New Ceramides from the Hypotensive Extract of a Sea Anemone, Paracondylactis indicus

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J. Nat. Prod., **1994**, 57 (3), 393-395• DOI: 10.1021/np50105a011 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 3, pp. 393-395, March 1994

NEW CERAMIDES FROM THE HYPOTENSIVE EXTRACT OF A SEA ANEMONE, *PARACONDYLACTIS INDICUS*

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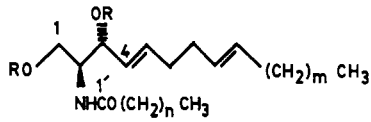
ABSTRACT.—A new ceramide isolated from a sea anemone, *Paracondylactis indicus*, has been characterized as *N*-palmityl-D-*erythro*-octadecasphinga-4(*E*),8(*E*)-dienine [**2**] by chemical and spectral studies. The presence of other *N*-acyl derivatives of the sphingadienine has also been demonstrated.

In continuation of the search in this laboratory for new secondary metabolites of marine species collected from Indian coastal water (1), the sea anemone *Paracondylactis indicus* Dave (2) was collected from the Bay of Bengal. One major and several minor new ceramides were isolated from its Me_2CO extract. The isolation and characterization of these ceramides is reported herein.

A molecular formula of $C_{34}H_{65}NO_3$ for the major component, coded Pi-1, was determined by hreims and confirmed by fabms. Its ir data indicated it to be a secondary amide (3290 and 1622 cm^{-1}) bearing hydroxyl(s) (3390 cm^{-1}). The ^1H- and ^{13}C-nmr spectral data of Pi-1 supported it as being a secondary amide (δ 6.36, 1H, d, J=7 Hz; δ 174.0, s) bearing two terminal methyls (δ 0.88, 6H, t, J=6 Hz; δ 14.0, q), four sp^2 methine carbons (see Experimental), a $COCH_2CH_2$ moiety (δ 1.62, 2H, br t and 2.22, 2H, t, J=7.5 Hz; δ 25.8 and 36.8, both t, β- and α-CH_2, respectively) and two hydroxyls (δ 2.96, 2H, br s, exchangeable), one primary (δ 62.4, t) and the other secondary (δ 74.3, d). The proton-proton and proton-carbon connectivities were then determined from ^1H-^1H COSY and ^1H-^{13}C XHCORR nmr experiments, respectively.

All the spectral information revealed that Pi-1 is a ceramide of the *N*-acylsphinga-4(*E*),8(*E*)-dienine [**1**] type. The *E* configuration for both the double bonds



1 m+n=22; R=H
2 m=8, n=14; R=H
3 m=8, n=14; R=Ac

became obvious from the appearance of a strong ir band at 962 cm^{-1} and the large coupling constants (15.3, 15.4 Hz) observed for the olefinic proton signals in its ^1H-nmr spectrum. The acid part of Pi-1 was identified as palmitic acid (C_{16}) by hydrolysis with MeOH-H_2SO_4 (3) when methyl palmitate was detected by gc as the only fatty acid methyl ester. This finding automatically established the length of the long-chain base as a C_{18}-amino alcohol, thereby settling the structure of Pi-1 as *N*-palmityl-octadecasphinga-4(*E*),8(*E*)-dienine [**2**]. The assigned structure received further support from the ir, ms, and ^1H-nmr spectral data of the derived diacetate [**3**]. Earlier, another ceramide encompassing the sphinga-4(*E*),8(*E*)-dienine skeleton was reported from a sea anemone, *Anemonia sulcata* (4). It differs in the chain-lengths of both the amino alcohol and the acid parts from Pi-1.

