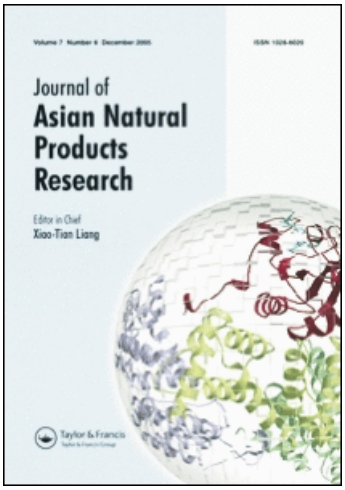

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New ceramides from the sponge *Cinachyra cavernosa*

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Two new ceramides **1** and **2**, and tetillapyrone **3** have been isolated from the Indian sponge *Cinachyra cavernosa*. The structures of **1**, **2**, and **3** were determined by spectroscopic and chemical analyses.

Keywords: new ceramide **1** and **2**; tetillapyrone; sponge, *Cinachyra cavernosa*

1. Introduction

Sponges are reported to contain secondary metabolites that are shown to possess diverse pharmacological activities. There is little chemical information on the sponges of the family Tetillidae, order Spirophorida, class Demospongia. Previous workers [1] reported the isolation of a very small amount of the potent cytotoxic macrolide, cinachyrolide A from *Cinachyra* species and two cholest-4-ene-3, 6-diones [2] from *Cinachyra tarentina* in addition to three common 3β-hydroxysterols and three cholest-4-en-3-ones.

2. Results and discussion

As a part of our continuing studies on bioactive metabolites from marine organisms, the sponge *Cinachyra cavernosa* collected from Tamil Nadu Coasts, India, was extracted with methanol and then with methanol–chloroform (1:1, v/v). Both the extracts were combined after concentration and subjected to solvent partition to afford hexane, chloroform, and *n*-butanol-soluble and -insoluble fractions. The *n*-butanol-soluble fraction was chromatographed over silica gel column to give a mixture of two new ceramides **1**, **2** and a pyrone previously reported [3] from the sponge *Tetilla*. The mixture of ceramides was rechromatographed over silica gel (100–200 mesh) to give pure compounds **1** and **2** as white amorphous powders.

Compound **1**, $[\alpha]_D^{27}$ + 10.0 (*c* 0.1, MeOH), has the molecular formula of $C_{41}H_{83}NO_4$ that was determined by FAB-MS [*m/z* 654 $(M + H)^+$]. The IR absorption bands at 1640 and 1540 cm^{-1} and a ^{13}C NMR signal at δ 173.4 suggested the existence of a secondary amide group. The absorption bands at 3400 cm^{-1} showed the presence of hydroxyl group. The ^1H NMR spectrum (Table 1) indicated the presence of a D_2O exchangeable doublet at δ 8.63 ($J = 8.4$ Hz) for the NH of an amide function. Five protons were observed between δ 5.12 and 4.28 and were assigned to the protons attached to a carbon bearing a heteroatom. The strong signal at δ 1.25 indicated that **1** is a lipid. Two aliphatic chains were suggested by the presence of signals for the methylene groups at δ 2.16–1.94 and 1.48, a large signal at δ 1.25–1.32, and three methyl groups at δ 0.88,

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Table 1. ^{1}H and ^{13}C NMR spectral data for ceramides **1** and **2** in pyridine-d_5.

	1		**2**	
Position	$^{1}H^{a}$	$^{13}C^{a}$	^{1}H	^{13}C
1	4.50	62.2	4.50	61.9
	4.48		4.39	
2	5.12	53.7	5.12	52.9
3	4.42	76.7	4.35	76.6
4	4.28	73.0	4.28	72.9
5	1.94, 2.16	33.9	2.18, 1.91	34.1
$(CH_2)n$	1.25–1.32	29.6–32.1	1.25–1.32	29.6–32.1
CHiPr	1.48	22.7	1.48	28.2
$(CH_3)_2$iPr	0.88	22.7	0.86	22.7
NH	8.63		8.58	
1’		173.4		175.3
2’	2.49	36.9	4.62	72.4
3’	1.87	26.4	1.97	35.6
$(CH_2)n$	1.20–1.32	29.6–32.1	1.25–1.32	29.6–32.1
CH_3	0.87	14.3	0.86	14.3

[a] Recorded at 200 MHz. Coupling constants in Hz are in parenthesis.

0.87 including a doublet (6H), and a triplet (3H). Analysis of $^{1}H-^{1}H$ COSY and $^{13}C-^{1}H$ COSY spectra of compound **1** allowed us to assign ^{1}H and ^{13}C chemical shifts (Table 1) and construct a ceramide-like structure that comprised a 4-hydroxy-sphingosine containing a non-hydroxy saturated fatty acid as indicated by the presence of a triplet at δ 2.49 (2H, $J = 7.4$ Hz) for the methylene protons adjacent to a carbonyl group.

