Bioactive compounds from the Indian ocean gorgonian *Subergorgia suberosa* (Pallas)

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Recently, we have reported the isolation of a new pregnane derivative from the red type gorgonian *S. suberosa* collected from the Mandapam coast, Tamil Nadu1. We report herein the isolation of other secondary metabolites of the same specimen and their antimicrobial activities.

Chromatography over silica gel of the EtOAc solubles of the above gorgonian gave a monohydroxy sterol mixture; subergorgic acid 2, batyl alcohol 3, and a new ceramide mixture in which N-hexadecanoyl-2-amino-1,3-dihydroxyoctadec-4-ene 6 is the major isomer, have been isolated. Compounds 1, 3, 6 and one of the pregnane mixtures exhibit antibacterial and antifungal activities.

**Keywords:** gorgonian, *S. suberosa*, antimicrobial activity, secondary metabolites

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The structure of compound B was established by its acid hydrolysis with 1.2M H2SO4 in 85% methanol, giving a sphingosine 4 and a fatty acid methyl ester 5. The fatty acid methyl ester on GC-MS analysis showed that it was composed of methyl esters of \(-\)C16, \(-\)C17, \(-\)C18, \(-\)C20 and \(-\)C22 carboxylic acids in the ratio 68 : 8 : 18 : 3 : 3. The size of the sphingosine part of compound B could not be ascertained from the sphingosine 4 obtained from acid hydrolysis. Due to paucity of the sample further analysis could not be done. However, its molecular weight could be derived from the mass spectrum of compound B. The FAB mass spectrum of compound B showed an ion at m/z 520. This corresponds to the (M+1)-H2O peak of C34 H52O3 N. Since the alkanoyl part of the major component of compound B was found to be C10H13O, its sphingosine part should be C18H37O2N, i.e. a C-18 sphingosine with one double bond. All naturally occurring sphingosines with one double bond have this bond in the \(\Delta^4\) position. Thus, the double bond in the sphingosine part of compound B may also be at \(\Delta^4\) and, on the basis of the IR band at 971 cm\(^{-1}\), with \(E\)-geometry. Compound B is thus a mixture of compounds 6-10 in the ratio 68:8:18:3:3, respectively. The spectral data of compound B fit into N-hexadecanoyl-2-amino-1,3-dihydroxyoctadec-4-ene 6, its major component (68%).
Antimicrobial activity

The isolated compounds were tested for their antimicrobial activities by the agar cup plate diffusion method. Compound B showed antibacterial activity against Bacillus subtilis, Bacillus pumilus, Proteus vulgaris and Escherichia coli; the pregnane derivative mixture (m.p. 154°C) and compound A showed activities against B. pumilus, P. vulgaris and E. coli; while compound 1 was active against P. vulgaris and E. coli. Compound B showed antifungal activity against Aspergillus niger and Rhizopus oryzae while compounds 1, A and the pregnane derivative mixture (m.p. 154°C) showed activities against R. oryzae only. Compound 1 was tested at 200 μg mL⁻¹ concentration and the other compounds at 500 μg mL⁻¹ concentration.

Extraction of the gorgonian and isolation of compounds

The gorgonian (3 Kg) was extracted with EtOH (7 × 10L) and the residue from the EtOH extract was taken up in EtOAc. Solvent was removed from the EtOAc extract and the resulting dark gum (80g) was chromatographed on a column (100 cm × 6 cm) of silica gel (200g). The column was eluted with increasing polarity from n-hexane through EtOAc.

n-Hexane eluates gave a mixture of aliphatic compounds (50g) and the 2% to 40% EtOAc in n-hexane eluates furnished the following compounds: a monohydroxy sterol mixture (4g); pregnane derivative mixture, m.p. 179°C (50mg); pregnane derivative mixture, m.p. 154°C (100mg); subergorgic acid 1 (3g); batyl alcohol 2 (2g); compound A (60mg) and compound B (50mg). The monohydroxysterol mixture was not examined further. Compounds 1 and 2 were identified by comparison with authentic samples.

Pregnane derivative mixture (mixture of Δ⁵β- and 5α-hydroxy-pregnan-20-one: colourless needles, m.p. 154°C, Rf 0.50 (hexane-EtOAc; 8:2); IR (CHCl₃): 3437, 3401, 1699, 1684, 1449, 1352, 1196, 1080 and 1038 cm⁻¹; ¹H NMR (90 MHz, CDCl₃, TMS): δ 5.38d, J = 5.00Hz (1H), 3.60m (W₁/₂=22Hz, 1H), 2.15s (3H), 2.12s (<3H), 1.02s (<3H), 0.85s (3H), 0.63s (3H); ¹³C NMR (22.40 MHz, CDCl₃, TMS): δ 209.6s, 141.0s, 121.3d, 71.2d, 63.9d, 56.9d, 54.3d, 50.1d, 44.9, 44.3, 39.1, 38.9, 38.2, 37.1, 35.6, 32.0, 31.5, 28.6, 24.4, 22.9, 21.3, 21.1, 13.5, 13.2q, 12.3q. These data were compared with the data reported for cholesterol and the 5α- and 5β-isomers of 3β-hydroxy-pregnan-20-one to deduce the major components of this mixture as indicated.

