Additional Bioactive Lyso-PAF Congeners from the Sponge *Spirastrella abata*

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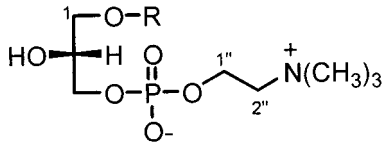
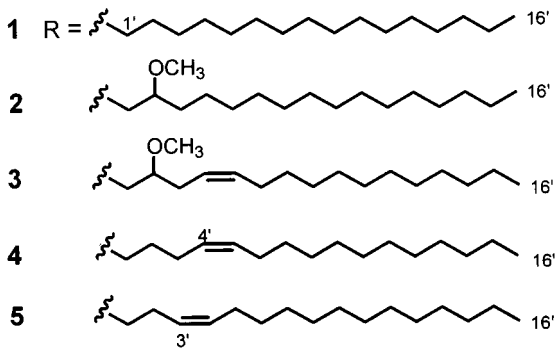
A known (**1**) and four new (**2**−**5**) lyso-PAF (platelet activating factor) derivatives were isolated from the sponge *Spirastrella abata*. Two of them are unprecedented in having a methoxy group at C-2′. The structures have been determined by combined spectroscopic methods. Their inhibitory effect on the biosynthesis of cholesterol and cytotoxicity against human solid tumor cell lines are reported.

Marine sponges of the genus *Spirastrella* are a rich source of structurally unique and biologically active compounds.[1−6] Lyso-PAF (platelet activating factor) congeners and lysophosphatidylcholines with a variety of ether-linked side chains at position *sn*-1 have already been isolated from *Spirastrella abata* (Spirastrellidae) in our laboratory.[7] Now we wish to report four new lyso-PAF congeners, two with 2′-methoxy substituents, from the same source. Previously, 2-methoxy fatty acids[8,9] have been reported from marine sponges, and their glycerol ethers were isolated from a brachiopod,[10] but the presence of 2-methoxy fatty acids in phosphocholines was not reported. 2′-Methoxy-substituted alkylglycerols, first isolated from shark liver oil,[11] have been shown to possess various biological activities such as antibacterial, antifungal, antitumor, and immune-stimulant activity.[12]

Activity-guided fractionation of the methanolic extract of *S. abata* followed by chromatographic separation resulted in the isolation of one known (**1**) and four new (**2**−**5**) lyso-PAF congeners. The known compound was identified as 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine by comparing physical properties and ^{1}H and ^{13}C NMR data with those reported previously from the same source.[7]





Compound **2** was isolated as light yellow, amorphous solid, and it showed a $[M + H]^+$ peak at *m*/*z* 512 in FABMS. The ^{1}H NMR spectrum featured a characteristic pattern of a phosphatidylcholine skeleton. Both ^{1}H and ^{13}C NMR data (see Experimental Section) were in agreement with a phosphatidylcholine gross structure and an alkyl side chain. The assignments were further confirmed by COSY and HMQC experiments. The ^{1}H NMR spectrum also featured a three-proton singlet at δ_H 3.40 correlated with a carbon at δ 57.8, which was assigned to a methoxy group. The respective carbinol proton appeared as a multiplet at δ 3.36 and showed connectivity with the carbon at δ 81.6 in the HMQC spectrum. In the COSY spectrum this proton signal was correlated with that of the protons resonating at δ 3.47, which were connected to a carbon at δ 74.5, a logical value for C-1′, and also with the proton signal at δ 1.48, which were further correlated with the alkyl chain protons. This confirmed that the methoxy group was present at C-2′. The stereochemistry at C-2 was deduced to be identical to that of **1**, which showed specific rotation similar to that reported earlier.[13] However, the stereochemistry at the methoxy site remains to be determined. The structure of **2** was elucidated as 1-*O*-(2′-methoxyhexadecyl)-*sn*-glycero-3-phosphocholine.

Compound **3** was also a light yellow, amorphous solid which showed a $[M + H]^+$ peak at *m*/*z* 510 in the FABMS. The ^{1}H NMR spectrum was similar to that of **2**, except for the presence of a two-proton multiplet at δ 5.43, a two-proton broad triplet at δ 2.27, and a two-proton broad quartet at δ 2.06. These findings coupled with the fact that its molecular weight was 2 units less than that of **2** suggested that **3** is a dehydro derivative of **2**. The COSY spectrum was helpful in assigning the position of the double bond. The carbinol proton signal at δ 3.36 was correlated with an allylic proton resonance at δ 2.27, which in turn was correlated with the olefinic proton absorption at δ 5.43. A correlation between the carbinol proton and H-1′ protons at δ 3.48 was also observed. Thus the double bond was indicated at C-4′, which was also confirmed by an enhanced peak due to the allylic cleavage at *m*/*z* 369 in the FAB-CID MS/MS. The ^{13}C NMR spectrum of **3** was also in agreement with the structure. The geometry of the double bond was deduced to be cis, as the allylic carbons resonated at δ 29.8 and 28.3. Thus the structure of **3** was determined to be 1-*O*-(2′-methoxy-4′*Z*-hexadecenyl)-*sn*-glycero-3-phosphocholine.

