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NEW GLYCOSPHINGOLIPIDS FROM THE MARINE SPONGE *HALICHONDRIA PANICEA*

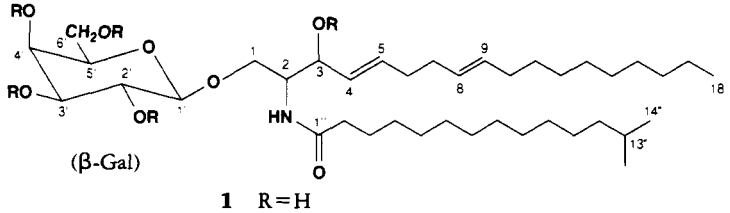
DALE G. NAGLE, WILLIAM C. MCCLATCHEY, and WILLIAM H. GERWICK*

College of Pharmacy, Oregon State University, Corvallis, Oregon 97331

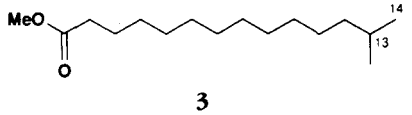
ABSTRACT.—A novel sphingolipid containing an iso-fatty acid, (4*E*,8*E*)-*N*-13′-methyltetradecanoyl-1-*O*-β-D-glucopyranosyl-4-sphingadiene, was isolated from the Oregon marine sponge *Halichondria panicea* and its structure determined using a combination of spectroscopic and chemical degradation techniques. A second galactosyl-ceramide, which contained an unusually long chain fatty acid amide component, was also isolated from *H. panicea*.

Sponges have long been recognized as a rich source of structurally novel lipids including unique sterols, fatty acids, phospholipids, and triglycerides (1–3). The major point of structural novelty in sponge sterols is the incorporation of additional carbon atoms as branches on the side chain. Further, the unique fatty acids, either in free form or as present in phospholipids and triglycerides, incorporate additional branch carbons; and many are of unusual length with unique patterns of unsaturation. These findings suggest that fundamental differences may exist between sponge membranes and those of all other animals. While the presence of sphingolipids in sponges was first suggested from tlc evidence over 20 years ago (4,5), structural proof for the existence of cerebrosides in sponges was not provided until the mid-seventies (6). Recently, *Halichondria japonica* has been shown to contain galactosylceramides with branches in the sphingosine base portion (7).

As part of our continuing evaluation of the chemistry of Pacific Northwest marine organisms (8), the lipid extract of the marine sponge *Halichondria panicea* Pallas (Homorrhaphidae, Demospongiae) was found to possess several interesting acid-charring compounds by tlc and appeared to warrant further investigation. Herein we report our isola-



1 R=H
2 R=Ac



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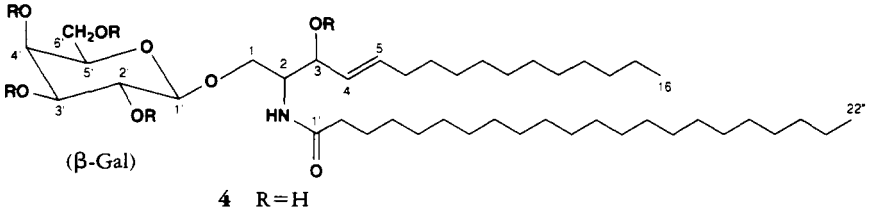

4 R=H
5 R=Ac

tion and structure elucidation of a novel galactopyranosyl-(β1-1′)-ceramide containing an iso-fatty acyl group from this temperate water sponge.

The $CHCl_3$-MeOH (2:1) extract of *H. panicea* was largely a complex mixture of homologous glycolipids (21%). These were separated following acetylation of the crude mixture (Ac_2O/pyridine) by repeated Si gel vacuum chromatographic separation and reversed-phase hplc. High field ^{1}H-nmr analysis of the glycolipid mixture before acetylation showed no acetate groups of natural origin. In this fashion, the polar acid-charring compound **1** was first isolated as the optically active peracetate derivative **2**, $[\alpha]_D$ −11.7°, and its molecular formula determined as $C_{49}H_{83}O_{13}N_1$ by negative ion hrfabms.

