SHORT PAPER

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A novel Ceramide from the Indian marine sponge *Fasciospongia cavernosa*†

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A new ceramide **1**, has been isolated along with a known compound 3,6-diacetoxy-cholest-7-en-3β,5α,6β-triol (**2**) from a marine sponge *Fasciospongia cavernosa* collected off the Mandapam coast in the Gulf of Manner, India. The structures of **1** and **2** were determined by spectroscopic analysis.

Keywords: Ceramide, Indian marine sponge

Marine organisms have proven to be a rich source of compounds, that might be useful for the development of new pharmaceutical agents.[1] In this context, a number of novel ceramides have been isolated and reported to possess interesting biological properties [DNA polymerase α-inhibitory,[2] antiviral,[3] antifungal and cytotoxic,[4] antihepatotoxic[5] activity]. A literature survey revealed that the genus *Fasciospongia* is a rich source of N-acyl-2-methylene-β-alanine methyl esters,[6] phenolic compounds,[7] trisnorditerpenes,[8] and manolides.[9]

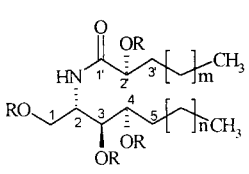
In our search for bioactive compounds from marine organisms,[11,12] we have examined the sponge *Fasciospongia cavernosa* and described the isolation of 2-methylene-β-alanine methyl ester derivatives with some common sterols.[10] In this paper we report on the isolation and characterisation of a new ceramide from the sponge *F. cavernosa*.

The 1:1 dichloromethane:methanol extract of the sponge *Fasciospongia cavernosa* was subjected to gel filtration chromatography on Sephadex LH-20 (1:1 dichloromethane: methanol) followed by silica gel chromatography. Elution with hexane through hexane: ethyl acetate mixtures to ethyl acetate afforded the known 2-methylene-β-alanine methyl ester derivatives, cholest-5-en-3β-ol, cholest-5, 22(*E*)-dien-3β-ol, 5α, 8α-epidioxy-cholest-6-en-3β-ol, crotonic acid and diphenylsufhone.[10] In the ethyl acetate eluate, ceramide **1** was obtained as a mixture with an intractable gum. Hence, the mixture was acetylated (Ac_2O/Py) and then the acetylated mixture was chromatographed on a silica gel column to obtained a ceramide **1a** (25 mg).

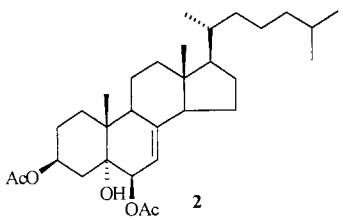
Compound **1a** was obtained as a semisolid, $[\alpha]_D^{25}$ 12.285 (*c* 0.455, $CHCl_3$) and analyzed for $C_{49}H_{91}NO_9$ by HRFABMS. The IR absorptions at 1746 and 1686 cm^{-1} indicated the presence of acetoxy and amide groups respectively. The ^1H NMR spectrum showed the signal due to a secondary amide proton at δ 6.58 (1H, d, *J* = 9.5 Hz), three methine protons bearing acetoxy groups at δ 5.12 (1H, m), 5.08 (1H, m) and 4.92 (1H, m), a methine proton bearing amide nitrogen at δ 4.45 (1H, m), and a methylene group bearing an acetoxy group at δ 4.02 (1H, dd, *J* = 3.5, 11.5 Hz) and 4.35 (1H, dd, *J* = 3.5, 11.5 Hz). Furthermore, the ^1H NMR spectrum showed four acetyl groups at δ 2.03 (3H, s), 2.06 (3H, s), 2.09 (3H, s) and 2.18 (3H, s), polymethylene chain at δ 1.25 (br s), and two terminal methyl groups at δ 0.85 (6H, t, *J* = 6.5 Hz). The forgoing spectral data suggested that compound **1a** was a tetra-acetoxy saturated ceramide. This is further supported by its ^{13}C NMR spectrum, which showed a signal for an amide carbonyl at δ 169.8 (s), four acetyl carbonyls at δ 170.0 (s), 170.0 (s), 170.8 (s), and 171.2 (s), three secondary carbon attached to acetyl groups at δ 72.3 (d), 72.7 (d) and 74.0 (d), a primary carbon attached to acetyl group at δ 62.4 (t), a secondary carbon attached to amide group at δ 47.8 (d), a polymethylene chain at δ 29.38 (t), 29.29 (t), 29.28 (t), and 28.59 (t), and two terminal methyl groups at δ 14.0 (q).