The protonated molecular ion $[M + H]^+$ of compound **4** appeared at *m*/*z* 480 in the FABMS. Its ^{1}H NMR spectrum was similar to that of compound **3** except for the absence of methoxy and the related carbinol proton signals. A quintet corresponding to two protons at δ 1.61 (J = 6.6 Hz)

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Table 1. In Vitro Cytotoxicities (ED_{50} μg/mL) of Compounds **1**−**5** against Human Solid Tumor Cells[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	5.5	4.9	5.4	4.9	5.4
2	5.8	5.7	5.2	4.5	5.0
3	6.3	5.9	3.8	4.0	3.7
4	>30	>30	>30	>30	>30
5	>30	>30	>30	>30	>30
cisplatin	0.7	1.2	2.3	1.0	1.1
doxorubicin	0.02	0.11	0.02	0.08	0.04

[a] A549: human lung cancer; SK-OV-3: human ovarian cancer; SK-MEL-2: human skin cancer; XF498: human CNS cancer; HCT15: human colon cancer.

was assigned to H-2′. A pair of broad quintets at δ 2.04 and 1.98 was assigned to H-3′ and H-6′, respectively. Characteristic signals for phosphatidylcholine (δ 4.28, 2H, m, H-1″; δ 3.90, 3H, m, H-2, H-3; δ 3.63, 2H, m, H-2″; δ 3.45, 4H, m, H-1, H-1′; δ 3.21, 9H, s, *N*-CH_3) observed in the ^{1}H NMR spectrum of **4** confirmed its gross structure. The position of the double bond was ascertained at C-4′ due to the prominent allylic cleavage at *m*/*z* 339 in the FAB-CID MS/MS. The geometry of the double bond was deduced to be cis, like other analogues of the same series isolated from the same source.[7] The structure of **4** was determined to be 1-*O*-(4′*Z*-hexadecenyl)-*sn*-glycero-3-phosphocholine.

Compound **5** showed a protonated molecular ion $[M + H]^+$ at *m*/*z* 480 in the FABMS. The ^{1}H NMR spectrum of **5** was similar to that of **4** with only slight differences. A quartet at δ 2.32 (J = 6.6 Hz) was assigned to H-2′, and a broad quintet at δ 2.05 was due to H-5′. The H-2′ proton resonated at δ 2.32 instead of at δ 1.61, indicating that the double bond was moved toward the ether end. The location of the double bond was confirmed based on the enhanced allylic cleavage at *m*/*z* 325 in the FAB-CID MS/MS. All the other important fragments were similar to that of **4**. Hence, the structure of **5** was determined as 1-*O*-(3′*Z*-hexadecenyl)-*sn*-glycero-3-phosphocholine.

The isolated compounds were assayed for inhibition of cholesterol biosynthesis in the Chang liver cell since the lyso-PAF and lysophosphatidylcholines were shown to be active in our previous study.[7] Compounds **4** and **5** were found to possess moderate activity with an IC_{50} value of 118 and 102 μg/mL, respectively, but compounds **1** and **2** were inactive and were rather cytotoxic to the cells. Compared to our earlier study,[7] this result showed that the congeners with shorter and unsaturated side chains are more active inhibitors of cholesterol biosynthesis. The presence of the 2′-methoxy group showed no effect on inhibitory activity. Cytotoxicity assay against five human tumor cell lines showed that compounds **1**−**3** were significantly cytotoxic, while compounds **4** and **5** were inactive (Table 1). It is noteworthy that these lyso-PAF congeners are cytotoxic and structurally related to the synthetic antineoplastic agents such as edelfosine (1-*O*-alkyl-2-*O*-methyl-*sn*-glycero-3-phosphocholine) and ilmofosine.[14]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC200, Bruker DMX600, and Varian Unity Plus 300 spectrometers. Chemical shifts were reported in reference to the respective residual solvent peaks (δ_H 3.3 and δ_C 49.0 for CD_3OD). FAB-CID MS/MS data were obtained using a JEOL JMS-HX110/110A (four-sector instrument with a E1B1E2B2 configuration).