Four spin systems were determined in **2** from analysis of the ^{1}H-^{1}H COSY (see Table 1). The first ^{1}H-^{1}H spin system was that of a sugar moiety, assigned as the tetraacetate ester of a β-galactopyranosyl residue from $^3J_{HH}$ values and a comparison to models (9,10). From observation of a correlation between the signals at δ 4.34 (m, H-2) and δ 5.72 (d, 4.2, N-H), a second spin system was established as the C-3 acetate ester of a mono-unsaturated sphingosine base unit.

A third spin system was assigned as a saturated ceramide acyl group. A 6H signal in the methyl region, δ 0.86 (d, 6.6) was correlated to a methine resonance at δ 1.51 (m) and suggested that either the sphingosine base or the acyl chain contained a terminal gem-dimethyl group. Overlap in the methylene region made placement of the gem-dimethyl group and chain length determination impossible from ^{1}H nmr alone. However, the positional attachments of the sugar, sphingolipid base, and fatty acid amide moieties were established from spectroscopic comparison with well characterized galactopyranosyl-(β1-1′)-ceramide analogues (6,10,11).

The length and branching pattern of

TABLE 1. Nmr Data for Derivative **2**.[a]

Position	^{1}H nmr	^{13}C nmr
	Sphingosine Base	
1_a	3.58 dd (10.4, 4.5)	67.14
1_b	3.94 dd (10.4, 4.2)	
2	4.34 m	50.52
3	5.26 t (6.8)	73.76
4	5.4 m	124.92
5	5.80 dt (15.3, 6.2)	136.25
6	2.1 m	32.44[b]
7	2.05 m	32.03[b]
8	5.39 m	128.98
9	5.41 m	131.28
10	1.96 q (6.6)	32.63
11	1.3 m	29.2–29.9[c]
12	1.26 m	29.2–29.9[c]
13	1.26 m	29.2–29.9[c]
14	1.26 m	29.2–29.9[c]
15	1.26 m	29.2–29.9[c]
16	1.26 m	29.2–29.9[c]
17	1.25 m	29.2–29.9[c]
18	0.88 t (6.5)	14.12
	Sugar	
1′	4.44 d (7.8)	101.03
2′	5.15 dd (10.4, 7.8)	68.88
3′	5.00 dd (10.4, 3.2)	70.76
4′	5.4 m	66.95
5′	3.90 t (6.6)	70.76
6′	4.13 dd (6.6)	61.24
	Fatty Acid	
N-H	5.72 d (4.2)	—
1″	—	172.68
2″	2.14 m	36.89
3″	1.62 m	25.72
4″	1.3 m	29.2–29.9[c]
5″	1.26 m	29.2–29.9[c]
6″	1.26 m	29.2–29.9[c]
7″	1.26 m	29.2–29.9[c]
8″	1.26 m	29.2–29.9[c]
9″	1.26 m	29.2–29.9[c]
10″	1.26 m	29.2–29.9[c]
11″	1.26 m	29.2–29.9[c]
12″	1.14 q (6.8)	39.09
13″	1.51 m	27.99
14″,15″	0.86 d (6.6) 6H	22.66 (2C)
	Acetate Esters	
	2.17 s	21.12[d] 170
	2.06 s	20.83[d] 170
	2.05 s	20.67[d] 170
	2.05 s	20.67[d] 170
	1.99 s	20.65[d] 170

[a]All ^{1}H-nmr spectra were recorded at 400 MHz and ^{13}C-nmr spectra at 100 MHz in $CDCl_3$ with 0.05% TMS as internal chemical shift reference.

