

Studies on the Secondary Metabolites from the Indian Gorgonian *Subergorgia suberosa*: Isolation and Characterization of Four Analogues of the Cardiotoxin Subergorgic Acid

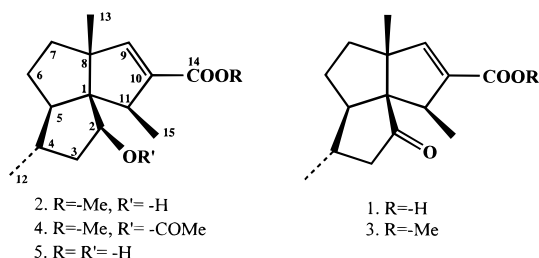
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Chemical investigation of the methanol extract of the Indian ocean gorgonian coral *Subergorgia suberosa* resulted in isolation and identification of four novel compounds **2–5**. Structural investigation revealed compound **1** to be subergorgic acid and the others its analogues.

As part of our continuing collaborative research effort on the isolation of novel bioactive metabolites from marine sources, we have isolated subergorgic acid (**1**) and four other metabolites from the Indian ocean gorgonian coral. The Gorgonian coral *Subergorgia suberosa* occurs widely in Indo-Pacific waters. Gro-weissz et al.¹ have isolated a novel tricyclopentanoid cardiotoxin, subergorgic acid from the Pacific gorgonian. Recently, subergorgic acid, mixtures of pregnane derivatives, 11 monohydroxy sterols, a trihydroxy sterol, and two secosteroids were reported from the coral collected from East Indian coast (Mandapam area).² Several reports on the total synthesis of subergorgic acid have been recently reported.^{3–5} The isolation and identification of subergorgic acid, with unique structural features, had prompted us to investigate in detail similar species collected from the Indian ocean near the west coast of India. Herein, we report isolation and identification of five compounds, subergorgic acid (**1**), its β -hydroxy analogue (**5**), its 2- β -hydroxy methyl ester (**2**), its methyl ester (**3**), and 2- β -acetoxy methyl ester (**4**), from the MeOH extract of this animal.



The gorgonian belongs to the genus *S. suberosa* PALLAS, (coelenterata, Gorgonacea, Subergorgiidae).⁶ The methanol extract of this coral on partition between aqueous methanol–hexane, and then aqueous methanol–chloroform, followed by repeated column chromatography of the hexane and chloroform fractions over silica using hexane–acetone and chloroform–methanol gradient systems yielded compounds **1–5**.

Compounds **2–4** were isolated as oils and **1** and **5** were white solids. All showed end absorption in the UV spectrum.

Compound **1** was a solid, mp 178 °C, which showed characteristic absorption bands at 1725, 1681, 1643, 2935 cm^{-1} , indicating it has both ketone and acid functionalities. The secondary ionization mass spectrometry (SIMS) showed a molecular ion m/z at 249 ($M + H$)⁺, and high-resolution mass measurements established the molecular formula $\text{C}_{15}\text{H}_{20}\text{O}_3$, identical to that of subergorgic acid. This information, along with the ¹H, ¹³C, COSY, HETCOR NMR data when compared with that available from the literature, proved compound **1** to be a subergorgic acid.

Compound **2** showed a molecular ion m/z 265 ($M + H$)⁺ in SIMS; high-resolution mass measurements revealed the molecular formula $\text{C}_{16}\text{H}_{24}\text{O}_3$. Its IR spectrum showed infrared absorptions at 3452, 2926, 2864, 1715, 1632, 1456, and 1254 cm^{-1} , suggesting the presence of hydroxy and ester functional groups. The ¹³C NMR spectrum showed 16 carbon chemical shifts, in agreement with the molecular formula obtained from HRMS. The carbon chemical shift at 165.6 ppm in combination with IR evidence confirmed the presence of an ester functionality. ¹³C NMR also suggested the presence of a trisubstituted olefin. The five unsaturations required by the molecular formula, when compared with the above functionalities, indicated **2** to have a tricyclic structural skeleton. The spectroscopic data of **2** were similar to those of subergorgic acid (**1**),¹ but **2** had an additional CH_4 unit. The ¹H NMR spectrum of **2** (Table 1) showed two additional signals as compared to that of subergorgic acid, a single proton doublet at δ 4.39, and another three proton singlet at δ 3.72. In the ¹³C NMR spectrum of **2** (Table 2), the carbon signal at 217.8 ppm due to ketone C-2 of subergorgic acid was missing. Instead, the ¹³C NMR spectrum showed a carbon signal (doublet) at 76.1 ppm and an additional quartet carbon signal at 52.0 ppm. These data suggested that, in **2**, the ketonic function of subergorgic acid must have been reduced to a hydroxy function and that it contains an –OMe group in place of an –OH group. The position of the –OMe

