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Marine Natural Products. X.[1] Pharmacologically Active Glycolipids from the Okinawan Marine Sponge *Phyllospongia foliascens* (Pallas)

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By monitoring pharmacological activities, an anti-inflammatory active galactolipid [designated as M-5 (**2**)] and a sulfonoglycolipid [designated as M-6 (**4**)] which shows resistant activity against a complement fixation reaction have been isolated from the Okinawan marine sponge *Phyllospongia foliascens* (Pallas). The structures of these pharmacologically active principles have been elucidated as **2** and **4**, respectively, on the basis of chemical and physicochemical evidence.

Keywords——marine sponge; *Phyllospongia foliascens*; glycolipid; galactolipid; sulfonoglycolipid; ^{13}C NMR

During the course of our investigations on marine natural products,[2] we have recently reported the characterization of an anti-inflammatory active principle from the lipid-soluble fraction of the Okinawan marine sponge *Phyllospongia foliascens* (Pallas). The compound, named foliaspongin, is a bishomosesterterpene and the chemical structure (**1**) was proposed in our preliminary paper.[3] In the continuing study on the more polar ingredients of the same sponge, we have isolated two glycolipids: a galactolipid designated as M-5 (**2**), which exhibits anti-inflammatory activity, and a sulfonoglycolipid designated as M-6 (**4**), which shows resistant activity against a complement fixation reaction. This paper deals with the structure elucidation of these glycolipids.[4]

The fractionation of bioactive principles was carried out while monitoring the above-mentioned pharmacological activities, as shown in Fig. 1. Repeated column chromatography, with polystyrene gel, silica gel, and Sephadex LH-20 as adsorbents, finally led to the isolation of two bioactive principles, M-5 (**2**) and M-6 (**4**), each of which showed a single spot on the thin-layer chromatogram (TLC).

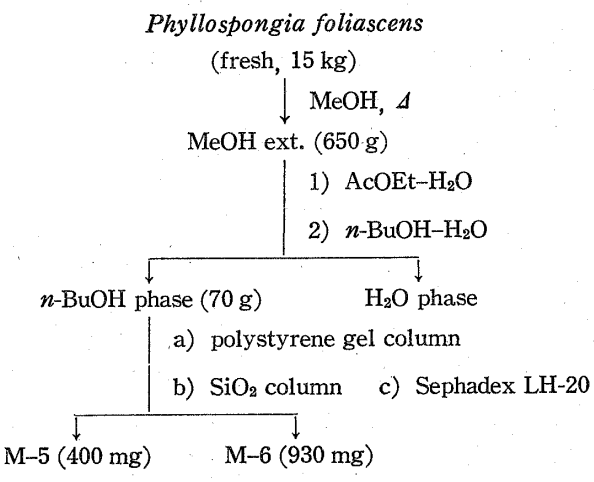


Fig. 1

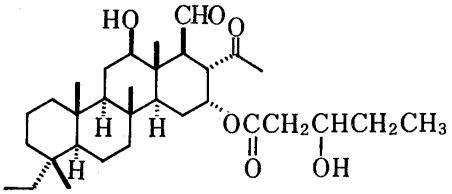
The infrared (IR) spectrum of M-5 (**2**) shows the presence of hydroxyl groups and ester functions (1744, 1719 cm^{-1}), while the proton magnetic resonance (^1H NMR) spectrum of M-5 shows signals which are characteristic of glycolipid: *e.g.* a deformed triplet at δ 0.87 (terminal methyl residue), a broadened signal at δ 1.25 (methylene chain in fatty acid moiety), and a mass of signals between δ 3.5 and δ 4.5 (sugar moiety).

Treatment of M-5 with dilute sodium methoxide in methanol furnished a glycerol galactoside (**3**), $[\alpha]_D$ −9° (H_2O), and a mixture of fatty acid methyl esters. The chemical-ionization (CH_4)

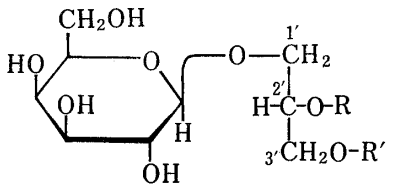
mass spectrum (CI–MS) of the glycerol galactoside showed an ion peak of m/z 255 ($M^+ +1$). Methanolysis of **3** liberated methyl galactoside and glycerol as identified by gas–liquid chromatography (GLC) of their trimethylsilyl (TMS) derivatives. The ^{13}C NMR spectrum (Table I) of **3** finally defined the structure as glycerol β-D-galactopyranoside.[5] In addition, the C-2′ configuration in the glycerol moiety of **3** is presumed to be *R* on the basis of a comparison of the specific rotation with the reported values, $[\alpha]_D$ (H_2O): $-7°$ for C-2′*R* and $+2°$ for C-2′*S*.[6]

