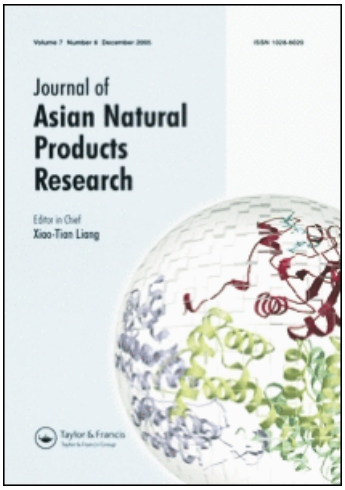


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Chemical Constituents of *Pseudopterogorgia* Species of the Indian Ocean

Mulabagal Vanisree[a]; Gottumukkala V. Subbaraju; Ch. Bheemasankara Rao[b]
[a] Department of Chemistry, Sri Venkateswara University, Tirupati, India [b] School of Chemistry, Andhra University, Visakhapatnam, India

To cite this Article Vanisree, Mulabagal , Subbaraju, Gottumukkala V. and Rao, Ch. Bheemasankara(2001) 'Chemical Constituents of *Pseudopterogorgia* Species of the Indian Ocean', Journal of Asian Natural Products Research, 3: 1, 23 — 29
To link to this Article: DOI: 10.1080/10286020108042835
URL: http://dx.doi.org/10.1080/10286020108042835

JANPR, Vol. 3, pp. 23–29
Reprints available directly from the publisher
Photocopying permitted by license only

CHEMICAL CONSTITUENTS OF *PSEUDOPTEROGORGIA* SPECIES OF THE INDIAN OCEAN

MULABAGAL VANISREE[a], GOTTUMUKKALA V. SUBBARAJU[a,*] and CH. BHEEMASANKARA RAO[b]

[a]*Department of Chemistry, Sri Venkateswara University, Tirupati - 517 502, India;*
[b]*School of Chemistry, Andhra University, Visakhapatnam - 530 003, India*

(Received 7 March 2000; In final form 15 April 2000)

A new ceramide, (2S,3R,4E)-1,3-dihydroxy-2-[(hexadecanoyl)amino]-nonadeca-4-ene (**1**), cholest-5-en-3β,7β,19-triol (**2**), identified as its, peracetyl derivative (**3**), and batyl alcohol (**4**) were isolated from *Pseudopterogorgia* species. **1** exhibited antibacterial activity against Gram-positive and Gram-negative bacteria.

Keywords: *Pseudopterogorgia* species; Gorgonian; Ceramide; Polyhydroxysterol

INTRODUCTION

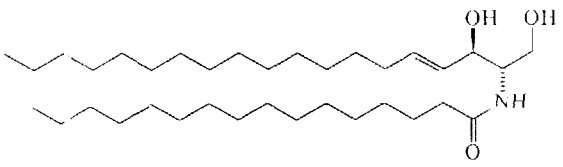
Gorgonians (Phylum: Coelenterata) have been recognized as a rich source of a variety of secondary metabolites, namely, novel sterols, prostaglandins, terpenoids, polyhalogenated compounds, phospholipids and ceramides [1]. In continuation of our studies on marine coelenterates [2–8], we have examined *Pseudopterogorgia* species collected from the Tuticorin coast of the Indian ocean and the results are reported in this note.

RESULTS AND DISCUSSION

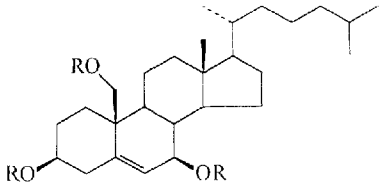
The residue from the ethyl acetate soluble portion of the methanolic extracts of the *Pseudopterogorgia* species, on extensive chromatography over silica

*Corresponding author. Tel.: 91-8574-49757. Fax: 91-8574-27499.

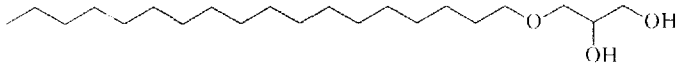
gel, gave compound **A[1]** and batyl alcohol (**4**) in addition to a polyhydroxysterol fraction.