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Table 1. ^1H NMR Chemical Shifts Observed in Subergorgic Acid and Its Analogs

carbon no.	subergorgic acid	1	2	3	4	5
1						
2		4.39 (d, $J = 2$ Hz)		5.35 (d, $J = 2$ Hz)		4.38 (d, $J = 2$ Hz)
3	2.37 (dd, $J = 18, 7$ Hz), 2.01 (dd, $J = 16.7, 12.6$ Hz)	1.43 (dt, $J = 2, 12$ Hz), 1.8 (ddd, $J = 2, 7$ Hz)	2.35 (dd, $J = 16, 7$ Hz), 1.95 (dd, $J = 18, 4$ Hz)	1.42 (ddd, $J = 4, 2, 12$ Hz), 1.84 (ddd, $J = 2, 7$ Hz)	2.00 (dt, $J = 17, 2$ Hz), 2.17 (dt, $J = 18, 7$ Hz)	1.40 (dt, $J = 14, 7$ Hz), 1.8 (ddd, $J = 2, 4, 14$ Hz)
4		1.95 (dd, $J = 2, 4$ Hz)		1.91 (dd, $J = 2, 4$ Hz)	1.66 (m)	1.95 (m)
5	2.09 (dd, $J = 8.8, 6.7$ Hz)	1.64 (m)	2.07 (dd, $J = 9, 2$ Hz)	1.6 (ddd, $J = 10, 2.5, 2$ Hz)	2.09 (dd, $J = 9, 7$ Hz)	1.65 (m)
6		1.34 (m), 1.58 (dt, $J = 11, 2$ Hz)	1.65 (m), 1.63 (m)	1.36 (m), 1.71 (dt, $J = 11, 2$ Hz)		1.35 (dt, $J = 12, 2$ Hz), 1.6 (ddd, $J = 12, 2$ Hz)
7		1.75 (m), 1.95 (m)	1.55 (m), 1.78 (dd, $J = 11, 5$ Hz)	1.8 (m), 1.95 (dd, $J = 11.5, 4$ Hz)	1.63 (dt, $J = 7, 2$ Hz), 1.80 (dd, $J = 2, 12$ Hz)	1.95 (m), 1.78 (m)
8						
9	6.43 (s)	6.42 (s)	6.3 (s)	6.4 (s)	6.43 (s)	6.42 (s)
10						
11	3.01 (q, $J = 7$ Hz)	2.69 (q, $J = 7.0$ Hz)	3.02 (q, $J = 7.0$ Hz)	2.78 (q, $J = 7.5$ Hz)	3.01 (q, $J = 7.0$ Hz)	2.68 (q, $J = 8$ Hz)
12	1.12 (d, $J = 6.4$ Hz)	1.04 (d, $J = 7.5$ Hz)	1.10 (d, $J = 7.5$ Hz)	1.03 (d, $J = 6.5$ Hz)	1.12 (d, $J = 6.5$ Hz)	1.05 (d, $J = 7$ Hz)
13	1.22 (s)	1.38 (s)	1.2 (s)	1.18 (s)	1.22 (s)	1.4 (s)
14						
15	1.13 (d, $J = 7.3$ Hz)	1.13 (d, $J = 7$ Hz)	1.10 (d, $J = 7$ Hz)	1.15 (d, $J = 7$ Hz)	1.14 (d, $J = 7.5$ Hz)	1.15 (d, $J = 8$ Hz)
O COCH_3						
OCO CH_3				2.07 (s)		
CH $_3$		3.72 (s)	3.72 (s)	3.75 (s)		

