Two New Antimicrobial Lysoplasmanylinositols from the Marine Sponge *Theonella swinhoei*

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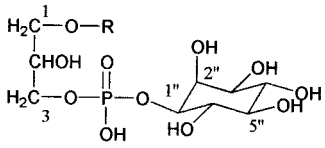
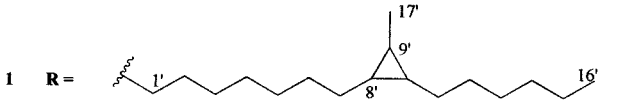
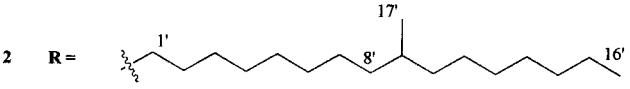
Two new antimicrobial lysoplasmanylinositols have been isolated from the marine sponge *Theonella swinhoei*. Their structures were elucidated on the basis of spectroscopic and chemical analysis.

The lithistid sponge *Theonella swinhoei* is a rich source of polyketides and nonribosomal peptides, many of which are structually unusual and highly bioactive.[1] We previously isolated bistheonellides[2] and theonellamides[3,4] from the Hachijo-jima Island collection of *T. swinhoei* GRAY (Theonellidae) with a white interior, while polytheonellamides,[5] cyclotheonamides,[6,7] and aurantosides[8,9] were isolated from the variety with yellow interior. Further investigation of the white variety resulted in two new antimicrobial lysoplasmanylinositols. Here we describe the isolation and structure elucidation of these lipids.

The aqueous alcoholic extract of the sponge (14 kg, wet wt) was partitioned between H_2O and ether, and the aqueous layer was further extracted with $CHCl_3$. The antimicrobial $CHCl_3$ fraction was repeatedly chromatographed on silica gel and ODS to afford lysoplasmanylinositols **1** and **2**, in yields of 5.0×10^{-6} and 5.7×10^{-6}% on the basis of wet sponge weight.







Lysoplasmanylinositol **1** had a molecular formula of $C_{26}H_{51}O_{11}P$, as established by high-resolution FAB mass spectrum. The ^1H NMR spectrum (Table 1) revealed signals for a terminal methyl at δ 0.78 (t, $J = 6.6$ Hz), a secondary methyl at δ 0.92 (d, $J = 6.6$ Hz), three oxygenated methylenes, seven oxygenated methines, and a long alkyl chain at δ 1.05−1.30 in addition to three shielded signals at δ −0.09 (1H, m), 0.36 (1H, m), and 0.27 (1H, m), which were assigned to be a 1,2,3-trisubstituted cyclopropane unit by interpretation of COSY data. The COSY spectrum also suggested the presence of a 1,3-disubstituted glycerol [δ 3.48 (1H, dd, $J = 10.2$ and 6.0 Hz), 3.53 (1H, dd, $J = 10.2$ and 5.4 Hz), 4.04 (1H, m), and 4.12 (2H)] and an inositol unit [δ 4.86 (1H, ddd, $J = 9.0$, 5.6 and 3.0 Hz), 4.49 (1H, t, $J = 3.6$ Hz), 4.11 (1H), 3.97 (1H, t, $J = 9.0$ Hz), 4.00 (1H, t, $J = 9.0$ Hz), and 4.16 (1H)]. One proton of the inositol and two of methylene protons in the glycerol unit were coupled to a phosphate group (δ_P 2.50). Thus, the element of $C_{17}H_{33}$ remained to be elucidated. Since one of carbons was oxygenated, the remaining portion must be a C_{16} alkyl chain.

