Journal of Natural Products
Vol. 58, No. 3, pp. 442-445, March 1995

A NOVEL SPHINGOSINE DERIVATIVE FROM THE SPONGE *SPIRASTRELLA INCONSTANS*[1]

H.S. GARG* and SHALINI AGRAWAL

Medicinal Chemistry Division, Central Drug Research Institute, Lucknow 226001, India

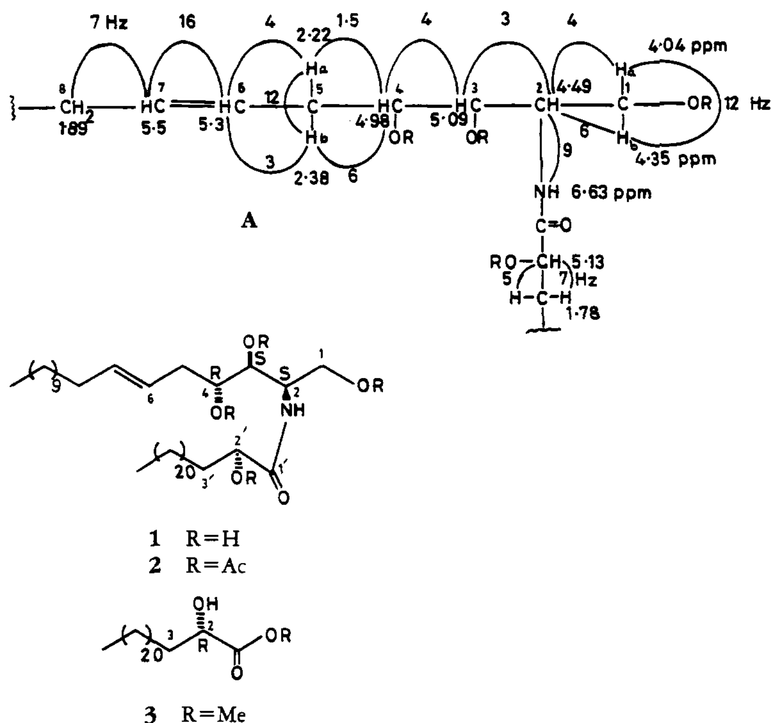
ABSTRACT.—An EtOAc-MeOH (2:1)-soluble fraction of the crude MeOH extract of the sponge *Sprirastrella inconstans*, collected from the eastern coast of India, has yielded a new sphingolipid. Its structure was established as (2*S*,3*S*,4*R*)-*N*-[2′-(*R*)-hydroxytetracosanoate]-1,3,4-trihydroxy-2-amino-octadeca-6-ene, on the basis of fabms and ^{1}H-nmr decoupling studies on its tetraacetate.

In the course of screening marine organisms of the Indian coast and nearby islands for bioactive substances (1), in vitro antiviral activity was noted for a MeOH extract of the fresh sponge *Spirastrella inconstans* (Dendy), family Spirastrellidae, collected from the eastern coast of India. Earlier a sesquiterpene picrotoxin (2) and fourteen saturated and Δ^5-unsaturated steroids (3) with C_8–C_{10} side-chains were obtained from this sponge. We report in this paper the isolation and structure elucidation of a new sphingosine derivative from the EtOAc-MeOH (2:1)-soluble fraction.

The EtOAc-MeOH (2:1)-soluble fraction, on chromatography over Si gel eluting with CH_2Cl_2, yielded a mixture of known triglycerides and sterols that was not further investigated. The fraction eluted with $CHCl_3$-MeOH (1:1) afforded the crystalline compound **1** by flash chromatography. The ir spectrum of **1** showed absorptions at 3350 and 1050 cm^{-1} (hydroxy); 3250 and 1640 cm^{-1} (amide); and 2900, 2850, and 1450 cm^{-1} (aliphatic). The fabms of **1** gave a $[M+H]^+$ ion at *m/z* 682, consistent with the molecular formula $C_{42}H_{83}NO_5$. The ^{1}H-nmr spectrum of **1** in $CDCl_3$ with a drop of CD_3OD showed the presence of terminal methyl groups at δ 0.89 (t), aliphatic methylenes (δ 1.2–1.4), carbinol protons (δ 2.6–4.2), olefinic protons (δ 5.4–5.64), and an amide -NH doublet (δ 7.3).