1



2 R=H
3 R=Ac



4

Compound **A** was obtained as an amorphous powder from hexane and ethyl acetate, m.p. 104–105°C, $[\alpha]_D$ - 6.0 (c 1.3, $CHCl_3$) and analysed for $C_{35}H_{69}NO_3$ [FABMS *m/z* 534 ($M^+ + H - H_2O$)]. Its IR (KBr) spectrum showed bands at 3320 (hydroxyls and NH) and 1620 cm^{-1} (amide carbonyl). Its ^1H NMR (Tab. I) spectrum exhibited signals at δ 3.92 (1H, *dd*, $J = 11.2$, 3.8 Hz), 3.68 (1H, *dd*, $J = 11.2$, 2.5 Hz) and 4.29 (1H, brs) corresponding to hydroxymethylene and hydroxymethine protons, respectively. The ^1H NMR spectrum also showed signals at δ 5.51 (1H, *dd*, $J = 15.4$, 6.5 Hz) and 5.77 (1H, *dt*, $J = 15.4$, 6.7 Hz) attributable to olefinic protons. δ 6.30 (1H, brd, $J = 4.6$ Hz), assignable to an amide NH and a series of signals at δ 2.2 (2H, *t*, $J = 7.8$ Hz); 1.61 (2H,*m*); 1.25 (48H, brs) and 0.87 (6H, *d*, $J = 6.9$ Hz) suggestive of a long fatty acid unit and an alkyl chain characteristic of ceramides [9]. The ^{13}C NMR (Tab. I) spectral data

TABLE I NMR data of compound **A(1)** (500 MHz for ^1H and 125 MHz for ^{13}C in $CDCl_3$)

Position	*δH**	*δC**	*HMBC*
1	3.68 (1H, *dd*, $J = 11.2, 2.5$ Hz) 3.92 (1H, *dd*, $J = 11.2, 3.8$ Hz)	62.4	–
2	3.88 (1H, *m*)	54.6	C-1, C-1′, C-3
3	4.29 (1H, brs)	74.4	–
4	5.51 (1H, *dd*, $J = 15.4, 6.5$ Hz)	128.8	C-3, C-6
5	5.77 (1H, *dt*, $J = 15.4, 6.7$ Hz)	134.2	C-3, C-6, C-7
6	2.04 (2H, *m*)	32.3	C-4, C-5, C-7
1′		174.0	–
2′	2.21 (2H, *t*, $J = 7.8$ Hz)	36.8	C-1′, C-3′, C-4′
3′	1.61 (2H, *m*)	25.8	C-1′, C-2′,C-4′
NH	6.30 (1H, *brd*, $J = 4.6$ Hz)	–	C-1′
19 and 16′	0.87 (6H, *t*, $J = 6.9$ Hz)	14.1	–
C-7 to C-18 and C-4′ to C-15′	1.25 (48H, brs)	29.1, 22.7, 29.2, 29.3, 29.4, 29.5, 29.5, 29.6, 29.7 and 31.9	–

* Assignments are supported by ^1H-^1H COSY and HMQC data.

supported the presence of an amide carbonyl (δ 174.0), hydroxymethylene (δ 62.4), hydroxymethine (δ 74.4) and two olefinic carbons (δ 128.8 and 134.2). It also exhibited signals at δ 54.6 assignable to a methine carbon connected to amide nitrogen. A series of signals at δ 36.8, 25.8, 22.7 and 14.1 in addition to a complex group of signals between δ 29.1 and 29.7 supports the presence of a long fatty acid and an alkyl chain of the ceramide [9]. The ^1H- ^1H COSY spectrum of the compound A showed correlations between signals located at δ 3.92 (1H, *dd*, $J = 11.2$, 3.8 Hz, Hb-1) and 3.68 (1H, *dd*, $J = 11.2$, 2.5 Hz, Ha-1); 3.92 (1H, *dd*, $J = 11.2$, 3.8 Hz, Hb-1) and 3.88 (1H, *m*, H-2); 3.88 (1H, *m*, H-2) and 3.68 (1H, *dd*, $J = 11.2$, 2.5 Hz, Ha-1); 4.29 (1H, brs, H-3) and 3.88 (1H, *m*, H-2); 5.51 (1H, *dd*, $J = 15.4$, 6.5 Hz, H-4) and 4.29 (1H, brs, H-3); 5.77 (1H, *dt*, $J = 15.2$, 6.7 Hz, H-5) and 5.51 (1H, *dd*, $J = 15.4$, 6.5 Hz, H-4) revealing the presence of hydroxyls at C-1 and C-3 and the double bond between C-4 and C-5. A correlation between an amide NH (δ 6.30, 1H, brd, $J = 4.6$ Hz, NH) and a methine proton (δ 3.88, 1H, *m*, H-2) was also noticed in the spectrum. The position of hydroxyls and the double bond received further support from HMBC data (Tab. I).

