

Secondary metabolites of a soft coral (*Nephthea* sp.) of the Bay of Bengal

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Dedicated to Prof. (Mrs.) A. Chatterjee to mark her 85th birthday and contribution¹
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Abstract

Chemical investigation of an extract of a soft coral, *Nephthea* sp. from the Bay of Bengal, showing antiviral activity against Ranikhet disease virus (*in vitro*) and Vaccina virus (*in vitro* and *in vivo*) afforded wax esters, cholesterol, 1-*O*-alkylglycerols, fatty acids and D(-)-2*S*,3*R*-2-aminooctadeca-4*E*,8*E*-diene-1,3-diol-*N*-palmitate.

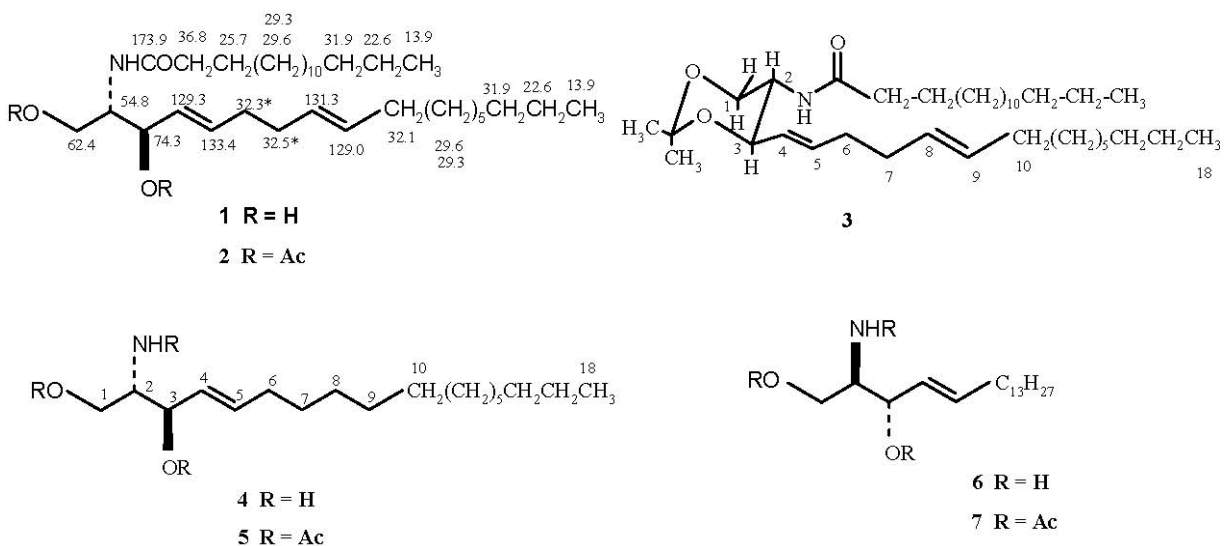
Keywords: *Nephthea* sp., wax ester, cholesterol, 1-*O*-alkylglycerol, fatty acids, (-)-2*S*,3*R*-2-aminooctadeca-4*E*,8*E*-diene-1,3-diol-*N*-palmitate

Introduction

A rich source of varied natural products² is found to be the soft corals that belong to the phylum Coelenterata (Cnidaria) comprised of marine invertebrates such as sea anemone, hydroids, corals, jelly fish and many other less distinguished animals. Chemical investigation of soft corals of the genus *Nephthea*³⁻²⁶ (i.e. *N. chabrolii*^{3-7, 14}, *N. brassica*,^{8,15,16} *N. tixera*,¹¹ *N. albida*^{11,12}, *N. erecta*¹³ and other *Nephthea* spp.^{9,10,17-22}) collected from various locations led to the isolation of varied extractives viz., sesquiterpenoids (guaianes^{3,4,6,7,8,22} and eudesmanes^{17,21}), tetraprenylbenzenoids^{9,21}, diphenylpropane-1,3-diol¹⁰, sterols (24-methylenecholesterols¹¹⁻¹⁶) and diterpenoids (bicyclic^{6,22}, brassicolides,⁸ xeniaphyllanes¹⁷, and cembranes^{18-20,23,24}). As a part of our search for bioactive components from marine sources a soft coral, *Nephthea* sp., was collected from the Bay of Bengal. Preliminary bioactivity studies involving CH₂Cl₂-MeOH (1:1) extract of the raw crushed organism showed antiviral activity against Ranikhet disease virus (*in vitro*; 88% protection at 0.05 mg per ml) and Vaccina virus (*in vitro*; 50% protection at 0.05 mg per ml and *in vivo*; 70% protection at 0.01 µg per ml) and also found to have hypotensive activity in rats. This observation prompted the present investigators to take up systematic chemical investigations of the aforesaid marine organism, the results of which is being reported herewith.