The connectivity of the structural fragments was established by 2D NMR methods, and the stereochemistry determined by comparison with known compounds. In the ^1H–^1H COSY spectrum the methine proton at δ 5.08 (2'-H) was coupled with the methylene protons at δ 1.80 (3'-H), which in turn were correlated with a methylene signal at δ 1.25 (–CH_2–) this suggested that the one segment of the molecule was a long-chain α-hydroxy acyl group. Furthermore, the methylene protons at δ 4.02 (1a-H) and 4.35 (1b-H) were coupled with the methine proton at δ 4.45 (C-2), which in turn was correlated with a methine proton at δ 5.12 (C-3). This was coupled with the methine proton at δ 4.92 (C-4), which in turn coupled with methylene protons at δ 1.60 (C-5). This suggested that the second segment of the molecule was a 1, 3, 4-triacetoxysphingosine. The ^{13}C chemical shifts of C-1–C-4, C-1', and C-2' of ceramide are especially suitable for determination of the relative stereochemistry of sphingosine moiety.[13,14] The carbon chemical shifts at δ 62.4 (C-1), 47.8 (C-2), 72.3 (C-3), 72.7 (C-4), 169.8 (C-1') and 74.0 (C-2') were virtually identical with those reported data of other (2S, 3S, 4R)-phytospingosine moieties.[14]

Further, the structure was confirmed by mass spectral studies (Scheme 1). The FABMS spectrum of **1a** showed peak at *m/z* 838 (M^++1) for $C_{49}H_{91}NO_9$ and which was underwent a McLafferty rearrangement to yield fragments A and B. Further the fragment B by loss acetic acid twice yielded fragment C at *m/z* 278 and the fragment A by loss of H_2O and acetic acid yielded fragments D at *m/z* 422 and E at *m/z* 362 respectively. This confirms the carbon chain lengths in the acid and base segments. Thus the structure of compound **1a**



1 . R = H, m = 21, n = 10
1a. R = Ac, m = 21, n = 10



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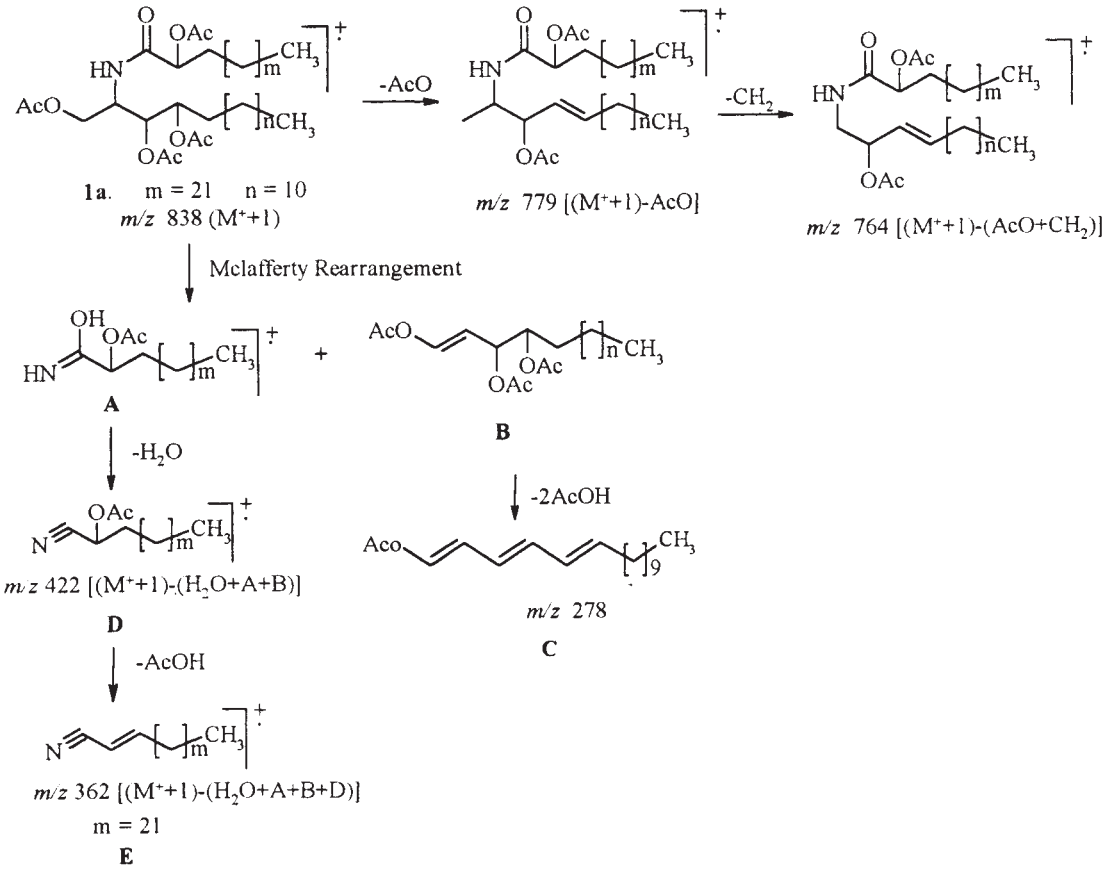
† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M).*