The fatty acid and sphingosine chain length of compound **1** were determined through acid methanolysis. Methanolysis of compound **1** using MeOH/10% HCl and extraction with *n*-hexane yielded fatty acid methyl ester. The ^{1}H NMR spectrum indicated that it possessed a normal aliphatic chain (one triplet at δ 0.87). Absence of any carbinol proton showed that it is a non-hydroxy fatty acid. The EI-MS analysis of the fatty acid methyl ester showed the presence of methyl ester of tricosanoic acid **1a** (*m*/*z* 368). The aqueous layer was neutralized with Ag_2CO_3 and purified by column chromatography over silica gel after acetylation, then yielded tetraacetylsphingamine **1b**, the long-chain base (LCB). Its ^{1}H NMR spectrum exhibited two methyl groups at δ 0.87 (doublet) corresponding to the isopropyl group. The EI-MS of the acetylated LCB showed one ion at *m*/*z* 426 for $[M\text{-AcO}]^{+}$, corresponding to the 1,3,4-triacetyl-2-acetaminoalkane **1b**. By comparison with the literature [4,5] data of natural and synthetic sphingamines [6], the optical rotation $[\alpha]_D^{27} + 27.0$ (c 0.05, MeOH) supports the 2*S*-, 3*S*-, and 4*R*-configurations. From these findings, the structure of **1** was determined as shown in Figure 1.

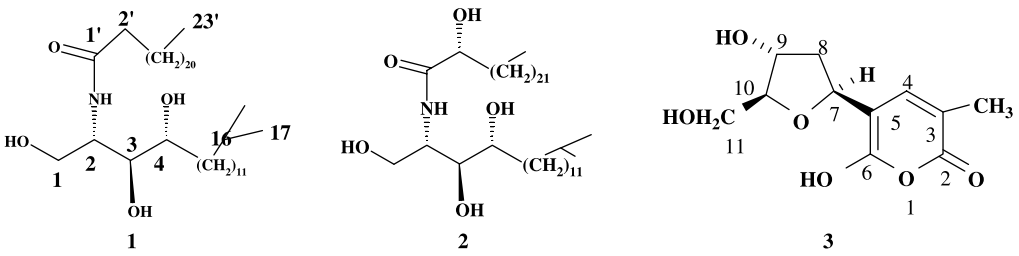


Figure 1. Structure of compounds **1**, **2**, and **3**.

Compound **2**, $[\alpha]_D^{27}$ + 28.0 (*c* 0.1, MeOH), was considered to be a congener of **1** by the comparison of its NMR (Table 1) with those of **1**. The molecular formula of $C_{42}H_{85}NO_5$ was determined by ESI-MS data. The ESI-MS spectra showed the molecular ion peak at *m/z* 684 $[M + H]^+$. The ^1H NMR revealed the presence of an additional proton attached to a carbon bearing a heteroatom at δ 4.62, and the absence of the triplet at δ 2.49 led to the proposal that **2** is a 4-hydroxy-sphingosine containing a 2′-OH fatty acid, which was supported by COSY and $^1H-^{13}C$ COSY spectra. The carbon chain length was determined by acid methanolysis. Purification of the methanolysis product by column chromatography afforded two compounds. The first component, which eluted with chloroform, corresponded to the methyl ester of α-hydroxy acid. The ^1H NMR spectrum indicated that it also possessed normal aliphatic chain (one triplet at δ 0.85). The EI-MS analysis, after acetylation, showed the presence of methyl ester of acetoxy-2-tetracosanoic acid **(2a)** (*m/z* 440). The optical rotation $[\alpha]_D^{27}$ − 2.7 indicated the *R* configuration. The second more polar component corresponded to the sphingamine moiety, which was found identical to the sphingamine moiety of **1**. The stereochemistry of the sphingamine moiety of **2** was also assigned as (2*S*, 3*S*, 4*R*). These findings allowed us to assign the structure of compound **2** as shown in Figure 1.

3. Experimental

3.1 General experimental procedure

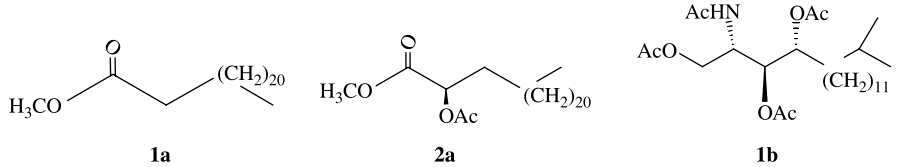
All the melting points were taken in open capillaries in a Complab melting point apparatus and are uncorrected. The IR spectra were recorded on Perkin-Elmer RX-1 spectrophotometer using KBr pellets or neat. The FAB-MS was recorded on Jeol SX 102/DA-6000 and ESI-MS on QUATTRO-II Mass Spectrophotometer. The NMR spectra were recorded on Bruker-300 NMR Spectrometer. Silica gel (60–120 mesh or 230–400 mesh) for column chromatography and thin layer chromatography on pre-coated silica gel 60 F_{254} plates were used. HPLC were performed on Shimadzu LC-20 AD system. $[\alpha]_D$ were measured Rudolf Autopol III polarimeter.