Results and Discussion

Chromatographic resolution of the extract of the *Nephthea* species had afforded wax esters, cholesterol, 1-*O*-alkylglycerols, fatty acids and a new ceramide^{25,26} (**1**). The amorphous material **1** displayed a broad IR absorption band at 3100-3500 cm⁻¹ characteristic for *NH* and *OH* groups and an absorption band at 1650 cm⁻¹ for an amide *CO*. Further **1** readily afforded a diacetate (**2**). The proton networks in both **1** and **2** were established by extensive homodecoupling as well as COSY-90 experiments involving them. The ¹H NMR spectrum of the ceramide showed signals at δ 6.26 (1H, br. d, very slowly exchanged by D₂O) corresponding to an amide proton coupling with a signal at δ 3.91 (m). The methylene protons of hydroxymethyl group resonated at δ 3.93 (1H, dd, *J* = 10.5, 3.3 Hz) and 3.69 (1H, br. d, *J* = 10.5 Hz). The methine proton multiplet at δ 3.91 also coupled with the proton resonating at δ 4.31 (1H, t). The latter proton, geminal to a hydroxyl group, was coupled to an olefinic proton resonating at δ 5.54 (1H, dd, *J* = 15.5 and 6.4 Hz) which in turn was coupled to the resonance at δ 5.78 (1H, dt, *J* = 15.5 and 6.4 Hz).



The latter signal was further coupled to a signal at δ 2.10 (4H, m, allylic methylenes) which again coupled with the resonance at δ 5.42 (1H, dt, *J* = 15.0 and 5.8 Hz). The latter interacted with the signal at δ 5.36 (1H, dt, *J* = 15.0 and 5.8 Hz). Thus a -CONHCH(CH₂OH)-CH(OH)-CH=CH-(CH₂)₂-CH=CH-CH₂- system was present. Again, ¹H NMR spectrum also displayed signals at δ 2.23 (2H, t, *J* = 7.6 Hz) for -CO-CH₂-CH₂-, 1.64 (2H, m) for CO-CH₂-CH₂-CH₂-, 1.25 (huge, br. s) for several methylene protons of long chain fatty alkyl moiety and 0.88 (6H, t, *J* = 6.6 Hz) for methyl protons of two terminal ethyl groups. EIMS and FAB MS of the ceramide conclusively established its structure as 2-aminooctadeca-4*E*,8*E*-diene-1,3-diol-*N*-palmitate (**1**). EIMS spectrum displayed base peak at *m/z* 281 (C₁₈H₃₃O₂⁺) and a prominent one at 298 for [C₁₅H₃₁CONH=CHCH₂OH]⁺. The positive ion LISIMS using *m*-O₂N-C₆H₄CH₂OH/CH₂Cl₂

matrix in presence of Na^+ displayed ion peaks at m/z 558.6, 536.6, 520.5 and 518.6 corresponding to $(\text{MNa})^+$, $(\text{MH})^+$, $(\text{M-CH}_3)^+$, $(\text{MH-H}_2\text{O})^+$ ions respectively. Again negative ion LISIMS using $m\text{-O}_2\text{N-C}_6\text{H}_4\text{CH}_2\text{OH/CH}_2\text{Cl}_2$ matrix showed ion peak at m/z 534.1 for $(\text{M-H})^-$ ion.

For conclusive carbon signal assignments (displayed around structure **1**) one-bond ^{13}C - ^1H correlation studies were made. X-H correlation spots between (i) δ_{C} 13.9 and δ_{H} 0.88 (t, H₃-16' and H₃-18), (ii) δ_{C} 22.6 and δ_{H} 1.25 (m, H₂-15' and H₂-17), (iii) δ_{C} 25.7 and δ_{H} 1.64 (m, H₂-3'), (iv) δ_{C} 29.3 and 29.6 and δ_{H} 1.25 (m, several methylene protons), (v) δ_{C} 31.9 and δ_{H} 1.25 (m, H₂-14' and H₂-16), (vi) δ_{C} 32.1 and δ_{H} 1.95 (m, H₂-10), (vii) δ_{C} 32.3 and 32.5 and δ_{H} 2.10 (m, H₂-6 and H₂-7), (viii) δ_{C} 36.8 and δ_{H} 2.23 (t, H₂-2'), (ix) δ_{C} 54.8 and δ_{H} 3.91 (m, H-2), (x) δ_{C} 62.4 and δ_{H} 3.63 and 3.93 (br. d and dd respectively, H₂-1), (xi) δ_{C} 74.3 and δ_{H} 4.31 (t, H-3), (xii) δ_{C} 129.0 and 129.3 and δ_{H} 5.36 and 5.54 (dt each, H-9 and H-4 respectively), (xiii) δ_{C} 131.3 and δ_{H} 5.42 (dt, H-8) and δ_{C} 133.4 and δ_{H} 5.78 (dt, H-5) in the ^{13}C - ^1H correlation spectrum established the structure **1** for the ceramide.