The length of the fatty acid and alkyl chains was determined by combination of mass spectral data and hydrolysis. The FABMS of compound A exhibited ions at *m/z* 534 ($M^+ + H - H_2O$), 520 ($M^+ - CH_2OH$), 502 ($M^+ - CH_2OH - H_2O$) in addition to intense ion

at *m/z* 282. EIMS spectrum of compound A showed a very weak ion at *m/z* 533 ($M^+ - H_2O$) along with a base peak at *m/z* 281. Formation of ion *m/z* 281 in EIMS spectrum is diagnostic for the presence of the palmitic acid unit in ceramides [9]. Further, the acid methanolysis of compound **A** gave palmitic acid methyl ester [EIMS: *m/z* 270, M^+]. The relative stereochemistry of C-2 and C-3 could be assigned, tentatively, as 2S, 3R based on comparison of carbon chemical shifts (C-1, C-2 and C-3) and optical rotation values of compound **A** with those of other naturally occurring ceramides [10–12]. Configuration of the double bond between C-4 and C-5 has been deduced as E based on coupling constant ($J = 15.4$ Hz) between H-4 and H-5. Therefore, compound **A** could be derived as (2S, 3R, 4E)-1,3-dihydroxy-2-[(hexadecanoyl)amino]-nonadeca-4-ene (**1**) which is a new ceramide.

1 exhibited weak antibacterial activity against Gram-positive (*Bacillus pumilis*, *Bacillus subtilis* and *Staphylococcus epidermis*) and Gram-negative (*Escherichia coli* and *Pseudomonas aerogenosa*) bacteria at a concentration of **1** mg/mL. MIC of **1** against *Staphylococcus epidermis* was found to be 100 μg/mL.

The polyhydroxysterol fraction was found to contain impurities and its ^1H NMR spectrum revealed that the sterol fraction contained no acetoxyl group. Hence, the sterol fraction was acetylated with Py/Ac_2O and the acetyl derivative was purified by chromatography over silica gel to give compound **B(3)**.

Compound **B** was obtained as colourless liquid and analysed for $C_{33}H_{52}O_6$ [EIMS, *m/z* 400 ($M^+ - 2\,X\,C_2H_2O - CH_3COOH$), 382 ($M^+ - 2\,X\,CH_3COOH - C_2H_2O$). Its IR spectrum showed bands at 1740 cm^{-1} indicating the presence of an ester carbonyl. The ^1H NMR spectrum of compound **B** exhibited signals at δ 0.70 (3H, *s*) corresponding to a tertiary methyl and δ 0.848, 0.852 (each 3H, *d*, $J = 6.6$ Hz) and 0.90 (3H, *d*, $J = 6.4$Hz) assignable to three secondary methyls, in addition to signals at δ 2.08 (6H, *s*), 2.01(3H, *s*) and 5.48 (1H, brs) corresponding to three acetoxy methyls and an olefinic proton, respectively. A multiplet at δ 4.60 and a doublet at δ 4.99 (1H, brd, $J = 7.4$ Hz) characteristic of 3β,7β-diacetoxy system was also observed. In addition, the presence of an acetoxymethylene signals at δ 3.94 (1H, brd, $J = 11.8$ Hz) and 4.60 (1H, brd, $J = 11.8$ Hz) coupled with the conspicuous absence of the signal assignable to C-19 methyl in the ^1H NMR spectrum strongly suggests that compound **B** contain primary 19- CH_2OAc.