3.2 Collection of marine sponge

The marine sponge *Cinachyra cavernosa* was collected from Gujarat coast of Indian ocean in the month of January 2005 by the Botany Division of the Institute. It was washed and soaked in methanol and filled in a steel container, transported to the Institute from the site of collection with voucher specimen No. 216. The specimen has been preserved in the Botany Division of the Institute with the specimen No. 216.

3.3 Extraction and isolation

The methanol in which the sponge was preserved during transportation was drained off and the sponge was cut into small pieces (2 kg) and was percolated in methanol (5 × 3 l) at room temperature. The methanol extract and the drained methanol were combined and concentrated under reduced pressure below 50° to yield the methanol extract (50 g). The residual organism was again extracted



1a **2a** **1b**

Figure 2. Structure of compounds **1a**, **2a**, and **1b**.

with 50% methanol–chloroform (5 × 2 l) and the combined 50% methanol–chloroform extract was also concentrated under reduced pressure below 50°. The 50% methanol–chloroform extract (20 g) was obtained on drying. The methanol and 50% methanol-chloroform extracts showed similar TLC pattern and therefore were combined and fractionated by successive partitioning with hexane (4 × 500 ml), chloroform (4 × 500 ml), and *n*-butanol (4 × 500 ml) to get, respectively, the hexane-soluble (10 g), chloroform-soluble (5 g), and *n*-butanol-soluble (20 g) and -insoluble (35 g) fractions. The *n*-butanol-soluble fraction was investigated chemically to isolate the pure compounds reported in this communication. The *n*-butanol-soluble fraction (20 g) was chromatographed over silica gel (60–120 mesh) packed in ethyl acetate and eluted with increasing polarity of methanol as stepwise gradient. A total of 150 fractions of 250 ml each were collected and monitored by TLC plates of silica gel. The fractions with identical TLC pattern were combined. Visualization of spots was carried out by exposing to iodine vapors or spraying with 10% sulfuric acid solution, the identical fractions were purified by repeated column chromatography or by HPLC on reverse phase column RP-18 using acetonitrile–water (80:20, v/v) to give compounds **1** (28 mg), **2** (30 mg), and **3** (20 mg).

3.3.1 Compound *1*

It was obtained as white amorphous solid (28 mg), mp 193–195°C, $[\alpha]_D^{27}$ + 10.0 (*c* 0.1, MeOH). IR (KBr) ν_{max} 3400, 1040, 2940, 1640, 1540, and 1435 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1, FAB-MS *m/z* 654 $[M + H]^+$.

3.3.2 Methanolysis of compound *1*

Compound **1** (10 mg) was treated with 2 ml 82% aqueous MeOH and 0.9 N HCl (0.4 ml) and refluxed (FAME). Methyl tricosanoate (FAME) ESI-MS, *m/z* 368 (M^+), 337(M-31), 354, 310 98, 84, 74. ^1H NMR (200 MHz, $CDCl_3$, δ values) 0.86 (t), 1.26 (br s), 1.57 (m), 2.20 (t), 3.65 (s, OCH_3).

3.3.3 Compound *2*

It was obtained as white amorphous solid (30 mg), mp 105–107°C, $[\alpha]_D^{27}$ + 28.0 (*c* 0.1, MeOH), IR (KBr) ν_{max} 3400, 1040, 1640, 2950, 1640, 1540, and 1450 cm^{-1}. ^1H and ^{13}C NMR data, see Table 1. ESI-MS *m/z* 684 $[M + Na]^+$ *m/z* 707.

3.3.4 Methanolysis of compound *2*

Compound **2** (10 mg) was treated with 2 ml of 82% aqueous MeOH and 0.4 ml of 0.9 N HCl, and refluxed for 10 h. The reaction mixture was then extracted with *n*-hexane to yield fatty acid methyl ester (FAME). Methyl 2-hydroxy-tetracosanoate (FAME): ESI-MS, *m/z* 398 $[M]^+$, 367 (M^+-31), 384, 370, 98, 84, 74. ^1H NMR (200 MHz, $CDCl_3$, δ values) 0.86 (t), 1.26 (br s), 1.57 (m), 2.20 (t), 3.65 (s, OCH_3).

3.3.5 Compound *3*

Compound **3** was obtained by column chromatography of the combined fractions (65–106). It was crystallized from acetone as colorless needles with mp 192–193°C and $[\alpha]_D^{27}$ + 27.0 (*c* 0.1, acetone); NMR and MS spectral data of compound **3** were found completely identical to the data reported in the literature [3].

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