Relative stereochemistry of the chiral centers at C-2 and C-3 in ceramide **1** was derived from detailed NMR spectral studies on the acetonide^{25,26} (**3**) obtained by treatment of **1** with dry acetone and anhydrous CuSO_4 . A fairly large coupling constant (8.7 Hz) between H-2 and H-3 in acetonide immediately established the *erythro* configuration at C(2)-C(3) unit of acetonide **3** and hence in the parent ceramide **1**.

The proton resonance assignments for the acetonide **3** were supported from its homodecoupling experiment. Irradiation of the doublet for $>\text{NH}$ at δ 5.01 simplified the multiplet at δ 3.79 for H-2. Decoupling of the signal at δ 4.08 (1H, dd, H-3) clearly collapsed the signal at δ 5.44 (dd, H-4) to a doublet. Again irradiation of the signal at δ 3.79 (1H, m, H-2) affected the signals at δ 3.65 (1H, t, H_{ax}-1) and 4.00 (1H, dd, H_{eq}-1). Further irradiation of the triplet signal at δ 3.65 (H_{ax}-1) affected both the signals at δ 4.00 (dd) and 3.79 (m). Decoupling of the signal at δ 2.09 (4H, m, H₂-6 and H₂-7) simplified signals at δ 5.74 (1H, dt, H-5) and 5.39 (1H, m, H-8).

It is documented²⁸ that D-sphingosine i.e. *trans*-2-amino-octadeca-4-ene-1,3-diol (**4**), $[\alpha]_{\text{D}} - 2.8^\circ$ (CHCl_3) and triacetate **5**, $[\alpha]_{\text{D}} - 11.4^\circ$ (CHCl_3) possess *2S,3R-erythro*-configuration while L-sphingosine (**6**), $[\alpha]_{\text{D}} + 2.8^\circ$ (CHCl_3) and its triacetate **7**, $[\alpha]_{\text{D}} + 12.1^\circ$ (CHCl_3) have *2R,3S-erythro*-configuration. The ceramide **1**, $[\alpha]_{\text{D}} - 4.0^\circ$ (CHCl_3) isolated by present investigators and its diacetate **2**, $[\alpha]_{\text{D}} - 10.6^\circ$ (CHCl_3) possess similar functionalities as in **4** and **5** and also they exhibited similar optical rotation (levorotatory). Thus the ceramide from the soft coral belongs to the D-(*2S,3R*)-*erythro* series having the stereostructure **1** and accordingly its acetate has the structure **2**.

The isolation and structure elucidation of the ceramide D-*erythro*-2-amino-octadeca-4*E*,8*E*-diene-1,3-diol-*N*-palmitate as a new metabolite from the soft coral *Nephthea* sp. were presented elsewhere.^{25,26} Subsequently the *dextro* (L) variety of the ceramide **1** has been reported from the sea anemone *Paracondylactis indicus*^{29,30} and the *levo* variety from a gorgonian *Acabaria undulata*³⁰. This is, however, the first report of the occurrence of a ceramide in soft coral of the genus *Nephthea*.

Experimental Section

General Procedures. Column chromatography was carried out with silica gel (60-120 mesh) and tlc was performed on silica gel G plates. IR spectra (KBr) were recorded on a Perkin Elmer 782 spectrophotometer. All NMR spectra were recorded on a Bruker AM 300L supercon spectrometer equipped with ASPECT 3000 computer fitted with an array processor using programme version DISR87.1 or DISR94.1 in CDCl_3 as solvent at 300.13 MHz for proton and at 75.47 MHz for carbon. The chemical shifts values are in δ (ppm) downfield from TMS. Standard procedures were used for two-dimensional NMR experiments. Optical rotations were measured in a Perkin Elmer M241 electronic polarimeter in CHCl_3 at 25 °C. Gas chromatographic experiments were done with Hewlett Packard Model M5890, Series II gas chromatograph fitted with a Hewlett Packard integrator M3394A using appropriate experimental conditions. Mass spectra were taken in a Hitachi RMU 6L spectrometer operating at 70 eV.