[b–d]Values with the same superscript may be interchanged.

the fatty acyl chain were determined by base hydrolysis of derivative **2** (10% KOH in $EtOH/H_2O$), methylation (CH_2N_2 in Et_2O), and gc-ms. Although the ms fragmentation pattern for this fatty acid methyl ester **3** gave a 93% match with that of a standard sample of methyl *n*-pentadecanoic acid, the presence of a gem-dimethyl group in **3** was confirmed by ^{1}H-nmr analysis which established its structure as the methyl ester of 13-methyl-tetradecanoic acid (iso-pentadecanoic acid).

The trans (*E*) configuration of the C-4 and C-8 double bonds in derivative **2** was assigned by a combination of ^{1}H coupling constants, ^{13}C chemical shifts, and comparison with known C-4, C-8 diene sphingolipids (10–12). The length of the sphingosine backbone was deduced from consideration of the molecular composition of derivatives **2** and **3** and the partial structures described above, thus defining the structure of **1** as (4*E*,8*E*)-*N*-13′-methyl-tetradecanoyl-1-*O*-β-D-glucopyranosyl-4-sphingadiene.

Further study of *H. panicea*'s polar lipids resulted in the partial isolation of a number of sphingolipid constituents including compound **4**. These isolation efforts were complicated by the occurrence of a number of structural homologues within each structure class of sphingolipid. Comparison of ^{1}H-nmr, ^{13}C-nmr, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR, and fabms established compound **4**, isolated as its peracetate derivative **5**, as a structural analogue of derivative **2**, differing in the absence of a gem-dimethyl grouping, C-8 olefin, and the length of the fatty acid amide and sphingolipid base chains (see Experimental). The principal methyl ester (82%) of the fatty acyl chain obtained from **5** upon base hydrolysis and methylation was shown to be methyl docosanoate by gc-ms (*m/z* 354 $[M]^+$). Correspondingly, the sphingolipid backbone chain length was deduced from hrms, yielding the structure of **4** as (4*E*)-*N*-docosanoyl-1-*O*-β-D-glucopyranosyl-4-hexadecasphinganine. The fatty acid amide chain lengths of several structurally related minor impurities in this preparation of derivative **5** were determined from gc-ms of their corresponding methyl ester derivatives (eicosanoate, 10%; tetracosenoate, 6%; tricosanoate, 1.7%; see Experimental).

Djerassi and coworkers have suggested that the unusual branched and elongated fatty acyl chains found in sponge phospholipids may be the result of necessary adaptations to impart fluidity deep within the membrane bilayer, and may have evolved in conjunction with the "fluidizing" methylated sterols found in these marine animals (13). If these unusual sterol and phospholipid structural modifications are important to functional characteristics of sponge membrances, it is conceivable that other membrane components, including the sphingolipids, may have evolved similar methylation and chain elongation pattens. This study of the novel sphingolipid chemistry of *H. panicea*, which contains these unusual methylation and chain length modifications, supports this hypothesis. Compound **1** is, to our knowledge, the first reported ceramide to contain an iso-fatty acid at C-2.

EXPERIMENTAL

SPECTROSCOPIC DATA.—Ir spectra were recorded on a Beckman Acculab 7 spectrophotometer. Optical rotation(s) was measured on a Perkin Elmer Model 141 polarimeter using a 10-cm microcell. Nmr spectra were obtained at 400 MHz for ^{1}H and 100 MHz for ^{13}C on a Brucker AM 400 nmr spectrometer, and all shifts are reported relative to an internal TMS standard. Low resolution gc-eims of methylated fatty acid fragments were obtained on a Finnigan 4023 spectrometer, and low and high resolution mass measurements (hrms) of acetylated glycosphingolipids were obtained on a Kratos MS 50 TC. Hplc employed a Waters M-6000 pump, U6K injector, R 401 differential refractometer, and tlc used Merck aluminum-backed tlc sheets (Si gel 60 F_{254}) with 50% H_2SO_4 as a spray indicator. All solvents were distilled from glass prior to use.

SPONGE MATERIAL.—*H. panicea* (collection SH 15VII87-1: voucher available from WHG) was collected from exposed low intertidal rock surfaces (−1.0 to −0.5 m) at Strawberry Hill on

the Oregon coast in July 1987. The sponge material was immediately frozen at the site in CO_2 (s) and stored at −20°.

