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Two Ring-A-Aromatized Bile Acids from the Marine Sponge *Sollasella moretonensis*

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Abstract

Two ring-A-aromatized bile acids, **1** and **2**, were isolated from the sponge *Sollasella moretonensis*, collected from the seabed of northern Queensland. Structures were assigned on the basis of extensive 1D and 2D NMR studies, as well as analysis by HRESIMS. Compound **2** has previously been produced synthetically, though this marks its first isolation from a natural source.

Keywords

marine natural product; sponge; *Sollasella moretonensis*; ring-A-aromatized bile acids

As part of a collaborative project with the Queensland Museum to investigate the taxonomy, molecular genetics and intraspecific chemical variability of marine sponges sampled from the Great Barrier Reef [1], we studied *Sollasella moretonensis* collected from the inter-lagoon seabed area in northern Queensland. No chemistry had been reported previously for this species, suggesting this specimen would be an attractive source for chemical investigation. As a result, two ring-A-aromatized bile acids, **1** and **2**, were isolated. The isolation and structure elucidation are described herein.

Compound **1** gave the molecular formula $C_{23}H_{30}O_3$, as deduced from HRESIMS ($[M + Na]^+$ *m/z* 377.2113; Δ -5 ppm). The IR spectrum indicated the presence of a hydroxyl group (3274 cm^{-1}) and an *α,β*-unsaturated carboxyl group (1685 cm^{-1}). The ^1H NMR spectrum exhibited five downfield protons at δ_H 5.75 (d, *J* = 15.3 Hz), 6.50 (d, *J* = 15.3 Hz), 6.46 (d, *J* = 1.9 Hz), 6.53 (dd, *J* = 8.5, 1.9 Hz) and 7.06 (d, *J* = 8.5 Hz), which clearly indicated the existence of a *trans* double bond and a 1,3,4-trisubstituted benzene ring. The upfield region of the ^1H NMR spectrum displayed a singlet methyl at δ_H 0.77, a doublet methyl at δ_H 1.12 (d, *J* = 6.6 Hz) and several methines and methylenes. In addition to the benzene ring system, the COSY experiment revealed two partial structures, $CH{=}CHCH(CH_3)CHCH_2CH_2$ and $CH_2CH_2CHCHCH_2CH_2$, corresponding to the C-23, C-22, C-20, C-21, C-17, C-16, C-15 and C-12, C-11, C-9, C-8, C-7, C-6 fragments, respectively (Figure 1). Connectivity between fragments was established

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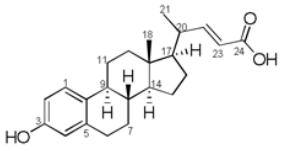

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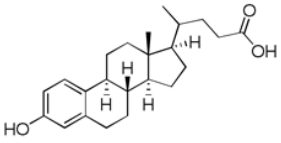

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through analysis of the HMBC spectrum (Figure 1). HMBC correlations from H-15 to C-13 and C-14, and from H-8 to C-13 and C-15 allowed connection of these two fragments. HMBC correlations from H_3-18 to C-12, C-17 and C-13, and from olefinic protons H-22 and H-23 to C-24 established the locations of Me-18 and the carboxyl group. Finally, HMBC correlations from the aromatic proton H-1 to C-9 and from H-4 to C-6 established the manner of attachment of thex aromatic ring to the alicyclic core, suggesting that compound **1** was a previously undescribed ring-A-aromatized bile acid, namely 3-hydroxy-19-nor-1,3,5(10),22-cholatetraen-24-oic acid.

The relative configuration of **1** was determined using NOESY (Figure 2). H-8 displayed a NOESY correlation with H_3-18, and H-9 showed a correlation with H-14. Similarly, H-9 exhibited correlations with H-12$_\beta$ and H-7$_\beta$, whereas H-8 showed correlations with H-11$_\alpha$ and H-6$_\alpha$. These data indicated that the B/C and C/D ring junctures were both *trans* with H-8, H_3-18, H-11$_\alpha$, and H-6$_\alpha$ on one face of the ring system and H-9, H-12$_\beta$, H-7$_\beta$, and H-14 on the opposite face. In addition, NOESY correlations from H-16$_\beta$ to both H-14 and H-17 suggested that the latter is located on the same face as H-9.

Compound **2** showed a pseudomolecular ion peak at *m/z* 357.2426 ($[M + H]^+$; Δ +1 ppm) in the HRESIMS, suggesting a molecular formula of $C_{23}H_{32}O_3$, which differs from **1** only by the addition of two hydrogen atoms. The ^{1}H NMR spectrum was very similar to that of **1**, the major difference being the absence of the olefinic protons at δ_H 5.75 (d, J = 15.3 Hz), and 6.50 (d, J = 15.3 Hz). In combination with the difference in the molecular formula, this suggested that **2** was the dihydro analogue of **1**, as confirmed by detailed analysis by 2D NMR (gCOSY, gHSQC and gHMBC). The relative configuration of **2** was determined to be the same as **1** based on analysis of the NOESY spectrum, thus identifying it as 3-hydroxy-19-nor-1,3,5(10)-cholatrien-24-oic acid, a compound that has been produced synthetically [2,3], though this is the first report of its isolation from a natural source.

Early interest in ring-A aromatized bile acids arose when they were discovered as metabolites produced by microbes in the human intestinal flora from bile acids [4-7], raising the question of whether they might play a role in development of colon cancer [8]. However, synthetic compound **2** was found to lack mutagenic activity on its own [3], thus discounting a significant role for these compounds in carcinogenesis in the colon [9].