Table 2. ^{13}C NMR Chemical Shifts Observed in Subergorgic Acid and Its Analogs

carbon no.	subergorgic acid	1	2	3	4	5
1	68.5 (s)	68.0	68.5	66.9	68.5	68.2
2	217.8 (s)	76.1	217.8	79.2	217.7	76.0
3	49.9 (t)	45.7	50.0	42.8	49.9	45.7
4	33.3 (d)	39.7	33.4	39.8	33.3	39.7
5	62.6 (d)	63.7	62.8	63.9	62.7	63.7
6	28.3 (t)	30.0	28.4	30.1	28.3	30.4
7	38.2 (t)	40.1	38.4	39.9	38.3	40.1
8	61.7 (s)	59.1	61.7	59.0	61.8	59.4
9	152.1 (d)	153.6	149.6	152.9	152.3	156.2
10	136.6 (s)	137.1	137.0	137.1	136.5	136.2
11	51.6 (d)	50.7	51.5	51.4	51.6	50.6
12	19.9 (q)	20.4	20.0	20.0	19.9	20.5
13	23.4 (q)	22.4	23.6	21.9	23.4	22.3
14	169.6 (s)	165.6	165.0	170.3	169.5	168.7
15	17.7 (q)	17.6	17.9	17.7	17.7	17.5
-OCO CH_3				177.7		
-OCO CH_3				21.7		
-CH $_3$		51.3	52.0	50.9		

group as an ester was established by IR, leading to the structural assignment of **2** (excluding stereochemistry at C-2).

Compound **3** exhibited similar properties, and SIMS showed a molecular ion m/z at 263 ($M + H$) $^+$. High-resolution mass measurements established the molecular formula, $\text{C}_{16}\text{H}_{22}\text{O}_3$, two protons less than **2**. The IR spectrum showed infrared absorptions at 2950, 1720, 1717, 1645, and 1250 cm^{-1} suggesting the presence of a five-membered ketone and ester functionalities. The ^1H NMR spectrum did not show the doublet proton signal at δ 4.39 as observed in **2**. The ^{13}C NMR spectrum showed a carbon signal at 217.8 instead of a peak at 76.1 ppm as seen in **2**. These data suggested the presence of a ketone instead of alcohol as in **3**.

The IR spectrum of **4** showed infrared absorptions at 2924, 1737, 1717, 1635, 1243, and 1022 cm^{-1} , suggesting two ester functionalities. The SIMS showed molecular ion m/z at 307 ($M + H$) $^+$, and high-resolution mass measurements established the molecular formula,

$\text{C}_{18}\text{H}_{26}\text{O}_4$, suggesting an additional COCH_2 or 42 mass units compared to **2**. The ^1H NMR spectrum revealed an additional three-proton singlet at δ 2.1, and the ^{13}C NMR spectrum showed two additional carbon atoms at 170.3 and 17.7 ppm. Also, the single proton doublet at δ 4.39 in **2** has shifted to δ 5.35 in this compound. These data revealed **4** as an acetate of **2**.

Compound **5** was a solid, mp 146 $^\circ\text{C}$, and showed absorptions at 3400 and 1685 cm^{-1} due to $-\text{OH}$ and α,β -unsaturated acid moiety. The molecular ion m/z at 251 ($M + H$) $^+$ revealed by SIMS and the molecular formula $\text{C}_{15}\text{H}_{22}\text{O}_3$ established by high-resolution mass measurements revealed two additional protons in **5** compared to subergorgic acid. A proton doublet at δ 4.4 in ^1H NMR and a carbon signal at 76.0 instead of 217.8 ppm seen in subergorgic acid suggested this compound to be a dihydrosbergorgic acid (**5**). The stereochemistry of the hydroxy group in **5** (and by implication in **2** also) was assigned as β by comparison with the proton chemical shift of the 2-H proton with that of reduced compound produced from subergorgic acid on LiAlH_4 reduction.¹

Experimental Section

General Experimental Procedures. Melting points were determined on a Laboratory Device's Mel-temp melting point apparatus and are uncorrected. IR spectra were taken on a ATI Mattson Genesis Series FTIR instrument. Ultraviolet spectra were obtained using a Hewlett-Packard 8450 A UV-vis spectrophotometer. NMR spectra were recorded on a Varian XL-400 spectrometer (400 MHz), XL-300 (300 MHz) spectrometer, with chemical shifts (δ) expressed downfield to TMS. HMBC studies were performed on a Varian XL-300 instrument using TMS as an internal standard. SIMS and HRMS were recorded on a VG-70-SE instrument using glycerol and thioglycerol matrix.