EXTRACTION AND ISOLATION OF GLYCOSPHINGOLIPIDS.—The sponge (1 gallon) was broken into small pieces and extracted with 2 liters of $CHCl_3$-MeOH (2:1) to produce 10.9 g of a dark green-brown oil. Vacuum chromatography of this extract over normal phase Si gel and eluting with mixtures of EtOAc in isooctane gave 10 fractions. Tlc showed that fractions 5 and 6 contained a nearly equal distribution of extremely polar non-uv-active acid-charring substances. These fractions were combined (2.3 g) and acetylated over an 18-h period at room temperature in 6 ml of Ac_2O-pyridine (1:1). The reaction was terminated with the addition of ice and then H_2O and extracted with Et_2O (4×). The combined Et_2O layers were sequentially washed with 5% HCl (3×), saturated $NaHCO_3$ (3×), and distilled H_2O (2×), and then dried over $MgSO_4$. Following filtration the solvents were removed in vacuo to yield 2.0 g of oil. The oil was then subjected to additional vacuum chromatography over Si gel and eluted with mixtures of EtOAc in isooctane to give 9 fractions (1–9). Fractions 3′ and 4′ were shown by tlc to contain nearly all of the acetylated material. Reversed-phase hplc (10 mm × 25 cm LiChrosorb® 7 μ C-18 column, 5% H_2O in MeOH) of fraction 3′ (0.652 g) resulted in the separation of 15 structurally related peracetate derivatives including derivative **2** (44.8 mg). Reversed-phase hplc (conditions as above) of fraction 4′ (0.652 g) resulted in the isolation of a similar, if not identical, set of related derivatives including derivative **5** (23.8 mg).

PREPARATION OF FATTY ACID DERIVATIVES.—The fatty acyl components of the glycosphingolipids were obtained by reaction of the peracetylated glycosphingolipid derivatives with 10% KOH in EtOH-H_2O (4:1) at 25° for 20 h. The hydrolysate was adjusted to pH 4 with 5% HCl and partitioned between $CHCl_3$ and H_2O (3×) after removal of the EtOH in vacuo. The organic extract was then washed repeatedly with distilled H_2O to remove any salts. The fatty acids were methylated (1–2 ml CH_2N_2 in Et_2O, 1 h) and purified by hplc (4.1 mm × 30 cm Versapack® Si 10 μ column, 2% EtOAc in isooctane) or analyzed directly by gc-ms.

Derivative **2**.—Oil: ir (CCl_4) 3100, 3004, 2390, 1720, 1661, 1346, 1225, 1070 cm^{-1}; $[\alpha]D$ −11.7° (c = 1.56, $CHCl_3$); fabms (3-nitrobenzyl alcohol, negative ion) *m/z* $[M+3\text{-NBA}]^-$ 1046.6 (52), 939.6 (38), $[M-H]^-$ 892.6 (11), 850.6 (47), 808.6 (12.5), 746.5 (5), 500.4 (11), 472.4 (5), 240.2 (2.5), 153.1 (100); hrfabms *m/z* (negative ion) $[M+3\text{-NBA}+H]^-$ 1047.6352 ($C_{56}H_{91}O_{16}N_2$) 1.6 mmu (±); deduced molecular formula of derivative **2** $C_{49}H_{83}O_{13}N_1$; ^1H and ^{13}C nmr see Table 1.

Derivative **3**.—Fatty acid methyl ester of derivative **2**: oil; gc-eims *m/z* $[M]^+$ 256 (55), $[M-Me]^+$ 241 (2), 225 (13), $[M-CHMe_2]^+$ 213 (39), 199 (16), 185 (11), 171 (11), 157 (17), 143 (44), 129 (21), 97 (30), 87 (62), 83 (33), 74 (100), 69 (40); ^1H nmr (400 MHz, $CDCl_3$) δ 3.68 (3H, s, -OMe), 2.31 (2H, t, J = 7.5, C-2″), 1.65 (2H, m, C-3″), 1.55 (1H, m, C-13″), 1.26 (16H, s, H-4″ to H-11″), 1.18 (2H, m, C-12″), 0.86 (6H, d, J = 6.6, C-14″ and C-15″).