Table 1. ^1H and ^{13}C NMR Spectral Data for **1**

carbon no.	^1H (mult., J in Hz)	^{13}C (mult.)	HMBC data
1	3.48 (dd, 10.2, 6.0)	73.3 (t)	C-2, 3, 1′
	3.53 (dd, 10.2, 5.4)		C-2, 3, 1′
2	4.04 (m)	71.1 (dd)[b]	C-1, 3
3	4.12[a]	68.6 (dt)[c]	C-1, 2
1′	3.34 (t, 6.6)	72.5 (t)	C-1, 2′, 3′
2′	1.45 (m)	30.6 (t)	C-1′
3′	1.05−1.30	27.1 (t)	
4′-7′	1.05−1.30	30.2−30.6	
8′	−0.09 (m)	27.3 (d)	C-17′
9′	0.36 (m)	17.8 (d)	
10′	0.27 (m)	24.2 (d)	
11′-13′	1.05−1.30	30.2−30.6	
14′	1.05−1.30	32.8 (t)	
15′	1.05−1.30	23.6 (t)	
16′	0.78 (t, 6.6)	14.6 (q)	C-14′, 15′
17′	0.92 (d, 6.6)	13.5 (q)	C-8′, 9′, 10′
1″	4.86 (ddd, 9.0, 5.6, 3.0)	77.9 (dd)[d]	
2″	4.49 (t, 3.6)	72.5 (d)	C-1″
3″	4.11[a]	72.4 (d)	C-4″
4″	3.97 (t, 9.0)	75.0 (d)	
5″	4.00 (t, 9.0)	75.2 (d)	C-4″, 6″
6″	4.16[a]	72.2 (dd)[e]	C-4″

[a] J_{H-H} couplings could not be assigned due to their overlapped signals. [b−e] J_{C-P} coupling values were 6.8, 6.6, 5.6, and 4.5 Hz, respectively.

One of the three high-field methine signals (δ 0.36, m) was coupled to a methyl signal in the COSY spectrum, while the other two signals [δ −0.09 (1H, m) and 0.27 (1H, m)] were correlated with a methylene unit; thereby a trisubstituted cyclopropane ring was placed in the middle of the long alkyl chain. The alkyl chain was connected to C-1 of glycerol, which was supported by an HMBC cross-peak between H_2-1′ and C-1. The position of the cyclopropane ring was evident from intense fragment ions at *m*/*z* 483 and 429 in FAB MS/MS data (Figure 1). The *myo*-inositol unit was assigned on the basis of the $^1H-^1H$ coupling constants.[10] The absolute stereochemistry of **1** could not be determined due to the paucity of material as well as its instability to acid hydrolysis.

Lysoplasmanylinositol **2** had a molecular formula of $C_{26}H_{53}O_{11}P$, as established by HRFABMS. The ^1H NMR spectrum was almost superimposable on that of **1**, except

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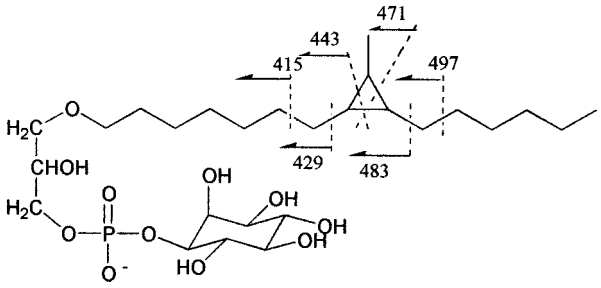


Figure 1. Fragment ions in the FAB MS/MS spectrum of **1**.