The gas–liquid chromatography–mass spectral (GC–MS) analysis of the above-mentioned fatty acid methyl esters defined the composition as a mixture of methyl myristate, methyl 8-hexadecenoate, and methyl palmitate in a ratio of 1: 10: 10. Since signals ascribable to olefinic carbons of the 8-hexadecenoyl residue in M-5 (**2**) are observed at δ_c 130.0 and 129.8 (both doublets: C-8″, C-9″) and allylic carbons at δ_c 27.4 (triplet: C-7″, C-10″),[7] the unsaturated fatty acid residue can be identified as a *cis*-8-hexadecenoyl group (*cf.* **b**).

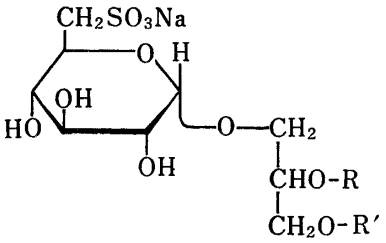
Detailed comparison of the ^{13}C NMR data for M-5 (**2**) and **3** has shown the fatty acid residues to be attached to C-2′ and C-3′ in M-5 (Table I). Thus, the signal due to C-1′ of the glycerol moiety in M-5 (**2**) is observed at higher ppm (2.9 ppm)[5] as compared to that in **3**, while signals ascribable to C-2′ and C-3′ for M-5 (**2**) and **3** are observed at similar chemical shifts.



foliaspongin (**1**)



M-5 (**2**): R, R′=(**a**: **b**: **c**=1: 10: 10)
3: R=R′=H



M-6 (**4**): R, R′=(**b**: **c**=1: 2)
Ant-1 (**5**): R=H, R′=(**a**: **c**=4: 96)
6: R, R′=H

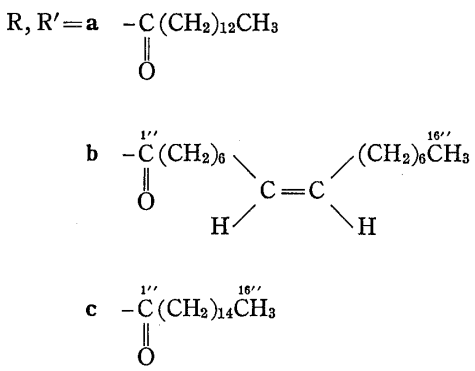


Chart 1

Consequently, the chemical structure of M-5 has been expressed as **2**, in which a mixture of fatty acid residues (**a**, **b**, and **c** in a ratio of 1: 10: 10) is attached to C-2′ and C-3′ of the glycerol moiety.

The IR spectrum of M-6 (**4**) shows the presence of hydroxyl, ester (1740 cm^{-1}, br), and sulfonate (1178 cm^{-1}, br) groups, while the ^1H NMR spectrum shows characteristic signals due to glycolipid, as observed in the case of M-5 (**2**): a deformed triplet at δ 0.88 and broadened signals at δ 1.29 both due to fatty acid residues, a mass of signals at δ 3.5—5.0 (sugar moiety) and a broad singlet at δ 5.26 (anomeric proton).

Alkaline hydrolysis of M-6 (**4**) as carried out for M-5 (**2**) furnished a glycerol sulfonoglycoside and a mixture of fatty acid methyl esters. The sulfonoglycoside thus isolated has been identi-

Table I. ^{13}C NMR Data for M-5 (2), 3, M-6 (4) and 6

Carbon	M-5 (2)[c]	3	M-6 (4)	6
1	105.5(d)	104.5(d)	99.9(d)	99.5(d)
2	72.5(d)	72.2(d)	72.7(d)	72.6(d)
3	75.0(d)	74.3(d)	74.4(d)	74.2(d)
4	70.4(d)	70.0(d)	74.0(d)	73.7(d)
5	76.9(d)	76.4(d)	69.6(d)	69.5(d)
6	62.6(t)	62.2(t)	53.7(t)	53.4(t)
1′	68.9(t)	71.8(t)	67.4(t)	70.2(t)
2′	71.9(d)	72.0(d)	72.0(d)	71.9(d)
3′	64.2(t)	63.8(t)	64.6(t)	64.0(t)
1″	175.0(s)		175.3(s)	
2″	35.1(t)		35.0(t)	
3″	26.3(t)		26.0(t)	
4″—13″[b]	30.4, 30.5 30.7, 30.8		30.4, 31.0	
14″	33.2(t)		33.1(t)	
15″	24.0(t)		23.7(t)	
16″	14.8(q)		14.9(q)	