The above data suggested that **1** was a fatty acid amide of a sphingosine derivative. Due to the poor solubility of **1** in most organic solvents, its structure elucidation was carried out on the acetyl derivative, **2**. Treatment of **1** with Ac_2O/pyridine at 80–90° for 4 h yielded the tetraacetate **2**. The fabms of **2** showed a $[M+H]^+$ ion at *m/z* 850, consistent with the tetraacetate composition $C_{50}H_{91}NO_9$. The ^{1}H-nmr spectrum of **2** showed the presence of four acetyl methyl singlets between δ 2.0 and 2.2 (Table 1) supporting the presence of four free hydroxy groups in **1**. The ^{1}H-nmr spectrum of **2** was similar to that of the tetraacetate of UF-131, a tetrahydroxy sphingosine *N*-palmitate reported earlier from *Ulva faciata* (4), differing only in the presence of two additional olefinic proton signals at δ 5.3 (ddd, J=16, 4, and 3 Hz) and δ 5.5 (dt, J=16 and 7 Hz). The sphingosine amide nature of **1** was also evident from the ^{13}C-nmr spectrum of **2** (Table 1) showing the presence of an amide functionality (δ 170.1 and 49.1), along with four carbinol carbons, two olefinic carbons, terminal methyls, and aliphatic carbons. The assignment of ^{1}H-nmr signals and the couplings of the core portion (**A**) of the molecule were determined by ^{1}H-nmr decoupling experiments and from the ^{13}C-nmr spectrum of **2**. The amide proton signal in **2** appeared at δ 6.63 (d, J=9 Hz) and was exchangeable with D_2O. Taking this signal as the starting point, the sequence in which these substituents were present in **1** was deter-

[1]CDRI Communication No. 5296.



mined by the following ^{1}H-nmr decoupling experiments.

Irradiation of the proton at δ 4.49 resulted in a change of the amide proton to a singlet, and the two quartets at δ 4.04 and 4.35 for $H_{1\alpha}$ and $H_{1\beta}$ (ABX system) both collapsed to doublets (geminal coupling 12 Hz), and the other signal at δ 5.09 was also changed to a doublet (J=3 Hz). This was in good agreement with the usual 1,3-dihydroxy-2-amido grouping of sphingosine derivatives. Irradiation of the proton at δ 4.98 collapsed the signal of the carbinol proton at δ 5.09 to a narrow doublet (J=3 Hz), while the signal of the aliphatic proton at δ 2.38 changed to a quartet (J=12 and 6 Hz) from a broad doublet. The rest of the protons were unaffected. Because none of these protons showed couplings with the olefinic protons, the double bond must be isolated from the hydroxyl groups.

Irradiation of the most downfield carbinol proton at δ 5.13 showed no effect on any signal for the carbinol or olefinic protons, but the methylene proton signal at δ 1.78 was affected. Irradiation of the latter signal changed the signal at δ 5.13 into a singlet. This suggested that there is a hydroxyl group in the molecule which is isolated with respect to the 1,2,3,4-substituted groups. Its chemical shifts and coupling suggested that it was part of an α-hydroxy acid moiety.

Compound **1**, on methanolysis using aqueous H_2SO_4/MeOH and extraction with hexane yielded the methyl ester of (2*R*)-hydroxytetracosanoic acid [**3**], $[M]^-$ *m/z* 398. The identification of **3** was established by comparing its spectral data with those reported (5). This fully supported the decoupling experiments as above; thus, the signal at δ 5.13 was assigned to the H-2′ proton and that at δ 1.78 to the H-3′ proton in **2**.

The placement of the double bond at Δ^6 in the base part of the molecule was established by further ^{1}H-nmr decoupling experiments. The olefinic proton that resonated at δ 5.5 (dt) was found to be adjacent to a methylene signal at δ 1.89, since irradiation of this latter proton changed the olefinic proton to a doublet (J=16

TABLE 1. ^{1}H- and ^{13}C-Nmr Assignments of **2**.