Pregnan derivative mixture (mixture of Δ¹-5α-pregnan-3,20-dione and 3β-hydroxy-5α-pregnan-20-one: colourless plates (MeOH), m.p. 179°C, Rf 0.50 (hexane-EtOAc; 8:2); IR (CHCl₃): 3437, 3401, 1699, 1684, 1449, 1352, 1196, 1080 and 1038 cm⁻¹; ¹H NMR (90 MHz, CDCl₃, TMS): δ 5.38d, J = 5.00Hz (<1H), 3.60m (W₁/₂=22Hz, 1H), 2.15s (3H), 2.12s (<3H), 1.02s (<3H), 0.85s (3H), 0.63s (3H); ¹³C NMR (22.40 MHz, CDCl₃, TMS): δ 209.6s, 141.0s, 121.3d, 71.2d, 63.9d, 56.9d, 54.3d, 50.1d, 44.9, 44.3, 39.1, 38.9, 38.2, 37.1, 35.6, 32.0, 31.5, 28.6, 24.4, 22.9, 21.3, 21.1, 13.5, 13.2q, 12.3q. These data were compared with the data reported for cholesterol and the 5α- and 5β-isomers of 3β-hydroxypregnan-20-one to deduce the major components of this mixture as indicated.
CDCl₃, TMS): δ 209.0s, 158.1d, 127.5d, 71.3d, 63.9d, 63.7, 56.8, 56.6, 54.4, 50.0, 44.4, 41.0, 39.2, 38.9, 38.2, 37.1, 35.7, 35.6, 32.1, 31.5, 28.7, 27.6, 24.5, 22.9, 21.3, 13.6q, 13.5q, 13.0q, 12.4q. These NMR data were compared with the data reported for Δ⁵α-pregnane-3,20-dione¹⁰, 5α-pregna-1,20-dien-3-one¹¹ and related steroids⁹ to deduce the major compounds of this mixture as indicated.

Compound A (Thymine 3). Obtained from the 30% EtOAc in hexane eluates as a light cream coloured solid. Did not melt even at 300°C (lit⁶ m.p. 318°C). Insoluble in CHCl₃ but soluble in DMSO and water. ¹H (90 MHz, DMSO, TMS) and ¹³C NMR (22.40 MHz, DMSO, TMS) data given in Text. FABMS (m-nitrobenzyl alcohol matrix): m/z 127 (M+1 of C₅H₆O₂N₂, 100%).

Compound B (Ceramide mixture 6-10): colourless microprisms (MeOH), m.p. 79-81°C, Rf 0.55 (CHCl₃-MeOH; 9:1); IR (CHCl₃): ¹H (90MHz, CDCl₃, TMS) and ¹³C NMR (22.40 MHz, CDCl₃, TMS) data given in Text. FABMS (m-nitrobenzyl alcohol matrix): m/z 520 [(M+1)-H₂O of C₃₅H₆₇O₃N, (15)], 296 (15), 282 (30), 264 (100%).

Acid hydrolysis of compound B. A solution of compound B (10mg) in 1.2M H₂SO₄ in 85% methanol (5mL) was refluxed for 4hr. The reaction mixture was then treated with water (10mL) and extracted with n-hexane (2 × 10mL). The hexane layer was seperated, dried over MgSO₄ and solvent removed to give the fatty acid methyl ester 5 as a colourless oil (5mg). GC-MS analysis: Shimadzu QP5050A; EI mode, DB5 column (0.25mm OD × 30 meter), He-flow rate 1.5ml/min, programme: 80°C (2 min)-260°C at 40°C/min and held at 260°C for 25 min, solvent n-hexane, showed the following compounds: (RT min, %): methyl n-hexadecanoate C₁₇H₃₄O₂ (8.20, 68), methyl n-heptadecanoate C₁₈H₃₆O₂ (8.73, 8), methyl n-octadecanoate C₁₉H₃₈O₂ (9.4, 18), methyl eicosanoate, C₂₁H₄₂O₂ (11.2, 3) and methyl docosanoate, C₂₃H₄₆O₂ (12.5, 3).

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References