The ^{13}C NMR spectrum of compound **B** supported the presence of three acetoxyls (δ170.3, 170.5, 171.0, 21.0, 21.3 and 21.5) and two olefinic carbons

(δ 126.4 and 139.1). The proton and carbon chemical shifts of compound **B** were found to be corroborative with those of cholest-5-en-3β,7β-19-triol 3,7,19-triacetate [13].

Based on the above, compound **B** could be identified as cholest-5-ene-3β,7β,19-triol 3,7,19-triacetate (**3**) and the original sterol as cholest-5-ene-3β,7β,19-triol (**2**). **2** was isolated earlier from the soft corals *Antipathes subpinnata* [13] and *Lytophyton viridis* [14].

Batyl alcohol was isolated as an amorphous solid (m.p. 68°C) and its identity was confirmed by m.m.p. and co-TLC with the authentic sample [4].

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined on a Mel-Temp apparatus and are uncorrected. IR spectra on a Perkin Elmer-1600 spectrometer, ^{1}H, ^{13}C and 2D NMR spectra were recorded on a GE-500 or 400 MHz NMR spectrometer, mass spectra on a VG micromass 70-70H spectrometer and JEOL SX 102/DA-600 spectrometer. Optical rotations were measured on an Autopol III automatic polarimeter. Separation and purifications were performed by column chromatography over Acme silica gel (finer than 200 mesh or 100–200 mesh).

Collection of the Gorgonian

Specimens of the gorgonian *Pseudopterogorgia* species (TT-98-01) were collected from the Tuticorin coast of the Indian Ocean during March, 1998. The specimens were authenticated as *Pseudopterogorgia* speices by Dr. Thomas, Central Marine Fisheries Research Institute, Trivandrum, India. Voucher specimens are on deposit at the Departments of Chemistry, Sri Venkateswara University, Tirupati and Andhra University, Visakhapatnam, India.

Extraction and Isolation

Specimens of the gorgonian (ca. 4 kg, wet wt.) were dried under shade and milled as course powder. The powder (ca. 1.5 kg) was extracted repeatedly with methanol in a Soxhlet apparatus. The combined methanolic solution was concentrated under reduced pressure and the concentrate (ca. 2L) was

fractionated with ethyl acetate. Removal of the solvent from the ethyl acetate extractives gave dark brown residue (ca. 25 g).

The residue was chromatographed over silica gel column using solvents of increasing polarity *n*-hexane through ethyl acetate. Rechromatography of selected fractions over silica gel column yielded **1** (25 mg) and **4** (80 mg) in addition to a polyhydroxysterol fraction (20 mg). The polyhydroxysterol fraction was acetylated with Py/Ac_2O under usual conditions and the acetyl derivative was chromatographed over silica gel to give **3** (10 mg) as an oil.

(2S,3R,4E)-1,3-dihydroxy-2[(hexadecanoyl)amino]-nonadeca-4-ene-(**1**). Amorphous solid from a mixture of hexane and ethyl acetate, 25 mg, m.p. 104–105°C, $[\alpha]_D$ -6.0 (c 1.3, $CHCl_3$) : IR (KBr) ν_{max} 3320 and 1620 cm^{-1}. ^1H NMR, ^{13}C NMR and HMBC spectral data (see Tab. I). FABMS *m/z* [$M^+ + H - H_2O$], 534 (29), [$M^+ - CH_2OH$), 520 (65), [$M^+ - CH_2OH - H_2O$], 502 (10), 296 (20), 282 (69), 264 (100), 154 (20), 136 (28), 43 (80), 29 (33); EIMS *m/z* [$M^+ - H_2O$], 533 (13), [$M^+ - H_2O - CH_2OH$], 502 (54), 298 (42), 281 (100), 250 (30), 207 (29).