^{1}H nmr			Decoupled	^{13}C nmr	
Proton	δ_H	J (Hz)	Signal, J (Hz)	Carbon[a]	δ_C
Sphingosine moiety				Sphingosine moiety	
H-1a	4.04 (dd)	12, 3	d,[b] 12	C-1	64.5
H-1b	4.35 (dd)	12, 5	d,[b] 12	C-2	49.1
H-2	4.49 (m)	—	[b] —	C-3	72.95
H-3	5.09 (dd)	4, 3	d,[b] 4	C-4	72.15
H-4	4.92 (dt)	6, 4		C-5	31.8
H-5a	2.22 (dd)	12, 4, 1.5		C-6	134.8
H-5b	2.38 (br d)	12, 6, 3		C-7	123.8
H-6	5.3 (d, dd)	16, 4, 3	dd,[c] 16, 3	C-8	31.9
H-7	5.5 (dt)	16, 7	d,[c] 16	C-9	29.35
H-8 (2H)	1.89 (m)	—	[c]	C-10 to C-17	29.4
-NH-	6.63 (d)	9	s[b]		29.4
H-18 (3H)	0.89 (t)	7.5			22.7
COCH_3×3	2.02 (s)	—		C-18	14.1
	2.05 (s)	—		COCH_3×4	20.8–21.1
	2.10 (s)	—		COCH$_3$×4	170.03
					170.05
					170.10
					170.02
N-Acyl moiety				*N*-Acyl moiety	
H-2′	5.13 (dd)	7, 5	s[d]	C-1′	170.15
H-3′ (2H)	1.78 (m)	—	[d]	C-2′	72.3
H-24′ (3H)	0.89 (t)	7, 5		C-3′	30.0
COCH_3 (3H)	2.18 (s)			C-4′	24.9
				C-5′ to C-23′	29.7
					24.9
					20.8
				C-24′	14.1

[a]Assignments were made by DEPT studies.
[b]Denotes changes in ^{1}H-nmr signals on irradiation of the proton signal at 4.49 ppm.
[c]At 1.89 ppm.
[d]At 1.78 ppm.

Hz) showing couplings with the adjacent olefinic proton at δ 5.3. Irradiation of the proton at δ 2.22 resulted in a change of the signal at δ 5.3 to a broad double doublet (J=16 and 3 Hz) and at the same time the carbinol proton at δ 4.98 (H-4) collapsed to a narrow double doublet (J=6 and 4 Hz), showing H-4 and H-5a couplings as 1.5 Hz and H-4 and H-3 couplings as 4 Hz. The proton signal at δ 2.38 (br d) also changed to a narrow double doublet (J=3 and 6 Hz; H-5b–H-6 and H-5b–H-4 coupling). The disubstituted double bond was assigned the *E* geometry on the basis of the observed $J_{6\text{-}7}$ coupling of 16 Hz. The above decoupling studies led to partial structure '**A**' for **1**.

Since the *N*-acyl moiety was identified as (2*R*)-hydroxytetracosanoic acid, the sphingosine moiety of **1** must be assigned as 2-amino-1,3,4-trihydroxy-octadeca-6-ene. The above data suggested the sphingolipid is a fatty acid amide of 1,3,4-trihydroxy-2-amino-octadeca-6-ene, an analogue of sphingosine. The identical coupling constants and chemical shifts along with the optical rotations reported for the synthetic ceramide part of the cerebroside agelasphin (5) and the synthetic D-ribo-C_{18}-phytosphingosine (6), further suggested a (2*S*,3*S*,4*R*)-configuration of the sphingosine moiety in **1**. Its proposed structure is thus (2*S*, 3*S*,4*R*)-*N*-[(*R*)-2′-hydroxytetracosanoyl]-

1,3,4-trihydroxy-2-amino-octadeca-6-ene [**1**].

This is the first report of a sphingosine derivative isolated from a marine source (sponge) in which the double bond has migrated to the C-6 position, allylic to the 4-hydroxyl group.

Recently, a number of ceramide agelasphins having 2-hydroxy fatty acid amides of triene and/or saturated trihydroxy sphingosine derivatives showing antitumor activity have been reported from the Okinawan sponge *Agelas mauritianus* (5). The presence of glycosidic derivatives of **1** in *S. inconstans* has been considered, and attempts are being made to isolate such compounds from the polar extracts of the sponge.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were run on a Perkin-Elmer 881 instrument as KBr disks, and mass spectra were recorded on a JEOL JMS-D-300 (70 eV) instrument and fabms on a JEOL 5×102 FAB instrument with a JMA-DA Good Data System. Optical rotations were recorded on an Autopol III automatic polarimeter in MeOH and/or $CHCl_3$. The ^{13}C- and ^{1}H-nmr and ^{1}H-nmr decoupling experiments were recorded on a Bruker-WM (400 MHz) instrument equipped with an Aspect 2000 computer, using TMS as internal reference.

