

## A New Sphingolipid from the Sponge *Ietrochota Baculifera* of the Indian Ocean

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A new sphingolipid was isolated from the ethyl acetate solubles of the alcoholic extract of the sponge *Ietrochota baculifera*. The compound was characterized as (2S, 3S, 4R)-2-[(2'R, 4'E)-2'-hydroxy-4'-tetracosenoylamino]-1,3,4-trihydroxyoctadecane (**1**) by means of NMR and FABMS data.

**Key Words:** Sphingolipid, *Ietrochota baculifera*, Sponge, Indian Ocean.

### INTRODUCTION

In continuation of our work on bioactive metabolites from marine organisms,<sup>1-3</sup> the marine sponge *Ietrochota baculifera* collected from the Tuticorin coast of the Indian Ocean is investigated. Earlier we reported the isolation of a few sphingolipids<sup>1</sup> and we now report the isolation of another sphingolipid (**1**) from the same organism.

### EXPERIMENTAL

<sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker DRX spectrometer operating at 300 and 75 MHz, respectively. The chemical shift values were reported in parts per million units and the coupling constants were in Hz. Positive ion FABMS was recorded on a JEOL-Sx-120/DA-6000 mass spectrometer using a beam of argon/xenon (2–8 keV) and *m*-nitrobenzyl alcohol as the matrix. Optical rotations were taken on a JASCO DIP-370 polarimeter. Elemental analysis was carried out on a Carlo-Erba 1108 analyzer. IR spectra were recorded on a Perkin-Elmer 881 instrument. Melting points were recorded on Boitus melting point apparatus and were uncorrected. Column chromatography was carried out using silica gel (finer than 200#, ACME) and MPLC was performed on Buchi B-688 MPLC system.

#### Animal Material

Specimens of the sponge *Ietrochota baculifera* were collected from the coast near Tuticorin, India (8°45'N, 78°12' E) at a depth of 10–15 m and preserved in methanol until extraction. A voucher specimen was deposited in the Marine

Organisms' Museum, Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India (Voucher No. AU2-152).

### Extraction and Isolation

The collected organism was washed thoroughly with fresh water and preserved in methanol at the site. The organism was extracted with methanol and concentrated. The residue was partitioned with EtOAc. The EtOAc soluble fraction was concentrated and chromatographed over silica gel (ACME, finer than 200#) which resulted in the isolation of compound 1 (10 mg).

**(2S,3S,4R)-2-[(2'R,4'E)-2'-Hydroxytetracos-4'-enoylamino]-1,3,4-trihydroxyoctadecane (1):** Colourless crystals, m.p. 148°C. Anal. Found: C 73.88, H 12.15, N 2.03%;  $C_{42}H_{83}NO_5$  requires: C 73.94, H 12.27, N 2.05%.  $[\alpha]_D^{28} + 8.9^\circ$  (c = 0.1, pyridine). IR ( $cm^{-1}$ ) ( $CHCl_3$ ):  $\nu_{max}$  3440, 2910, 2850, 1640, 1540, 1500, 1270, 1060 and 730.  $^1H$  and  $^{13}C$  NMR data given in Table-1. FABMS (positive mode) m/z (%) 683  $[M + 2H]^+$  (12), 682  $[M + H]^+$  (18), 300 (15), 282 (28), 264 (90).

TABLE-I  
 $^1H$  AND  $^{13}C$  NMR DATA OF THE COMPOUND 1 ( $C_5D_5N$ , TMS STANDARD)

Assignment	$^1H$ NMR (300 MHz) $\delta$ (multiplicity, J in Hz)	$^{13}C$ NMR (75 MHz) $\delta$ (multiplicity)
1a	4.53 (dd, 3.3, 12.0)	62.0
1b	4.49 (dd, 4.2, 12.3)	—
2	5.05 (m)	53.0
3	4.35 (m)	76.8
4	4.29 (m)	72.5
5	1.95 (m)	34.2
6-16	1.29 (br s)	30.4-26.7
17	1.29 (br s)	23.0
18	0.85 (t, 7.2)	14.3
NH	8.62 (d, 8.7)	—
1'	—	174.6 (s)
2'	4.69 (m)	73.0
3a'	3.04 (m)	33.7
3b'	2.91 (m)	—
4'	5.97 (dt, 7.5, 17.4)	126.3
5'	5.64 (dt, 7.5, 17.7)	132.5
6'	2.17 (m)	32.2
7'	1.69 (m)	30.4
8'-23'	1.29 (br s)	30.2-23.0
24'	0.85 (t, 7.2)	14.3

Assignments were made on the basis of  $^1H$ - $^1H$  COSY and HMQC spectra.

## RESULTS AND DISCUSSION

Compound 1 was obtained as colourless crystals. The molecular formula was established as  $C_{42}H_{83}NO_5$  by positive ion FABMS and elemental analysis. The presence of absorption bands for hydroxyl at 3410, 1040  $cm^{-1}$ , amide carbonyl at 1640  $cm^{-1}$  and aliphatic chain at 2940, 1450  $cm^{-1}$  in IR spectrum suggested the fatty acid amide nature of the compound.