The relative stereochemistry of Pi-1 at C-2/C-3 could not be determined with certainty, because the value of $J_{H\text{-}2/H\text{-}3}$ necessary for this determination (5) could not be ascertained from the ^1H-nmr spec-

tra of Pi-1 and its diacetate. Nevertheless, a 2*S*,3*R*, i.e., a D-*erythro*-configuration, was assumed for Pi-1 on the grounds that (i) all naturally occurring sphingosine-type amino alcohols possess this configuration (6) and (ii) the ^{13}C-nmr data for C-2 (δ 54.7) and C-3 (δ 74.3) agree well with those (δ 54.7 and 73.1, respectively) reported for a synthetic sample of *N*-octadecanoyl-D-*erythro*-sphingosine (7).

The ^{1}H-nmr spectrum of the residue obtained from the mother liquor left after precipitation of Pi-1 displayed qualitative similarity to that of Pi-1, indicating the presence of other *N*-acyl derivatives of the same sphingadienine. Acid hydrolysis (MeOH-H_2SO_4) of the residue produced methyl esters of palmitic, palmitolic, stearic, pentadecanoic, heptadecanoic, myristic, linoleic, and oleic acids, identified by gc, in decreasing order of concentration. This experiment demonstrated the presence of the respective ceramides incorporating the same sphinga-4,8-dienine skeleton in the Me_2CO extract of *P. indicus*.

The present note constitutes the first report of the isolation of new sphingadienine-derived ceramides from a marine species collected from Indian coastal water.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The mp is uncorrected. Ir spectra (KBr) were recorded on Perkin-Elmer IR-782 (for **2**) and Jasco IR-700 (for **3**) spectrophotometers. ^{1}H Nmr (300 MHz), ^{13}C nmr (75 MHz; multiplicities determined by DEPT 135 experiment), ^{1}H-^{1}H COSY and ^{1}H-^{13}C XHCORR nmr spectra were recorded in $CDCl_3$/TMS on a Bruker AM 300L spectrometer. Eims, hreims and positive-ion fabms (using 3-nitrobenzyl alcohol/CH_2Cl_2 matrix) were obtained with Hitachi RMU-6L, VG 70-SE, and Varian MAT 311A mass spectrometers, respectively. The optical rotation was measured on a Jasco DIP-360 digital polarimeter using a 5 cm cell. Gc spectra were recorded on a Hewlett-Packard 5840A Analytical Gas Chromatograph using a 15% DEGS/Chromosorb WHP (100–120 mesh) column (6′×1/8″) and N_2 as carrier gas at 35 ml/min at 200° at the column, FID and injection port. Si gel (60–120 mesh; Qualigens, India) was used for cc.

ANIMAL MATERIAL.—The marine species was collected from the Bay of Bengal, off the coast of Digha, latitude 21°37′N and longitude 87°31′30″E, in the Midnapore District of West Bengal, India in August 1988. This species was identified as *Paracondylactis indicus* by the Zoological Survey of India, Calcutta, by comparing its description with that reported for *P. indicus* by Dave (2).

EXTRACTION AND ISOLATION.—Immediately after collection, the sea anemones (3.5 kg) were dipped into Me_2CO (8 liters). The residue (7.8 g) from the Me_2CO extract of the macerated anemones showed hypotensive activity. It was partitioned between Et_2O and H_2O. The Et_2O-soluble part (2.5 g) was chromatographed over Si gel. The 2% MeOH/CH_2Cl_2 eluate furnished a solid residue (20 mg; 0.8% of the Et_2O-soluble part) that could not be induced to crystallize. It was purified by repeated precipitation from Et_2O-CH_3CN to give Pi-1 [**2**] as an amorphous solid, $[\alpha]_D$ +10.6° (*c*=1.7, MeOH); hreims *m/z* 535.4942 ([M]$^+$, <1%; calcd for $C_{34}H_{65}NO_3$, 535.4948), 517 (1), 281 (27), 280 (18), 250 (14), 82 (100); fabms *m/z* 536 [M+H]$^+$ (12), 518 (40), 280 (35), 262 (100), 250 (20); ^{1}H nmr ($CDCl_3$, additional data) δ 1.25 (38H, br s, $(CH_2)_{19}$), 1.96 (2H, br q, *J*=5.8 Hz, H-10), 2.09 (4H, m, H-6 and H-7), 3.68 (1H, br d, *J*=8.4 Hz, H-1), 3.90 (1H, m, H-2), 3.93 (1H, m, H-1), 4.29 (1H, br, H-3), 5.36 (1H, dt, *J*=15.3 and 5.7 Hz, H-8), 5.43 (1H, dt, *J*=15.3 and 5.7 Hz, H-9), 5.53 (1H, dd, *J*=15.4 and 6.1 Hz, H-4), 5.78 (1H, dt, *J*=15.3 and 6.1 Hz, H-5); ^{13}C nmr ($CDCl_3$, additional data) δ 133.4, 131.3, 129.3, 128.9, all d, C-5, -8/-9, -4, -9/-8, respectively; 32.6, 32.3, 32.1, 31.9 (×2), 29.7 (×n), 29.5 (×n), 29.3 (×n), 29.2, 22.6 (×2), all t, CH_2.