Animal material. The soft coral *Nephthea* sp. (Phylum : Cnidaria, Class : Anthozoa, Order: Alcyonacea, Family: Nephthidae) was collected from the Bay of Bengal about 20-30 km off coast from Digha (latitude 21°37' N, longitude 87°31'30" E; about 180 km west of Kolkata), West Bengal and stored in a freezer until extraction.

Extraction and isolation. The raw organism (10 kg) frozen in liquid N_2 was crushed mechanically, treated with CH_2Cl_2 -MeOH (1:1) (10L) and kept at ~ 5 °C for 15 days. Organic solvent and water from the liquid phase collected by filtration were removed under reduced pressure. Column chromatography of the extracted material and subsequent preparative TLC of the appropriate fractions afforded wax esters, cholesterol, 1-*O*-alkyl-glycerol, fatty acids and ceramide (**1**). First four components were characterized from their spectral (IR, ^1H and ^{13}C NMR) properties and similarity with the data for those isolated from *Cavenularia* sp.²⁷

Wax esters (Fatty ester of fatty acids)²⁷. Colourless semisolid mass (105 mg), R_f 0.8 in light petrol-chloroform (20:80). Wax esters fraction was hydrolyzed under basic condition to the acid and alcohol parts. Acid part was converted to methyl ester (FAME) by treatment with CH_2N_2 and the alcohol part to acetate (FAAC). Gas chromatography of FAME over a 1.8 m x 2mm glass column of 10 % DEGS in liquid phase supported on 80-100 mesh chromosorb W (HP) isothermally at 196 °C employing inlet temperature at 250 °C, FID detector at 250 °C and nitrogen flow rate at 30 ml per minute. FAME was composed mainly of methyl esters of palmitic acid (19.4%), stearic acid (14%), eicosanoic acid (5.2%), tricosanoic acid (5.7%), tetracosanoic acid (3.5%) as well as of 16:1 ω 9-hexadecenoic acid (9.4%), 18:1 ω 9-octadecenoic acid, 18;3 ω 3-octadecatrienoic acid and/or eicosanoic acid (4.6%) along with small amounts (< 2% each) of some other saturated and unsaturated fatty acids. Again from GC analysis over a glass column (1.8 m x 2mm) of 3% OV 17 in liquid phase supported on 80-100 mesh chromosorb W (HP) in gradient fashion (180-330 °C) with increase of temperature at the rate of 10 °C per minute, employing inlet temperature 350 °C, detector at 380 °C and flow rate of N_2 at 30 ml per minute

FAAC fraction was found to be consisted mainly of the acetates of hexadecanol (27.7%), heptadecanol (8.6%), octadecanol (43.4%), small amounts of tetradecanol (2.1%), hexadecenol (3.0%), eicosanol (2.0%), hexacosanol (2.4%) and of traces (< 1.5% each) of a few other saturated fatty alcohols.)

D(-)-2S,3R-2-Aminooctadeca-4E,8E-diene-1,3-diol-N-palmitate (1). Colourless amorphous mass (60 mg), $C_{34}H_{65}NO_3$, R_f 0.6 in $CHCl_3$ -MeOH (90:10); $[\alpha]_D -6.8^\circ$ (c 0.21, MeOH); $[\alpha]_D -4.0^\circ$ (c 0.32, $CHCl_3$); IR: ν_{max} 3500-3100, 2960, 2930, 2860, 1650, 1630, 1550, 1470, 1380, 1060 and 970 cm^{-1} ; δ_H 6.26 (1H, d, $J = 7.0$ Hz, NH), 5.78 (1H, dt, $J = 15.5$ and 6.4 Hz, H-5), 5.54 (1H, dd, $J = 15.5$ and 6.4 Hz, H-4), 5.42 (1H, dt, $J = 15.0$ and 5.8 Hz, H-9), 5.36 (1H, dt, $J = 15.0$ and 5.8 Hz, H-8), 4.31 (1H, m, H-3), 3.93 (1H, dd, $J = 10.5$, 3.3 Hz, H_A -1), 3.91 (1H, m, H-2), 3.69 (1H, br d, $J = 10.5$ Hz, H_B -1), 2.23 (2H, t, $J = 7.6$ Hz, H_2 -2'), 2.10 (4H, m, H_2 -6 and H_2 -7), 1.95 (2H, m, H_2 -10), 1.64 (2H, m, H_2 -3'), 1.25 (huge, br s, x CH_2) and 0.88 (6H, t, $J = 6.6$ Hz, 2 x $-CH_2CH_3$); m/z 535 (< 1%), 534 (< 1), 517 (2), 504 (1), 486 (1), 368 (1.5), 366 (2), 352 (3), 351 (3), 337 (1), 320 (2), 299 (8), 298 (45), 281 (100), 280 (10), 262 (6), 250 (10), 239 (5) and 238 (4).