Derivative **5**.—Oil: ir (CCl_4) 3100, 2960, 2930, 1750, 1680, 1225, 1070 cm^{-1}; fabms (3-NBA, negative ion) *m/z* $[M+3\text{-NBA}]^-$ 1119 (69), 1012 (62), $[M-H]^-$ 965 (22), 923 (86), 53 (38), 380 (100); ^1H nmr (400 MHz, $CDCl_3$) δ 5.75 (1H, dt, J = 15.6, 6.6, H-5), 5.7 (1H, d, J = 10, N-H), 5.4 (1H, m, H-4′), 5.4 (1H, m, H-4), 5.28 (1H, t, J = 7.0, H-3), 5.15 (1H, dd, J = 10.6, 8.0, H-2′), 5.00 (1H, dd, J = 10.6, 3.2, H-3′), 4.44 (1H, d, J = 7.9, H-1′), 4.35 (1H, m, H-2), 4.14 (1H, d, J = 6.6, H-6′), 3.93 (1H, dd, J = 10.0, 3.7, H_a-1), 3.92 (1H, t, J = 6.6, H-5′), 3.58 (1H, dd, J = 10.0, 4.2, H_b-1), 2.17 (3H, s, -OAc), 2.15 (2H, m, H-2″), 2.06 (3H, s, OAc), 2.05 (3H, s, -OAc), 2.04 (3H, s, -OAc), 2.04 (2H, m, H-6), 1.99 (3H, s, -OAc), 1.6 (2H, m, H-3″), 1.35 (2H, m, H-7), 1.3 (2H, m, H-4″), 1.26 (32H, m, H-5″ to H-21″), 1.26 (18H, m, H-8 to H-16), 0.88 (6H, t, J = 6.6, H-16 and H-22″); ^{13}C nmr (100 MHz, $CDCl_3$) δ 172.70 (C-1″), 170.32 (-OCOMe), 170.19 (-OCOMe), 169.92 (-OCOMe), 170.0 (-OCOMe), 169.59 (-OCOMe), 137.07 (C-5), 124.67 (C-4), 100.95 (C-1′), 73.75 (C-3), 70.80 (C-5′), 70.72 (C-3′), 68.85 (C-2′), 67.07 (C-1), 66.91 (C-4′), 61.18 (C-6′), 50.50 (C-2), 39.05, 36.80 (C-2″), 36.60, 34.39, 31.90, 30.02, 29.93, 29.69, 29.54, 29.49, 29.40, 29.32, 28.97, 27.95, 27.40, 27.10, 25.09, 22.66, 21.08 (-OCO*Me*), 20.80 (-OCO*Me*), 20.61 (2C, -OCO*Me*), 19.20 (-OCO*Me*), 14.14 (C-16), 14.12 (C-22″).

Major fatty acid methyl ester of derivative **5**.—Oil: gc-eims *m/z* $[M]^+$ 354 (87) (for $Me(CH_2)_{20}CO_2Me$), 323 (9), 311 (19), 297 (3), 283 (1), 269 (4), 255 (10), 241 (3), 227 (1.5), 213 (3), 199 (12), 185 (6), 171 (2), 157 (4), 143 (37), 129 (12), 115 (3), 101 (7), 97 (11), 87 (75), 74 (100), 55 (21), 43 (31).

Eicosanoate methyl ester of derivative **5**.—Gc-eims *m/z* $[M]^+$ 340 (93) (for $Me(CH_2)_{19}CO_2Me$), 309 (10), 297 (21), 283 (2), 255 (5), 241 (9), 199 (9), 185 (6), 157 (3), 143 (34), 129 (11), 111 (5), 97 (12), 87 (76), 74 (100), 69 (16), 55 (21), 43 (29).