ANIMAL MATERIAL.—The sponge *Spirastrella inconstans* (fresh wt 10 kg) was collected from the eastern coast of India and transported to the laboratory dipped in MeOH. A voucher specimen is preserved at the National Institute of Oceanography, Goa, India, voucher number CDR-179.

EXTRACTION AND ISOLATION.—The material was chopped into small pieces and extracted with MeOH (3×4 liters) to yield 40.2 g of the extract. This was fractionated into EtOAc-soluble (F003, 2.8 g) and EtOAc-MeOH (2:1)-soluble (F004, 15.5 g) fractions. Cc of F004 (12.0 g) was performed over Si gel using CH_2Cl_2 as the eluent followed by a $CHCl_3$/MeOH gradient (20–100% MeOH). The fractions eluted with CH_2Cl_2 yielded a mixture of triglycerides and sterols (ca. 0.4%) which was not investigated further. The fraction eluted with $CHCl_3$-MeOH, 1:1 (1.03 g; 0.1% dry wt of the sponge) was subjected to flash chromatography over Si gel (200–400 mesh) and eluted with a $CHCl_3$-MeOH gradient (3:7) to yield **1** (35.0 mg). Compound **1** was obtained as microcrystals (MeOH): mp 121–123°; $[\alpha]^{27}D$ +9.2° (c=0.05, MeOH); ir (film) ν max 3350, 3250, 2900, 2850, 1640, 1450, 1050 cm^{-1}; ^{1}H nmr ($CDCl_3+CD_3OD$) δ 0.89 (CH_3), 1.28 (CH_2×28), 2.22, 2.64 (2H), 3.58 (2H), 3.68, 3.80, 3.95, 4.23 (OH), 5.4, 5.64, and 7.3 (N*H*), fabms m/z 682 (M+H).

ACETYLATION OF **1** TO **2**.—Compound **1** (20 mg) in pyridine (0.5 ml) and Ac_2O (1.2 ml) was left at room temperature overnight and heated for 4 h at 80°. The usual workup and crystallization yielded **2**, mp 52°; $[\alpha]^{27}D$ +7.2° (c=0.03, $CHCl_3$); ir (film) ν max 3250, 2900, 2850, 1640, 1450, 1050 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Table 1; fabms m/z 850 $[M+H]^+$; $C_{50}H_{91}NO_9$ requires m/z 849 $[M]^+$.

Methanolysis of **1**.—Compound **1** (10 mg) was treated with 1.2 M H_2SO_4 in 80% aqueous MeOH (1–2 ml) for 8 h at 80°. The reaction mixture was extracted with hexane and the hexane layer was passed through Si gel to yield (2*R*)-hydroxytetracosanoic methyl ester [**3**]; $[\alpha]^{27}D$ −1.7° (c=0.03, $CHCl_3$); eims m/z 398 $(M)^+$ ($C_{25}H_{50}O_3$); ^{1}H nmr ($CDCl_3$) δ 4.20 (1H, br), 3.68 (3H, s, OCH_3), 1.76 (2H), 1.28 (CH_2×16), 0.89 (3H, t, J=7.5 Hz). The data were identical to those reported (5).

ACKNOWLEDGMENTS

This research was supported by a grant DOD/II-MRDF I/15/NP90-DD-II-10/10 from the Department of Ocean Development (India) and financial assistance to one of us (S.A.) is gratefully acknowledged. Thanks are also due to Dr. Raja Roy for proton decoupling nmr experiments and the Director, CDRI, for his interest in the work.

LITERATURE CITED

1. B.N. Dhawan, H.S. Garg, A.K. Goel, R.C. Srimal, M.N. Srivastava, and D.S. Bhakuni, *Ind. J. Exp. Biol.*, **31**, 505 (1993).
2. S.N. Sharma, M. Rambabu, A.S.R. Anjaneyulu, and C.B.S. Rao, *Ind. J. Chem.*, **26B**, 189 (1987).
3. R.S.H.S.N. Kalidindi, C.B.S. Rao, T. Akihisa, T. Tamura, and T. Matsumoto, *Ind. J. Chem.*, **27B**, 160 (1988).
4. H.S. Garg, M. Sharma, D.S. Bhakuni, B.M. Pramanik, and A.K. Bose, *Tetrahedron Lett.*, **33**, 1641 (1992).
5. T. Natori, M. Morita, K. Akimoto, and Y. Koezuka, *Tetrahedron*, **50**, 2771 (1994).
6. J. Mulzer and C. Brand, *Tetrahedron*, **42**, 5961 (1986).

Received 15 July 1994