Animal Material. The sponge S*pirastrella abata* was collected by hand using scuba at a 13 m depth in November 1996, off Cheju Island, Korea, and has been described elsewhere.[7]

Extraction and Isolation. The frozen sponge (5.25 kg) was extracted and fractionated into seven fraction (F1−F7) as described in our previous report.[7] Fraction F3 was eluted with 85% MeOH−H_2O and was very active in brine shrimp lethality assay.[15] This fraction was further chromatographed on a reversed-phase HPLC (YMC-Pack CN, 250 × 10 mm, S-5 μm, 120 Å) using CH_3CN−H_2O (1:1) to afford compounds **1**−**5** in semipurified form. They were further purified by repeated HPLC using 50−60% MeOH−H_2O as the mobile phase to yield compounds **1** (14 mg), **2** (9 mg), **3** (1.2 mg), **4** (0.8 mg), and **5** (0.8 mg).

1-*O*-Hexadecyl-*sn*-glycero-3-phosphocholine (1): amorphous solid; $[\alpha]^{18}_D$ −3.0 (*c* 0.50, MeOH); ^{1}H NMR (CD_3OD, 200 MHz) δ 4.27 (H-1″), 3.87 (H-2, H-3), 3.62 (H-2″), 3.45 (H-1, H-1′), 3.21 (*N*-CH_3), 1.56 (H-2′), 1.25−1.35 (H-3′−H-15′), 0.89 (H-16′); ^{13}C NMR (CD_3OD, 50 MHz) δ 72.9, 72.7, 71.1 (d), 68.5 (d), 67.5 (dt), 60.4 (d), 54.7 (t), 33.1, 30.8−30.4, 27.2, 23.7, 14.4; FAB-CID MS/MS *m*/*z* 482 $[M + H]^+$ (100), 466 (0.4), 452 (0.3), 438 (0.5), 424 (0.5), 410 (0.5), 396 (0.5), 382 (0.5), 368 (0.5), 354 (0.5), 340 (0.5), 326 (0.5), 312 (0.5), 256 (2.0), 224 (5.0), 184 (9.0).

1-*O*-(2′-Methoxyhexadecyl)-*sn*-glycero-3-phosphocholine (2): amorphous solid; $[\alpha]^{18}_D$ +5.6 (*c* 0.27, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) δ 4.29 (2H, m, H-1″), 3.94 (1H, ddd, J = 10.0, 6.1 Hz, J_{HN} = 3.8 Hz, H-3), 3.90 (1H, quint, J = 4.7 Hz, H-2), 3.85 (1H, ddd, J = 10.0, 5.6 Hz, J_{HN} = 5.6 Hz, H-3), 3.64 (2H, m, H-2″), 3.56 (1H, dd, J = 10.1, 4.7 Hz, H-1), 3.52 (1H, dd, J = 10.5, 3.8 Hz, H-1′), 3.49 (1H, dd, J = 10.1, 5.6 Hz, H-1), 3.47 (1H, dd, 10.5, 5.9 Hz, H-1′), 3.40 (3H, s, OCH_3), 3.36 (1H, m, H-2′), 3.23 (9H, s, *N*-CH_3), 1.48 (2H, quint, J = 7.0 Hz, H-3′), 1.35−1.25 (m, H-4′−H15′), 0.90 (3H, t, J = 7.0 Hz, H-16′); ^{13}C NMR (CD_3OD, 150 MHz) δ_C 81.6 (C-2′), 74.5 (C-1′), 73.6 (C-1), 71.0 (d, J_{CP} = 7.6 Hz, C-2), 68.3 (d, J_{CP} = 6.0 Hz, C-3), 67.5 (dt, J_{CP} = 7.2 Hz, J_{CN} = 3.0 Hz, C-2″), 60.4 (d, J_{CP} = 5.1 Hz, C-1″), 57.8 (OCH_3), 54.7 (t, J_{CN} = 3.8 Hz, *N*-CH_3), 33.1 (C-14′), 32.3 (C-3′), 30.9−30.5 (C-5′−C-13′), 26.5 (C-4′), 23.7 (C-15′), 14.4 (C-16′) (J represents ^{1}H−^{1}H coupling unless otherwise indicated); FAB-CID MS/MS *m*/*z* 512 $[M + H]^+$ (100), 496 (1.0), 482 (1.0), 468 (1.0), 454 (0.9), 440 (0.9), 426 (1.0), 412 (1.0), 398 (1.0), 384 (1.0), 370 (0.9), 354 (1.1), 298 (1.0), 258 (3.0), 224 (6.5), 184 (8.0).