Tetracosenoate methyl ester of derivative **5**.—Gc-eims *m/z* $[M]^+$ 380 (9) [for $Me(CH_2)_nCH=CH(CH_2)_{20-n}CO_2Me$ ($n<20$)], 348 (100), 306

(12), 264 (14), 250 (8), 236 (6), 222 (5), 208 (5), 194 (4), 180 (4), 166 (5), 152 (9), 138 (8), 125 (13), 111 (22), 97 (42), 87 (29), 83 (43), 74 (39), 69 (47), 55 (53), 43 (27).

Tricosanoate methyl ester of derivative **5**.—Gc-eims *m/z* $[M]^+$ 368 (100) [for $Me(CH_2)_{21}CO_2Me$], 337 (3), 325 (19), 311 (2), 283 (3), 269 (7), 255 (2), 241 (2), 227 (2), 213 (2), 199 (9), 185 (6), 157 (3), 143 (37), 129 (12), 111 (6), 97 (13), 87 (78), 74 (98), 69 (18), 55 (22), 43 (36).

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LITERATURE CITED

1. W. Bergman and A.N. Swift, *J. Org. Chem.*, **16**, 1206 (1951).
2. R.W. Morales and C. Litchfield, *Lipids*, **12**, 570 (1977).
3. E. Ayanoglu, K. Kurtz, J.M. Kornprobst, and C. Djerassi, *Lipids*, **20**, 141 (1985).
4. N.K. Kochetkov, V.E. Vaskovsky, I.G. Zhukova, G.P. Smirnova, and E.Y. Kostetsky, *Dokl. Akad. Nauk SSSR*, **173**, 1448 (1967).
5. V.E. Vaskovsky, E.Y. Kostetsky, V.I. Svetashev, I.G. Zhukova, and G.P. Smirnova, *Comp. Biochem. Physiol.*, **34**, 163 (1970).
6. F.J. Schmitz and F.J. McDonald, *J. Lipid Res.*, **15**, 158 (1974).
7. A. Hayashi, Y. Nishimura, and T. Matsubara, *Biochem. Biophys. Acta*, **1083**, 179 (1991).
8. W.H. Gerwick, M.W. Bernart, M.F. Moghaddam, Z.D. Jiang, M.L. Solem, and D.G. Nagle, *Hydrobiologia*, **204/205**, 621 (1990).
9. K. Falk, K. Karlsson, and B. Samuelsson, *Arch. Biochem. Biophys.*, **192**, 164 (1979).
10. Y. Kawano, R. Higuchi, R. Isobe, and T. Komori, *Liebigs Ann. Chem.*, 19 (1988).
11. R.D. Sitrin, G.C. Chan, J. Dingerdissen, C. DeBrosse, R. Mehta, G. Roberts, S. Rottschaefer, D. Staiger, J. Valenta, K. Snader, R.J. Stedman, and J.R.E. Hoover, *J. Antibiot.*, **41**, 469 (1988).
12. K. Chebaane and M. Guyot, *Tetrahedron Lett.*, **27**, 1495 (1986).
13. R.D. Walkup, G.C. Jamieson, M.R. Ratcliff, and C. Djerassi, *Lipids*, **16**, 631 (1981).

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ERRATUM

For the paper by Findlay *et al.* entitled "Novel Sulfated Oligosaccharides from the Sea Cucumber *Cucumaria frondosa*," *J. Nat. Prod.*, **55**, 93 (1992), the authors request the following changes:

On page 96 in Table 2, the correct values for δ_H are as follows: Xyl I H-4, 5.180; Glc H-3, 4.267, Glc H-4, 3.827, Glc H-5, 4.258 and Glc H-6, 5.178 (6). The correct value for δ_C for compound **5** Xyl II C-5 is 67.01 and for compound **6** Xyl II C-5 is 66.83. The authors apologize for any inconvenience caused.