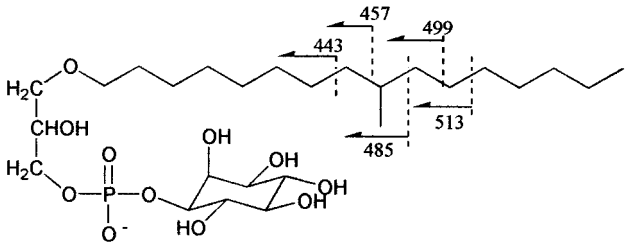
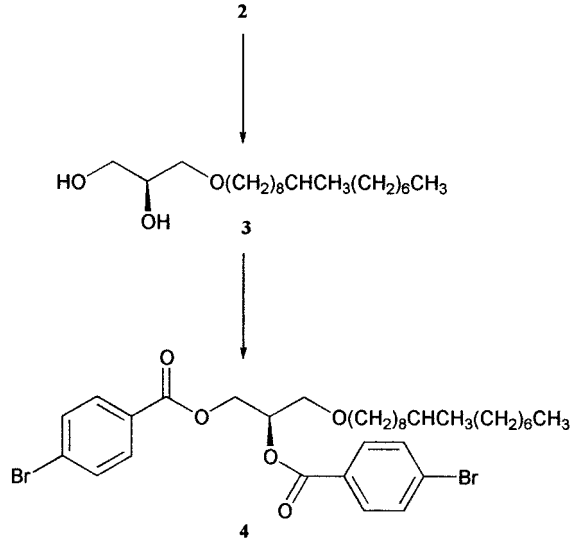


Figure 2. Fragment ions in the FAB MS/MS spectrum of **2**.

Scheme 1



for cyclopropane signals. A secondary methyl group could be placed at C-9′ of the alkyl chain on the basis of intense fragment ions at *m*/*z* 457 and 485 in FAB MS/MS (Figure 2). The absolute stereochemistry of the glycerol unit was determined as follows. Acid hydrolysis of **2** furnished glyceryl ether **3**, which was converted to di-*p*-bromobenzoate **4** (Scheme 1). The dibenzoate **4** exhibited split CD bands at 253 nm ($\Delta\epsilon$ −4.2) and 234 nm ($\Delta\epsilon$ 2.3), thereby indicating 2*R* stereochemistry.[11] Accordingly, compound **2** was 1-(9-methylhexadecyl)lysoplasmanylinositol.

Lysoplasmanylinositol **2** inhibited the growth of *Escherichia coli* at 50 μg/disk (12 mm inhibitory zone), whereas lysoplasmanylinositol **1** was only active in bioautography. They were not inhibitory against the fungus *Mortierella ramaniana*.

Although lysophosphatidyl inositols[10] have been isolated from the ascidian *Halocynthia roretzi* as antifungal constituents, this is the first report of lysoplasmanyl inositols from a marine organism.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a JEOL alpha 600 NMR spectrometer. Chemical shifts were referenced to solvent peaks: δ_H 3.30 and δ_C 49.0 for CD_3OD. FAB mass spectra were obtained with a JEOL SX-102 mass spectrometer. Triethanolamine or NBA/NaCl was used as the matrix. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. CD spectrum was measured on a JASCO J-720 CD/ORD spectrometer.

Animal Material. The sponge samples were collected by hand using scuba at a depth of 15 m off Hachijo-jima Island. The sponge was identified as *Theonella swinhoei* by Dr. Rob van Soest, Institute of Zoological Taxonomy, University of Amsterdam, The Netherlands. A voucher specimen (ZMA POR 15729) was deposited at the Zoological Museum of the University of Amsterdam.

Extraction and Isolation. The frozen sponge (14.0 kg) was extracted three times with 70% *n*-PrOH, and the combined extracts were concentrated and partitioned between water and ether. The aqueous layer was extracted with $CHCl_3$, leading to an extract that was antimicrobial against *E. coli*; it was further fractionated on a silica gel open column with a $CHCl_3$/MeOH/H_2O system. The fractions eluted with $CHCl_3$/MeOH/H_2O (80:20:1 and 70:30:5) were separated by ODS flash column chromatography with aqueous *n*-PrOH. The fraction eluted with 30% aqueous *n*-PrOH was purified by repeated HPLC on an ODS column (28−80% MeCN gradient elution and 45% MeCN containing 100 mM $NaClO_4$) to afford lysoplasmanylinositol **1** and **2** in yields of 5.0×10^{-6} and 5.7×10^{-6}% based on wet sponge.