Marine Sponge Material. The gorgonian coral (3 kg) was collected from Beyt Dwaraka (Gujarat) on the west coast of India, during the winter of 1989 by Cuba

10 m under the water surface and transported to the lab in methanol. It was classified to be from the genus *S. suberosa* PALLAS (coelenterata, Gorgonacea, Subergorgiidae).⁶ A specimen of sponge is on deposit in the Biology museum, at National Institute of Oceanography Institute at Dona Paula, Goa (India) (voucher no. NIO 484).

Extraction of *S. suberosa*. The sponge (3 kg) was soaked with MeOH. After 2 weeks, the solution was filtered, solvent was removed, and the residue was partitioned between aqueous MeOH–hexane and then aqueous MeOH–CHCl₃ to yield hexane, CHCl₃, and finally H₂O-soluble fractions. Chromatography of the hexane fraction on silica and eluting with a hexane–CH₃COCH₃ gradient system yielded a mixture of fatty acids, their methyl esters, monohydroxy sterols, and a mixture of oils having a camphor-like odor. The latter fractions were repeatedly purified over silica columns using hexane–CH₃COCH₃ gradient systems yielding mixtures of compounds **1** and **2**, **2** and **3**. The CHCl₃ fraction upon purification on silica column using CHCl₃–MeOH gradient systems yielded compound **4** and a mixture of **4** and **5**. The mixtures were further purified by preparative HPLC on Waters Deltapak columns, yielding 185, 130, 25, 135, and 20 mg of **1**, **2**, **3**, **4**, and **5**, respectively.

Subergorgic acid (1): mp 178 °C; $[\alpha]^{20}_D = -128.0$ (MeOH, *c* 1.0 mg/mL); IR(KBr) ν_{\max} 2935, 1725, 1681, 1643, 1422, 1285, 967 cm⁻¹; UV (MeOH) λ_{\max} 225 nm; SIMS *m/z* 249 (M + H)⁺.

2-β-Hydroxy methyl ester of subergorgic acid (2): oil, $[\alpha]^{20}_D = -49.4$ (MeOH, *c* 0.85 mg/mL); UV-(MeOH) λ_{\max} 225 nm; IR (neat) ν 3452, 2926, 2864, 1715, 1632, 1456, and 1254 cm⁻¹; SIMS *m/z* 265 (M + H)⁺; HRMS obsd 265.1801, calcd for C₁₆H₂₄O₃ 265.1804.

Subergorgic acid methyl ester (3): oil; $[\alpha]^{20}_D = -112.0$ (MeOH, *c* 1.0 mg/mL); UV(MeOH) λ_{\max} 224 nm; IR (KBr) ν_{\max} 2950, 1720, 1717, 1645, and 1250 cm⁻¹; SIMS *m/z* 263 (M + H)⁺; HRMS obsd 263.1800, calcd for C₁₆H₂₂O₃ 263.1803.

2-β-Acetoxy methyl ester of subergorgic acid (4): oil; $[\alpha]^{20}_D = -81.6$ (MeOH, *c* 1.0 mg/mL); UV(MeOH) λ_{\max} 224 nm; IR (KBr) ν_{\max} 2924, 1737, 1717, 1635, 1243 and 1022 cm⁻¹; SIMS *m/z* 307 (M + H)⁺.

2-β-Hydroxysubergorgic acid (5): mp 146 °C; $[\alpha]^{20}_D = -95.0$ (MeOH, *c* 1.0 mg/mL); UV(MeOH) λ_{\max} 225 nm; IR (KBr) ν_{\max} 3400, 2948, 2866, 1685, 1632, 1455, and 1257 cm⁻¹; SIMS *m/z* 251 (M + H)⁺.

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