Hydrolysis of Pi-1 [**2**].—The ceramide **2** (5 mg) was refluxed with 1 ml of 1.2 M H_2SO_4 in 85% aqueous MeOH in N_2 atmosphere for 3 h, then diluted with H_2O and extracted with distilled hexane (3×10 ml). The residue from the hexane extract obtained after usual workup (3) was subjected to gc under conditions as stated earlier, when methyl palmitate was detected as the only fatty acid methyl ester.

Acetylation of Pi-1 [**2**].—Pi-1 (**2**, 5 mg), dissolved in pyridine (5 drops), was treated with Ac_2O (10 drops) and left overnight. The usual workup, followed by cc over Si gel, furnished the diacetate [**3**] as white microneedles, mp 110° (petroleum ether-$CHCl_3$); ir ν max 3312 and 1646 (NHCO), 1733 (ester CO), 969 (*E*-double bond) cm^{-1}; eims *m/z* 619 [M]$^+$, 559, 499, 452, 393, 340, 332, 281, 280 (100%); ^{1}H nmr ($CDCl_3$) δ 0.88 (6H, t, *J*=6 Hz, 2×CH_2CH_3), 1.25 (38H, br s, $(CH_2)_{19}$), 1.58 (2H, m, H-3′), 1.96 (2H, q, *J*=6.3 Hz, H-10), 2.06 (6H, s, 2×OAc), 2.1 (4H,

m, H-6 and -7), 2.16 (2H, t, J=7.5 Hz, H-2′), 4.02 (1H, dd, 11.5 and 3.9 Hz), and 4.29 (1H, dd, J=11.5, 6.2 Hz, H_2-1), 4.45 (1H, m, H-2), 5.28 (1H, t, J=6.7 Hz, H-3), 5.34 (1H, dt, J=16.1 and 5.3 Hz, H-9), 5.4 (1H, m, H-8), 5.43 (1H, dd, J=14.4 and 6.7 Hz, H-4), 5.62 (1H, d, J=8.6 Hz, NHCO), 5.78 (1H, dt, J=14.9 and 6.3 Hz, H-5).

IDENTIFICATION OF OTHER CERAMIDES.—The residue from the mother liquor of Pi-1 was hydrolyzed and worked up as in the case of the hydrolysis of Pi-1. The resulting mass comprising the fatty acid methyl esters was analyzed by gc using the same conditions as before, when the methyl esters of myristic, pentadecanoic, palmitic, palmitolic, heptadecanoic, stearic, oleic, and linoleic acids were detected in the given order of elution.

ACKNOWLEDGMENTS

The authors thank the Office of the Naval Research, Government of the United States for funding the initial part of this work through an Indo-U.S. collaborative project and the C.D.R.I., Lucknow, India for conducting the biological screening experiments. We are grateful to Professor Dr. W. Wiegrebe, University of Regensburg, Germany for the fabms and Professor P.J. Scheuer, University of Hawaii at Manoa, Honolulu, for the hreims. Two of the authors (A.B. and A.K.B.) express their sincere thanks to the C.S.I.R., Government of India, for financial assistance.

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Received 28 July 1993