a) Abbreviations given in parentheses denote the signal patterns observed in off-resonance experiments: d=doublet, q=quartet, s=singlet, t=triplet.
b) Signals due to C-7″, 8″, 9″, 10″ in the fatty acid residue **b** are not shown here.
c) Signals due to the fatty acid residue **a** were not discriminated due to overlapping with signals due to **c**.

fied as glycerol α-sulfonoglycoside (**6**),[6] which was previously obtained by similar alkaline treatment of a sulfonoglycolipid Ant-1 (**5**) isolated from the sea urchin *Anthocidaris crassispina* A. Agassizi,[8] by TLC and by comparison of the ^{13}C NMR data.

The fatty acid composition in M-6 (**4**) was determined by GC–MS analysis of the above methyl esters as described for M-5 (**2**) to be a mixture (1: 2) of *cis*-8-hexadecenoic and palmitic acids. Furthermore, the ^{13}C NMR analysis of M-6 (**4**) in comparison with Ant-1 (**5**)[8] enabled us to identify the location of the fatty acid residues to be at C-2′ and C-3′ of the glycerol moiety (Table I).[9] Thus, as was observed in the case of M-5 (**2**), only the signal due to C-1′ of the glycerol moiety in M-6 was observed at 2.8 ppm higher position as compared with the signal of C-1′ in **6**.

Therefore, the chemical structure of M-6 can now be formulated as **4**, which possesses a mixture of fatty acid residues (in a ratio of **b**: **c**=1: 2) attached to C-2′ and C-3′ of the glycerol moiety in the glycerol sulfonoglycoside structure.

These two glycolipids, M-5 (**2**) and M-6 (**4**), seem to be unprecedented examples of the isolation of this type of compound from marine sponge. Since sulfonoglycolipid has been hitherto characterized from the sea urchin,[8,10] as mentioned above, and also isolated from the green alga *Ulva pertusa* Kjellman,[11] the physiological functions of these glycolipids seem worthy of investigation.

Experimental[12]

Isolation of M-5 (2) and M-6 (4)——The finely crushed marine sponge (fresh, 15 kg) was extracted with hot MeOH and the solvent was evaporated under reduced pressure to give 650 g of the extractive. The extractive was then partitioned into AcOEt–water and the water-soluble portion was then further partitioned into *n*-BuOH–water. The *n*-BuOH soluble portion (70 g), obtained by evaporation of the solvent under reduced pressure, was treated with MeOH. The MeOH-soluble portion was subjected to polystyrene gel column chromatography (Hitachi gel 3010, Hitachi Kasei) developing with 90% MeOH→MeOH. The fraction (20 g) eluted with MeOH was further purified by silica gel column chromatography (SiO_2 230—400 mesh, Merck) developing with $CHCl_3$–MeOH (10: 1→8: 1) to furnish two fractions containing M-5 (3.5 g) and

M-6 (2.7 g). Purification of the M-5 fraction by successive column chromatography on polystyrene gel (eluted with MeOH) and on Sephadex LH-20 (Pharmacia Fine Chemicals, eluted with MeOH) afforded M-5 (**2**, 400 mg). The M-6 fraction was purified by successive column chromatography on Sephadex LH-20 (MeOH), polystyrene gel (MeOH), and again on Sephadex LH-20 (MeOH) to afford M-6 (**4**) (930 mg).