Cholesterol²⁷. White crystalline solid (20 mg), m. p.147 ° ($CHCl_3$ -petrol), R_f 0.5 in $CHCl_3$ -MeOH (98:2); $[\alpha]_D +38^\circ$ (c 0.32, $CHCl_3$).

1-O-Alkylglycerol²⁷. Colourless semisolid mass (28 mg); R_f 0.4 in $CHCl_3$ -MeOH (95:5) consisted mainly of saturated long chain alkyl units with traces of unsaturated ones.

Fatty acids. White solid (32 mg), R_f 0.4 in $CHCl_3$ -MeOH (90:10), consisted mainly of saturated long chain alkyl units with traces of unsaturated ones.

Acetylation of ceramide. Acetylation of ceramide **1** (25 mg) with Ac_2O (0.5 ml) and pyridine (0.5 ml) followed by usual work-up afforded a crude acetate which was purified by column chromatography to yield the diacetate **2** (20 mg) as an amorphous powder, $C_{38}H_{69}NO_5$, $R_f \sim 0.5$ ($CHCl_3$ -MeOH, 98:2); $[\alpha]_D^{25} -10.6^\circ$ (c 0.14, $CHCl_3$); IR : ν_{max} (KBr) 3320, 2960, 2860, 1740, 1665, 1550, 1480, 1380, 1280, 1245, 1035 and 970 cm^{-1} ; δ_H 5.77 (1H, dt, $J = 15.2$, 6.3 Hz, H-5), 5.62 (1H, d, $J = 9.0$ Hz, $-CONH-CH$), 5.43 (1H, dd, $J = 15.2$ and 6.4 Hz, H-4), 5.40 (1H, m, H-8), 5.33 (1H, dt, $J = 14.8$ and 5.3 Hz, H-9), 5.28 (1H, t, $J = 6.4$ Hz, H-3), 4.45 (1H, m, H-2), 4.30 (1H, dd, $J = 11.5$ and 6.7 Hz, H_a -1), 4.03 (1H, dd, $J = 11.5$ and 3.8 Hz, H_b -1), 2.17 (2H, t, $J = 7.4$ Hz, H_2 -2'), 2.06 (6H, s, 2 x $OCOCH_3$), 2.06 (4H, m, H_2 -6 and H_2 -7), 1.95 (2H, m, H_2 -10), 1.60 (2H, m, H_2 -3'), 1.25 (huge, br. s, x CH_2), 0.88 (6H, t, $J = 6.9$ Hz, 2 x CH_2CH_3).

Acetonide 3. The crude **1** (20 mg) in dry acetone (10 ml) was treated with anhydrous $CuSO_4$ (150 mg) and the mixture was allowed to stand at room temperature for about 24 hours. Concentrated organic layer was subjected to preparative tlc separation using $CHCl_3$ as developer. Acetonide **3**. was obtained in fairly pure state as glassy mass (12 mg); $C_{37}H_{61}NO_3$; R_f 0.5 ($CHCl_3$); IR : ν_{max} 3400, 2960, 2920, 2840, 1650, 1560, 1550, 1470, 1400, 1370, 1210, 1160, 1090 and 970 cm^{-1} ; δ_H 5.74 (1H, dt, $J = 15.2$ and 5.7 Hz, H-5), 5.44 (1H, dd, $J = 15.2$ and 7.2 Hz, H-4), 5.41 (1H, m, H-9), 5.39 (1H, m, H-8), 5.01 (1H, d, $J = 7.8$ Hz, $-CHNHCO-$), 4.08 (1H, dd, $J = 8.7$ and 8.2 Hz, H-3), 4.00 (1H, dd, $J = 11.0$ and 5.1 Hz, H_{eq} -1), 3.79 (1H, m, H-2), 3.65 (1H,

t, J = 10.1 Hz, H_{ax}-1), 2.12 (2H, t, J = 6.9 Hz, H₂-2'), 2.09 (4H, m, H₂-6 and H₂-7), 1.96 (2H, m, H₂-10), 1.42 and 1.38 [3H each, s, -O-C(CH₃)₂-O-], 1.25 (huge, br. s, x CH₂), 0.88 (H₃-18 and H₃-16').

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References and Notes

1. This paper is dedicated to Professor (Mrs.) Asima Chatterjee to mark her 85th birthday and in recognition of her outstanding contribution to the chemistry of terrestrial natural products.
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