Table 1 NMR data of ceramides **1a**

Position	δ_H* *J* (Hz)	δ_C+	COSY	NOSEY
1a	4.02 dd (11.5, 3.5)	62.4 t	1b-H, 2-H	1b-H, 2-H
1b	4.35 dd (11.5, 3.5)	–	1a-H, 2-H	1a-H, 2-H
2	4.45 m	47.8 d	1a-H, 1b-H NH, 3-H	1a-H, 1b-H, NH, 3-H
3	5.12 m	72.3 d	4-H, 2-H	2-H, 4-H, NH
4	4.92 m	72.7 d	3-H, 5-H	3-H, 5-H, -CH2-
5	1.60 m	27.9 t	4-H, $-CH_2-$	4-H, -CH2-
NH	6.58 d (9.5)	–	2-H	3-H
1′	–	169.8 s	–	–
2′	5.08 m	74.0 d	3′-H	3′-H, -CH2-
3′	1.80 m	31.8 t	2′-H, $-CH_2-$	2′-H, -CH2-
$-CH_2-$	1.25 br s	29.38 (t), 29.29 (t), 29.28 (t), 28.59 (t)	$-CH_3$	CH_3
CH_3	0.85 (6.5) t	14.0 q (2 x C)	$-CH_2-$	$-CH_2-$
$COCH_3$	2.03 s	20.6 q, 170.0 s		
	2.06 s	20.7 q, 170.0 s		
	2.09 s	20.8 q, 170.8 s		
	2.18 s	20.9 q, 171.2 s		

* 400 MHz, $CDCl_3$; +50 MHz, $CDCl_3$



Scheme 1 Mass fragmentation of ceramide **1a**.

was confirmed as (2*S*, 3*S*, 4*R*, 2'*R*)-(2'-hydroxypentacosylamide)-1,3,4-hexadecanetriol-2',1,3,4-tetracetate.

Compound **2** was readily characterised as 3, 6-diacetoxycholest-7-en-3β,5α,6β-triol from a comparison of physical constants, ^{1}H NMR and mass spectral data with literature data[15]. This is the first report of its occurrence from the sponge *F. cavernosa*.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on Varian Unity 400 MHz and Varian Gemini 200 MHz spectrometers using TMS as internal standard. Chemical shifts are reported in parts per million, coupling constants (*J*) are expressed in Hertz, and ^{13}C NMR spectra are fully ^{1}H decoupled. Mass spectra were recorded on a VG Auto Spec-M instrument, IR spectra were recorded on Perkin-Elmer 240-C instrument. Optical rotations were measured on a JASCO DIP-370 polarimeter. Normal-phase thin-layer chromatography (TLC) was performed on silica gel G_{254} (Merck, Germany) with hexane/ethyl acetate (40:60).

Collection, extraction and isolation: Fresh specimens of the sponge *Fasciospongia cavernosa* were collected at 20 feet depth on the Mandapam coast, Tamilnadu during April 1996, and identified by Dr. P. A. Thomas, VRC, CMFRI, Thiruvananthapuram, India. The voucher specimen (IIC-239) has been deposited at the Museum of the National Institute of Oceanography, Goa, India. The sponge *F. cavernosa* was extracted with CH_2Cl_2–MeOH (1:1, 3 × 3 l) at room temperature and the combined extract was filtered and the solvent was removed under reduced pressure to yield a brownish gum (25 g). The

crude extract was partitioned between water and ethyl acetate. The organic layer was concentrated under vacuum and subjected to gel filtration chromatography (Sephadex LH-20; 1:1 dichloromethane-methanol) followed by silica gel chromatography eluting with hexane, hexane/ethyl acetate mixtures, and finally with ethyl acetate.