Lysoplasmanylinositol 1: colorless solid; $[\alpha]^{20}_D$ −10.0° (*c* 0.03, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; FABMS (negative) *m*/*z* 569 $(M - H)^-$ and 407 $(M - C_6H_{10}O_5)^-$; HRFABMS (negative) *m*/*z* 569.3065 (calcd for $C_{26}H_{50}O_{11}P$, 569.3092); FAB MS/MS (negative) *m*/*z* (relative intensity) 569 (100), 497 (11.8), 483 (21.3), 471 (6.2), 443 (4.6), 429 (10.0), 415 (11.9), 407 (79.1).

Lysoplasmanylinositol 2: colorless solid; $[\alpha]^{20}_D$ −8.9° (*c* 0.035, MeOH); ^{1}H NMR (600 MHz, CD_3OD/pyridine-d_5, 1:1) δ 4.58 (1H, dd, J = 3.0 Hz), 4.33 (1H, ddd, J = 9.0 5.6 and 3.0 Hz), 4.21 (1H, t, J = 9.0 Hz), 4.19 (2H, m), 4.07 (1H, t, J = 9.6 Hz), 4.07 (1H, m), 3.66 (1H, dd, J = 9.6 and 3.0 Hz), 3.55 (1H), 3.53 (1H), 3.48 (1H, dd, J = 9.6 and 6.6 Hz), 3.32 (2H, t, J = 6.6 Hz), 1.43 (2H, m), 1.26−1.12 (25H), 0.76 (3H, t, J = 6.6 Hz), 0.75 (3H, d, J = 7.2 Hz). ^{13}C NMR (150 MHz, CD_3OD/pyridine-d_5, 1:1) δ 78.4 (C-1″, dd), 76.8 (C-5″, d), 74.3 (C-4″, d), 73.7 (C-6″, d), 73.4 (C-2″ and 3″, d), 73.3 (C-1, d), 72.4 (C-1′, d), 71.1 (C-2, dd), 68.7 (C-3, dt), 38.0 (C-8′ and 10′, t), 33.7 (C-9′, d), 32.8 (C-14′, t), 30.9 (C-2′, t), 30.2−30.6(C-4′-7′ and 11′-13′), 27.1 (C-3′, t), 23.5 (C-15′, t), 20.2 (C-17′, q), 14.6 (C-16′, q); FABMS (negative) *m*/*z* 571 $(M - H)^-$ and 409 $(M - C_6H_{10}O_5)^-$; HRFABMS (negative) *m*/*z* 571.3276 (calcd for $C_{26}H_{52}O_{11}P$, 571.3248); FAB MS/MS (negative) *m*/*z* (relative intensity) 571 (100), 513 (3.9), 499 (8.9), 485 (8.5), 457 (12.5), 443 (6.2), 409 (27.3).

Preparation of Dibenzoate 4. Lysoplasmanylinositol **2** (0.7 mg) was treated with 2 N HCl (1.0 mL) at room temperature for 30 h. The reaction mixture was neutralized with 3 N NaOH (0.67 mL) and extracted with AcOEt. The organic phase was treated with *p*-bromobenzoyl chloride (2.0 mg) and DMAP (1.5 mg) in pyridine (200 μL) at room temperature for 20 h. The reaction mixture was diluted with water, extracted with ether, and purified by normal-phase HPLC to afford dibenzoate **4** (67 μg on the basis of UV absorption at 244.5 nm).

Compound 4: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.86 (2H, d, J = 8.4 Hz), 7.82 (2H, d, J = 9.0 Hz), 7.54 (2H, d, J = 9.0 Hz), 7.53 (2H, d, J = 7.8 Hz), 5.54 (1H, m), 4.63 (1H, dd, J = 12.0, 3.6 Hz), 4.56 (1H, dd, J = 12.0, 7.2 Hz), 3.68−3.74 (2H, m), 3.44 (2H, t, J = 7.0), 1.53 (2H, m), 1.19−1.13 (25H, m), 0.85 (3H, t, J = 6.6 Hz), 0.82 (3H, d, J = 6.6 Hz); FABMS (positive) *m*/*z* 719 $(M + Na)^+$.

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