8**. Fraction 7 (180 mg) was subjected to recycling HPLC (97% MeOH, 15 cycles) to give **3** (60 mg) and an unidentified compound (110 mg). Respective recrystallization of compounds **1**—**6** from AcOEt–MeOH (1 : 15) gave colorless needles, **3** (47 mg), **4** (290 mg), **2** (450 mg), **5** (140 mg), **1** (1.2 g) and **6** (40 mg). Compounds **7** and **8** were recrystallized from $CHCl_3$–MeOH (1 : 2) to give, respectively, a white powder, 23 and 53 mg.

3: Needles, mp 122—144 °C (dec.), $[\alpha]_D$ −4.9° (c=0.7, $CHCl_3$–MeOH, 1 : 1) [lit.,[7] $[\alpha]_D$ −2.4° (c=0.05, $CHCl_3$)]. FD-MS *m/z* (%): 558 $(M+choline+H)^+$ (38), 104 (100). FAB-MS *m/z* (%): 454 $(M+H)^+$ (75), 184 (100). ^{1}H-NMR (CD_3OD) δ: 0.89 (3H, t, J=7.0 Hz, H_3-14), 1.20—1.37 (22H, br s), 1.56 (2H, m, H_2-2), 3.22 (9H, s, N–Me × 3), 3.46 (2H, m, H_2-1), 3.48 (2H, m, H_2-1′), 3.63 (2H, m, H_2-2″), 3.85, 3.94 (each 1H, m, H_2-3′), 3.89 (1H, m, H-2′), 4.28 (2H, m, H_2-1″). ^{13}C-NMR (CD_3OD) δ: 14.2 (C-14), 23.5 (C-13), 27.0 (C-3), 30.3—30.5 (9C), 32.9 (C-2), 54.6 (N–Me × 3), 60.2* (C-1″), 67.4* (C-3′), 68.3* (C-2″), 70.9* (C-2′), 72.5 (C-1′), 72.7 (C-1).

4: Needles, mp 200—205 °C (dec.), $[\alpha]_D$ −2.3° (c=3.0, MeOH). FD-MS *m/z* (%): 572 $(M+choline+H)^+$ (40), 104 (100). FAB-MS *m/z* (%): 468 $(M+H)^+$ (75), 184 (100). ^{1}H-NMR (CD_3OD) δ: 0.88 (6H, d, J=7.0 Hz, 13-Me_2), 1.18 (2H, m), 1.20—1.37 (18H, br s), 1.53 (1H, sep, J=6.6 Hz, H-13), 1.56 (2H, m, H_2-2), 3.23 (9H, s, N–Me × 3), 3.45 (2H, m, H_2-1), 3.49 (2H, m, H_2-1′), 3.64 (2H, m, H_2-2″), 3.85, 3.94 (each 1H, m, H_2-3′), 3.89 (1H, m, H-2′), 4.29 (2H, m, H_2-1″). ^{13}C-NMR (CD_3OD) δ: 22.9 (13-Me_2), 27.1, 28.4, 29.0, 30.5—30.9 (8C), 40.1, 54.6 (N–Me × 3), 60.3* (C-1″), 67.4* (C-3′), 68.4* (C-2″), 71.0* (C-2′), 72.6 (C-1′), 72.8 (C-1).

5: Needles, mp 188—202 °C (dec.), $[\alpha]_D$ −4.0° (c=1.0, MeOH). FD-MS *m/z* (%): 586 $(M+choline+H)^+$ (35), 104 (100). FAB-MS *m/z* (%): 482 $(M+H)^+$ (70), 184 (100). ^{1}H-NMR (CD_3OD) δ: 0.88 (6H, d, J=7.0 Hz, 14-Me_2), 1.20—1.36 (24H, br s), 1.56 (2H, m, H_2-2), 3.22 (9H, s, N–Me × 3), 3.46 (2H, m, H_2-1), 3.48 (2H, m, H_2-1′), 3.64 (2H, m, H_2-2″), 3.85, 3.94 (each 1H, m, H-3′), 3.89 (1H, m, H-2′), 4.29 (2H, m, H_2-1″). ^{13}C-NMR (CD_3OD) δ: 23.1 (14-Me_2), 27.2, 28.5, 29.1, 30.6—31.0 (9C), 40.2, 54.7 (N–Me × 3), 60.4* (C-1″), 67.5* (C-3′), 68.5* (C-2″), 71.0* (C-2′), 72.7 (C-1′), 72.9 (C-1).