M-5 (**2**), a white powder, IR ν_{max}^{KBr} cm^{-1}: 3595, 3500, 3400 (br), 3265, 1744, 1719. ^1H NMR (90 MHz, $CDCl_3$, δ): 0.87 (deformed t-like), 1.25 (br), 2.30 (t-like), 3.5—4.5 (sugar protons), 5.36 (t-like, olefinic proton). ^{13}C NMR (25 MHz, CD_3OD, δ_c): as shown in Table I. ^{13}C NMR (25 MHz, $CDCl_3$, δ_c): 14.0 (q), 22.7 (t), 25.0 (t), 27.4 (t), 29.1 (t), 29.3 (t), 29.5 (t), 29.8 (t), 31.9 (t), 32.0 (t), 34.3 (t), 34.4 (t), 61.6 (t), 63.1 (t), 68.2 (t), 69.0 (d), 70.4 (d), 71.3 (d), 73.7 (d), 74.9 (d), 104.2 (d), 129.8 (d), 130.0 (d), 173.4 (s), 173.7 (s). TLC (silica gel 60 F_{254}, Merck, $CHCl_3$–MeOH–H_2O=65: 35: 10, lower phase): Rf=0.65.

M-6 (**4**), a white powder, IR ν_{max}^{KBr} cm^{-1}: 3450 (br), 1740 (br), 1178 (br), 1036. ^1H NMR (90 MHz, d_5-pyridine, δ): 0.88 (deformed t-like), 1.29 (br), 3.5—5.0 (sugar protons), 5.26 (br s, anomeric proton), 5.46 (t-like). ^{13}C NMR (25 MHz, CD_3OD+D_2O, δ_c): 28.3 (t, C-7″, 10″), 130.8 (d, C-8″, 9″), and other signals as given in Table I. TLC [as described for M-5 (**2**)]: Rf=0.35.

Alkaline Treatment of M-5 (2)——A solution of 1/6 N NaOMe–MeOH (4 ml) was added to M-5 (**2**, 55 mg) and the solution was left standing at 20°C for 4 h. The reaction mixture was neutralized with 2 N HCl–MeOH and partitioned into *n*-hexane–MeOH mixture. Evaporation of the solvent under reduced pressure from the MeOH phase yielded a residue which was purified by column chromatography on Sephadex LH-20 eluting with MeOH to furnish **3** (18 mg). From the *n*-hexane phase, a mixture of fatty acid methyl esters (12 mg) was obtained after similar treatment. **3**, $[\alpha]_D^{15}$ −9° (c=0.6, H_2O).[6] CI–MS (CH_4): m/z 255 (M^++1). ^{13}C NMR (25 MHz, D_2O+CD_3OD, δ_c): as given in Table I. GLC analysis of fatty acid methyl esters (3% SE-30 on Chromosorb WAW DMCS 80—100 mesh, 3 mm×1 m, column temp. 140°C, carrier gas N_2 at flow rate 20 ml/min): t_R (min)=**a** 7′50″, **b** 16′50″, **c** 19′00; **a**: **b**: **c**=1: 10: 10.

Methanolysis of 3——A solution of 2.5 N HCl–MeOH (1.5 ml) was added to **3** (5 mg) and the solution was heated under reflux for 1.5 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. Evaporation of the solvent under reduced pressure from the filtrate gave a residue which was dissolved in pyridine (0.2 ml) and treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (0.2 ml) at 20°C for 5 min. The TMS derivative thus obtained was shown to be a mixture of TMS-glycerol and methyl TMS-galactoside by GLC analysis (5% SE-52 on Chromosorb WAW DMCS, 80—100 mesh, column 3 mm×2 m): TMS-glycerol (column temp. 120°C, N_2 at flow rate 25 ml/min, t_R (min)=7′00″) and methyl TMS-galactoside (column temp. 150°C, N_2 at flow rate 32 ml/min, t_R (min)=21′10″, 24′50″, 28′45″).

Alkaline Treatment of M-6 (4)——A solution of 1/6 N NaOMe–MeOH (3 ml) was added to M-6 (**4**) (25 mg) and the mixture was left standing at 20°C for 1 h. The reaction mixture was neutralized with 2 N HCl–MeOH and the total solution was then partitioned into *n*-hexane–MeOH mixture. The MeOH phase was separated and treated as described above for M-5 (**2**) and the product was purified by Sephadex LH-20 column chromatography (eluting with MeOH) to furnish **6** (12 mg).[8] Concentration of the *n*-hexane phase gave a mixture of fatty acid methyl esters (12 mg). **6**, $[\alpha]_D^{15}$ +58° (c=0.6, H_2O). ^{13}C NMR (25 MHz, D_2O, δ_c): as given in Table I. TLC (silica gel 60 F_{254}, Merck, $CHCl_3$–MeOH–H_2O=9: 13: 3): Rf=0.50. GLC analysis [as described for M-5 (**2**)]: **b**: **c**=1: 2.

References and Notes

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12) The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our preceding paper.[1]