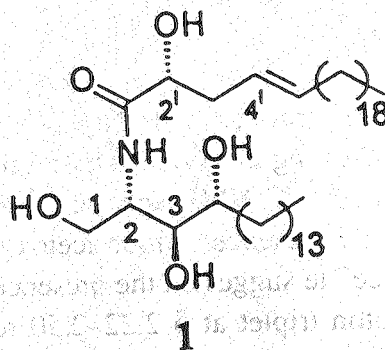
The presence of a doublet at  $\delta$  8.62 ( $J = 8.7$  Hz) for an amide proton, a broad singlet at  $\delta$  1.29 of unbranched long methylene chain protons and triplet at  $\delta$  0.85 ( $J = 7.2$  Hz) of two end methyl protons in  $^1H$  NMR spectrum and the presence of a carbonyl carbon at  $\delta$  174.6, long methylene chain carbons at  $\delta$  32.2–23.0 and end methyl carbons at  $\delta$  14.3 in  $^{13}C$  NMR spectrum indicated the sphingolipid nature of the compound<sup>1-5</sup>. The presence of four acetoxy methyls at  $\delta$  1.90–2.02 in  $^1H$  NMR spectrum of its acetate suggested the presence of four hydroxyls. The absence of characteristic proton triplet at  $\delta$  2.22–2.50 revealed the presence of oxygenated methine in between carbonyl carbon and long methylene chain<sup>5</sup>. The appearance of resonances at  $\delta$  5.97 (dt,  $J = 7.5, 17.4$  Hz), 5.61 (dt,  $J = 7.5, 17.7$  Hz) and at  $\delta$  132.5, 126.3 in  $^1H$  and  $^{13}C$  NMR spectra respectively indicated the presence of an olefinic bond in the compound. The carbon signals at  $\delta$  76.8, 73.0, 72.5 and 62.0 indicated the presence of three oxygenated methines and an oxygenated methylene.

The  $^1H$ - $^1H$  COSY spectrum showed correlation between NH proton at  $\delta$  8.62 and proton at  $\delta$  5.05 (m, H-2, overlapped with  $H_2O$  peak) which in turn showed cross peaks with three more protons at  $\delta$  4.53 (dd,  $J = 3.3, 12.0$  Hz), 4.49 (dd,  $J = 4.2, 12.3$  Hz) and 4.35 (H-3). The H-3 proton was further coupled with another oxygenated proton at  $\delta$  4.29 (m, H-4) and the latter proton showed correlation with methylene protons at  $\delta$  1.95 (m) which in turn has coupling with long methylene chain. The olefinic proton at  $\delta$  5.97 was correlated, in addition to other olefinic proton at  $\delta$  5.64, with two methylene protons at  $\delta$  3.04 (m, 1H, H-3a') and 2.91 (m, 1H, H-3b') which were correlated to each other and showed correlation with an oxygenated methine proton at  $\delta$  4.69 (m, H-2'). This methine proton has correlation only with the above methylene protons, which confirmed the presence of this oxygenated methine adjacent to carbonyl carbon and the presence of olefinic bond in fatty acyl chain of the sphingolipid.

The chain lengths of the fatty acyl and alkyl chains were determined by mass spectrum. The positive ion FABMS spectrum showed molecular ion peak at  $m/z$  683  $[M + 2H]^+$  and a base peak at  $m/z$  264 for  $[CH_2C(NH_2)=CHCH=(CH_2)_{12}CH_3]^+$  moiety,  $([M + 2H - COCH(OH)CH_2CH=CH(CH_2)_{18}CH_3 \cdot 3H_2O])^+$ , indicating the presence of  $C_{18}$  sphingosine base in the compound<sup>6</sup>. The fragment ions at  $m/z$  282, 300 supported the assignment and confirmed the presence of  $C_{18}$  base and thus  $C_{24}$  fatty acyl skeletons in the compound 1.

The stereochemistry was established as 2S, 3S, 4R for base and 2'R for the fatty acyl unit by comparing the spectral data with naturally occurring sphingolipids<sup>1-5,7</sup>. The large coupling constant (17 Hz) of olefinic protons in fatty acyl chain indicated *trans* (E) configuration for the double bond<sup>8</sup>.

Thus, the structure of the compound **1** was deduced as (2S, 3S, 4R)-2-[(2'R, 4'E)-2'-hydroxy-4'-tetracosenoylamino]-1,3,4-trihydroxyoctadecane. This is the aglycone of the glycosphingolipid, iotrорidoside-B which was reported earlier from the same sponge<sup>1</sup>. In this context, it may be noted that the aglycone may be formed due to hydrolysis of the glycosphingolipid or may be a precursor in the biogenetic pathway.



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