6: Needles, mp 245—260 °C (dec.), $[\alpha]_D$ −3.3° (c=3.6, MeOH). FD-MS *m/z* (%): 600 $(M+choline+H)^+$ (40), 104 (100). FAB-MS *m/z* (%): 496 $(M+H)^+$ (75), 184 (100). ^{1}H-NMR (CD_3OD) δ: 0.87 (6H, d, J=7.0 Hz, 15-Me_2), 1.18 (2H, m), 1.20—1.36 (24H, br s), 1.52 (1H, sep, J=7.0 Hz, H-15), 1.57 (2H, m, H_2-2), 3.23 (9H, s, N–Me), 3.46 (2H, m, H_2-1), 3.48 (2H, m, H_2-1′), 3.64 (2H, m, H_2-2″), 3.85, 3.94 (each 1H, m, H_2-3′), 3.89 (1H, m, H-2′), 4.29 (2H, m, H_2-1″). ^{13}C-NMR (CD_3OD) δ: 23.1 (15-Me_2), 27.2, 28.5, 29.1, 30.6—31.1 (10C), 40.2, 54.7 (N–Me × 3), 60.4* (C-1″), 67.5* (C-3′), 68.6* (C-2″), 71.0* (C-2′), 72.7 (C-1′), 72.9 (C-1).

7: Powder, mp 235—250 °C (dec.), $[\alpha]_D$ −3.0° (c=2.0, MeOH). FD-MS *m/z* (%): 600 $(M+choline+H)^+$ (38), 104 (100). FAB-MS *m/z* (%): 496 $(M+H)^+$ (75), 184 (100). ^{1}H-NMR (CD_3OD) δ: 0.79 (3H, t, J=7.0 Hz, H_3-17), 1.20—1.36 (28H, br s), 1.48 (2H, m, H_2-2), 3.13 (9H, s, N–Me × 3), 3.40 (2H, m, H_2-1), 3.47 (2H, m, H_2-1′), 3.52 (2H, m, H_2-2″), 3.77, 3.86 (each 1H, m, H_2-3′), 3.83 (1H, m, H-2′), 4.18 (2H, m, H_2-1″). ^{13}C-NMR (CD_3OD) δ: 14.5 (C-17), 23.7 (C-16), 27.2 (C-3), 30.5—30.8 (12C), 33.1, (C-2), 54.7 (N–Me × 3), 60.4* (C-1″), 67.5* (C-3′), 68.5* (C-2″), 71.1* (C-2′), 72.7 (C-1′), 73.0 (C-1).

8: Powder, mp 255—260 °C (dec.), $[\alpha]_D$ −4.0° (c=2.0, MeOH) [lit.,[11] $[\alpha]_D$ −3.84° (c=2.0, MeOH)]. FD-MS *m/z* (%): 614 $(M+choline+H)^+$ (40), 104 (100). FAB-MS *m/z* (%): 510 $(M+H)^+$ (80), 184 (100). ^{1}H-NMR (CD_3OD) δ: 0.80 (3H, t, J=7.0 Hz, H_3-18), 1.18—1.26 (30H, br s), 1.48 (2H, m, H_2-2), 3.13 (9H, s, N–Me × 3), 3.37 (2H, m, H_2-1), 3.40 (2H, m, H_2-1′), 3.54 (2H, m, H_2-2″), 3.76, 3.86 (each 1H, m, H_2-3′), 3.82 (1H, m, H-2′), 4.19 (2H, m, H_2-1″).

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