1-*O*-(2′-Methoxy-4′*Z*-hexadecenyl)-*sn*-glycero-3-phosphocholine (3): amorphous solid; ^{1}H NMR (CD_3OD, 300 MHz) δ 5.43 (2H, m, H-4′, H-5′), 4.28 (2H, m, H-1″), 3.91 (3H, m, H-2, H-3), 3.63 (2H, m, H-2″), 3.48 (4H, m, H-1, H-1′), 3.40 (3H, s, OCH_3), 3.36 (1H, m, H-2′), 3.21 (9H, s, *N*-CH_3), 2.27 (2H, br t, J = 6.3 Hz, H-3′), 2.06 (2H, br q, J = 6.9 Hz, H-6′), 1.35−1.25 (H-7′−H15′), 0.89 (3H, t, J = 7.5 Hz, H-16′); ^{13}C NMR (CD_3OD, 50 MHz) δ_C 133.2 (C-5′), 126.0 (C-4′), 81.6 (C-2′), 74.1 (C-1′), 73.6 (C-1), 71.1 (d, J_{CP} = 7.4 Hz, C-2), 68.4 (d, J_{CP} = 5.5 Hz, C-3), 67.5 (m, C-2″), 60.4 (d, J_{CP} = 5.2 Hz, C-1″), 57.7 (OCH_3), 54.7 (t, J_{CN} = 3.7 Hz, *N*-CH_3), 33.1 (C-14′), 30.8−30.4 (C-7′−C-13′), 28.3 (C-3′), 23.7 (C-15′), 14.4 (C-16′); FAB-CID MS/MS *m*/*z* 510 $[M + H]^+$ (100), 494 (1.0), 480 (0.9), 466 (0.9), 452 (0.8), 438 (0.8), 424 (0.7), 410 (0.5), 396 (0.6), 382 (0.6), 369 (2.0), 299 (2.5), 224 (8.0), 184 (20.0).

1-*O*-(4′*Z*-Hexadecenyl)-*sn*-glycero-3-phosphocholine (4): amorphous solid; ^{1}H NMR (CD_3OD, 300 MHz) δ 5.41 (2H, m, H-4′, H-5′), 4.28 (2H, m, H-1″), 3.90 (3H, m, H-2, H-3), 3.63 (2H, m, H-2″), 3.45 (4H, m, H-1, H-1′), 3.21 (9H, s, *N*-CH_3), 2.04 (2H, br q, H-3′), 1.98 (2H, br q, H-6′), 1.61 (2H, quint, J = 6.6 Hz, H-2′), 1.35−1.25 (H-7′−H-15′), 0.89 (3H, t, J = 6.9 Hz, H-16′); FAB-CID MS/MS *m*/*z* 480 $[M + H]^+$ (100), 464 (0.3), 450 (0.2), 436 (0.3), 422 (0.3), 408 (0.2), 394 (0.2), 380 (0.2), 366 (0.2), 352 (0.2), 339 (1.0), 256 (0.9), 224 (2.2), 184 (5.0).

1-*O*-(3′*Z*-Hexadecenyl)-*sn*-glycero-3-phosphocholine (5): amorphous solid; ^{1}H NMR (CD_3OD, 300 MHz) δ 5.42 (2H, m, H-3′, H-4′), 4.28 (2H, m, H-1″), 3.89 (3H, m, H-2, H-3), 3.64 (2H, m, H-2″), 3.45 (4H, m, H-1, H-1′), 3.21 (9H, s, *N*-CH_3), 2.32 (2H, q, J = 6.6 Hz, H-2′), 2.05 (2H, br q, H-5′),1.35−1.25 (H-6′−H-15′), 0.89 (3H, t, J = 6.9 Hz, H-16′); FAB-CID MS/MS *m*/*z* 480 $[M + H]^+$ (100), 464 (0.5), 450 (0.4), 436 (0.5), 422

(0.5), 408 (0.5), 494 (0.5), 380 (0.3), 366 (0.3), 352 (0.3), 338 (0.5), 325 (1.0), 256 (1.8), 224 (5.5), 184 (10).

Cytotoxicity Assay. Cytotoxicity was measured by SRB (sulforhodamine B) assay, which was developed for measuring the cellular protein contents of the culture.[16] In a typical assay the rapidly growing cells were harvested, counted, and inoculated into 96-well microtiter plates ((1−2) × 10^4 cells/well). After incubation for 24 h, the compounds dissolved in the culture medium were applied to the culture wells in triplicate followed by incubating at 37 °C for 48 h under 5% CO_2 atmosphere. The cultures were fixed with cold TCA and stained with 0.4% SRB dissolved in 1% acetic acid. After solubilizing the bound dye with 10 mM unbuffered Tris base by using a gyrotary shaker, the absorption was measured with a microplate reader (Dynatech MR 700) at a wavelength of 520 nm.

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