Cholest-5-en-3*β*,7*β*-19-triol 3,7,19-triacetate (**3**), Liquid, 10 mg; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.70 (3H, *s*, H-18), 0.848 and 0.852 (each 3H, *d*, $J = 6.6$ Hz, H-26 and H-27), 0.90 (3H, *d*, $J = 6.4$ Hz, H-21), 2.01 (3H, *s*, $OCOCH_3$), 2.08 (6H, *s*, 2 *X* $OCOCH_3$), 3.94 (1H, brd, $J = 11.8$ Hz, Ha-19), 4.60 (1H, brd, $J = 11.8$ Hz, Hb-19), 4.60 (1H, *m*, H-3) 4.99 (1H, brd, $J = 7.4$ Hz, H-7), 5.48 (1H, brs, H-6); ^{13}C NMR ($CDCl_3$, 22.5 MHz), 33.2 (C-1), 29.7 (C-2), 72.7 (C-3), 37.7 (C-4), 139.1 (C-5), 126.4 (C-6), 74.6 (C-7), 39.6 (C-8), 48.3 (C-9), 43.1 (C-10), 21.7 (C-11), 39.9 (C-12), 43.1 (C-13), 56.3 (C-14), 24.9 (C-15), 28.4 (C-16), 55.5 (C-17), 11.9 (C-18), 64.0 (C-19), 35.7 (C-20), 18.8 (C-21), 36.2 (C-22), 23.9 (C-23), 39.6 (C-24), 28.0 (C-25), 22.8 (C-26), 22.6 (C-27), 170.3, 170.5, 171.0, 21.0, 21.3 and 21.5 (3 *X* $OCOCH_3$); EIMS *m/z* [$M^+ - CH_3COOH - 2\,X\,C_2H_2O$] 400 (12), [$M^+ - 2\,X\,CH_3COOH - C_2H_2O$], 382 (13), 254 (16), 124 (25), 69(66), 55 (100).

Batyl alcohol (**4**) Amorphous powder from methanol, 80 mg, m.p. 68°C (Ref. [4] m.p. 69–71°C); IR(KBr): ν_{max} 3330, 2919, 1460, 1129, 870 cm^{-1}; EIMS [M^+] 344(4), 253(15), 125(17), 97(28), 57(100).

Acknowledgements

The authors thank Dr. P. A. Thomas, CMFRI, Trivandrum for identification of the gorgonian, Dr. Jorge I. Jimenez, University of Hawaii, USA for some of the spectral data. The research was partly funded by the Department of Ocean Development, Ministry of Science and Technology, Government of India to CBR.

References

[1] Faulkner, D. J. (1999). *Nat. Prod. Rep.*, **16**, 155–198 and earlier parts of the same series.
[2] Raju, B. L., Subbaraju, G. V., Reddy, M. C., Rao, D. V., Rao, C. B. and Raju, V. S. (1992). *J. Nat. Prod.*, **55**, 904–911.
[3] Raju, B. L., Subbaraju, G. V., Rao, C. B. and Trimurthulu, G. (1993). *J. Nat. Prod.*, **56**, 961–966.
[4] Rani, M. E., Subbaraju, G. V., Raju, B. L., Rao, C. B. and Rao, D. V. (1994). *Indian J. Nat. Prod.*, **10**, 3–8.
[5] Raju, B. L., Subbaraju, G. V., Reddy, K. V. and Rao, C. B. (1994). *Indian J. Chem.*, **33B**, 1033–1037.
[6] Raju, B. L., Subbaraju, G. V. and Rao, C. B. (1995). *Indian J. Chem.*, **34B**, 221–226.
[7] Vanisree, M., Subbaraju, G. V. and Rao, C. B. (1999). *Indian J. Chem.*, **38B**, 394–396.
[8] Vanisree, M., Subbaraju, G. V. and Rao, C. B. (2000). *J. Asian Nat. Prod. Res.*, **2**, 87–95.
[9] Venkateswarlu, Y., Reddy, N. S., Ramesh, P., Rama Rao, M. and Sivaram, T. (1998). *Indian J. Chem.*, **37B**, 1264–1268.
[10] Mancini, I., Guella, G., Debitus, C. and Pietra, F. (1994). *Helv. Chim. Acta*, **77**, 51–58.
[11] Shin, J. and Seo, Y. (1995). *J. Nat. Prod.*, **58**, 948–953.
[12] Hattori, T., Adachi, K. and Shizuri, Y. (1998). *J. Nat. Prod.*, **61**, 823–826.
[13] Bortolotto, M., Braekman, J. C., Daloze, D., Losman, D. and Tursch, B. (1976). *Steroids*, **28**, 461–466.
[14] Aiello, A., Fattorusso, E. and Menna, M. (1992). *J. Nat. Prod.*, **55**, 321–325.