Acetylation of ceramide **1:** To the mixture of ceramide **1** (30 mg).in dry pyridine (0.1 ml) was added acetic anhydride (0.5 ml) and the mixture was allowed to stand at room temperature for 12 hours. After the usual workup the reaction mixture was purified on a silica gel column eluting with hexane: ethyl acetate (8:2) to afford an acetyl derivative **1a** (25 mg).

Ceramide (**1a**): Obtained as a semisolid, $[\alpha]^{25}_{D}$ 12.285 (*c* 0.455, $CHCl_3$); IR ν_{max} (KBr): 2923, 2853, 1746, 1686, 1542, 1459, 1373, 1232, 1044 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (50 MHz, $CDCl_3$): see Table 1; HRFABMS, obsd. *m/z* 838.6782 [M+1, $C_{49}H_{92}NO_9$] requires *m/z* 838.6772. FABMS: *m/z* (%) 838(M^++1) (6), 779(12), 764(12), 736(4), 704(2), 422(2), 362(2), 324(4), 278(9) and 264(14).

3, 6-Diacetoxy-cholest-7-en-3β,5α,6β-triol (**2**): Semisolid (10 mg); $[\alpha]^{25}_{D}$ -131° (*c* 0.15, $CHCl_3$); IR ν_{max} (neat) : 3510, 1750, 610 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 0.61 (3H, s, H-18), 0.88 (3H, d, *J* = 6.5 Hz, H-26), 0.90 (3H, d, *J* = 6.5 Hz, H-27), 0.92 (3H, d, *J* = 6.5 Hz, H-21), 1.05 (3H, s, H-19), 2.02 (3H, s, $COCH_3$), 2.03 (3H, s, $COCH_3$), 4.80 (1H, br d, *J* = 5 Hz, 6α-H), 5.12 (1H, m, 3α-H), 5.20 (1H, br d, *J* = 5 Hz, 7-H); EIMS : *m/z* 442[M^+-AcOH], 382, 364, 251, 163, 149, 43.

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References

1 D.J. Faulkner, *Nat Prod Rep*, **93**, 1993, 1673; references cited therein.
2 J. Kobayashi, S. Mikami, H. Shigmore, T. Takao, Y. Shimonishi, S. Izuta and S. Yoshida, *Tetrahedron*, **51**, 1995, 10487.
3 H.S. Garg, M. Sharma, D.S. Bhakuni, B.N. Pramanik and A.K. Bose, *Tetrahedron Lett*, **33**, 1992, 1641.
4 H-Y. Li, S. Matsunaga and N. Fusetani, *Tetrahedron*, **51**, 1995, 2273.
5 S.Y. Ki, Y-H. Choi, H. Huh, J. Kim, Y.C. Kim and H.S. Lee, *J. Nat. Prod.*, **60**, 1997, 274.
6 Y. Kashman, L. Fishelson and I. Neeman, *Tetrahedron*, **29**, 1973, 3655.
7 M.R. Kernan, R.C. Cambie and P.R. Bergquist, *J. Nat. Prod.*, **54**, 1991, 269.
8 Y. Venkateswarlu and M.A. Farooq Biabani, *J. Nat. Prod.*, **52**, 1989, 1331.
9 A. Montagnac, M. Pais and C. Debitus, *J. Nat. Prod.*, **57**, 1994, 186.
10 Y. Venkateswarlu, P. Ramesh, K.V. Sridevi and T. Prabhakara Rao T, *Ind. J. Chem.*, **37(B)**, 1998, 832.
11 Y. Venkateswarlu, N. Srinivasa Reddy, P. Ramesh, M. Rama Rao and T. Siva Ram, *Ind. J. Chem.*, **37(B)**, 1998, 1264.
12 P. Ramesh, N. Srinivasa Reddy, Y. Venkateswarlu, M. Venkata Rami Reddy and D.J. Faulkner, *Tetrahedron Lett*, **39**, 1998, 8217.
13 (a) N. Takenori, K. Yauhiko and H. Tatsuo, *Tetrahedron Lett*, **34**, 1993, 5591; (b) A. Kohji, N. Takenori and M. Masahiro, *Tetrahedron Lett*, **34**, 1993, 5593.
14 (a) S. Sugiyama, M. Honda and T. Komori. Liebigs *Ann.Chem*, 1990, 1069–1078; (b) S. Sugiyama, M. Honda, R. Higuchi, and T. Komori. Liebigs *Ann.Chem*, 1991, 349–356.
15 F. Cafieri, E. Fattoruso, M. Garagnin and C. Santacroce, *J. Nat. Prod.*, **48**, 1985, 944.