Although sterols with aromatic A rings are common in terrestrial plants and in some animals as hormones (for example, estradiol), few have been reported from marine organisms. To our knowledge, only geodisterol [10] and two sulfated congeners [11] have been reported from marine sponges. One unusual feature of geodisterol among ring-A aromatized sterols is that the side chain is fully intact, whereas it is more common for the side chain to have undergone carbon-carbon bond cleavage, as in **1** and **2**. It is interesting to note that **2**, a compound thought to be an intermediate in the metabolism of bile acids by human gut bacteria [3], is apparent in the extract of a sponge.

Experimental

General experimental procedures

Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV spectra were acquired in spectroscopy grade methanol using a Hewlett-Packard 8452A diode array spectrophotometer. IR spectra were recorded on a JASCO FT/IR-420 spectrophotometer. NMR data were collected using a Varian INOVA 500 (^{1}H 500 MHz, ^{13}C 125 MHz) NMR spectrometer with a 3 mm Nalorac MDBG probe with a z-axis gradient and utilized residual solvent signals for referencing (δ_H 3.30 ppm, δ_C 49.00 ppm for CD_3OD). High-resolution ESIMS analyses were obtained using a Micromass Q-Tof Micro mass spectrometer. Analytical and semi-preparative HPLC was accomplished utilizing a Beckman System Gold 126 solvent module equipped with a 168 PDA detector.

Sponge material

Sollasella moretonensis van Soest, Hooper, Beglinger and Erpenbeck, 2006 was collected from an inter-lagoon seabed area of Torres Strait, far North Queensland (−19° 37′ 30.00″ N, 148° 8′ 6.00″ E) under permit [1]; a voucher specimen of G329277 is maintained at the Queensland Museum.

Extraction and isolation

The frozen sponge (150 g wet wt) was exhaustively extracted with MeOH to yield 6.0 g of crude extract that was then separated on HP20SS resin using a gradient of H_2O to IPA in 25% steps, and a final wash of 100% MeOH, yielding 5 fractions. The third fraction (50/50 H_2O/IPA) was further fractionated on Sephadex LH-20 with 1:1 CH_3Cl: MeOH to give 6 fractions (Fr. 3.1-3.6). Fr. 3.5 was chromatographed by HPLC using a Phenomenex Luna C18 column (250 × 10 mm) employing 80% CH_3CN/H_2O at 4 mL/min to yield compound **1** (0.9 mg, t_R = 4.5 min) and compound **2** (1.2 mg, t_R = 5.2 min).

3-Hydroxy-19-nor-1,3,5(10),22-cholatetraen-24-oic acid (1)

Colorless solid

$[\alpha]_D$: +27° (*c* 0.1, MeOH).

IR (film): 3274, 2927, 2866, 1685, 1652, 1557, 1505, 1456, 1403, 1287, 1254, 1209, 1181, 1140, 1029, 985, 928, 868, 844, 813, 786, 725 cm^{-1}.

UV/Vis λ_{max} (MeOH) nm (log ε): 206 (4.77), 222 (4.36), 284 (3.94).

^{1}H NMR and ^{13}C NMR: Table 1.

HRESIMS: *m/z* [M + Na^+] calcd for $C_{23}H_{30}O_3Na$: 377.20926; found: 377.2113.

3-Hydroxy-19-nor-1,3,5(10)-cholatrien-24-oic acid (2)

Colorless solid

$[\alpha]_D$: +29° (*c* 0.1, MeOH).

IR (film): 3274, 2925, 2866, 1731, 1558, 1499, 1455, 1445, 1415, 1378, 1286, 1240, 121, 1182, 1142, 1041, 928, 867, 843, 805, 786, 724 cm^{-1}.

UV/Vis λ_{max} (MeOH) nm (log ε): 206 (4.82), 222 (4.40), 284 (4.05).

^{1}H NMR (500 MHz, CD_3OD): 7.04 (1H, d, *J* = 8.4 Hz, H-1), 6.51 (1H, dd, *J* = 8.4,1.6 Hz, H-2), 6.45 (1H, d, *J* =1.6 Hz, H-4), 2.74 (1H, m, H-6$_\alpha$), 1.28 (1H, m, H-6$_\beta$), 1.85 (1H, m, H-7$_\alpha$), 1.30 (1H, m, H-7$_\beta$), 1.30 (1H, m, H-8), 2.10 (1H, m, H-9), 2.22 (1H, m, H-11$_\alpha$), 1.41 (1H, m, H-11$_\beta$), 2.12 (1H, m, H-12$_\alpha$), 1.39 (1H, m, H-12$_\beta$), 1.29 (1H, m, H-14), 1.29 (1H, m, H-15$_\alpha$), 1.86 (1H, m, H-15$_\beta$), 1.17 (1H, m, H-16$_\alpha$), 1.69 (1H, m, H-16$_\beta$), 1.20 (1H, m, H-17), 0.73 (3H, s, H_3-18), 1.46 (1H, m, H-20), 0.98 (3H, d, *J* = 6.7 Hz, H_3-21), 1.80 (1H, m, H-22$_\alpha$), 1.30 (1H, m, H-22$_\beta$), 2.22 (1H, m, H-23$_\alpha$), 2.05 (1H, m, H-23$_\beta$).

^{13}C NMR (125 MHz, CD_3OD): 127.0 (C-1), 113.5 (C-2), 156.7 (C-3), 115.9 (C-4), 139.7, (C-5), 30.8 (C-6), 28.9 (C-7), 40.4 (C-8), 45.2 (C-9), 133.5 (C-10), 27.8 (C-11), 41.3, (C-12), 44.6 (C-13), 57.0 (C-14), 29.2 (C-15), 24.8 (C-16), 57.4 (C-17), 12.4 (C-18), 37.2 (C-20), 18.9 (C-21), 34.0 (C-22), 36.3 (C-23), 167.5 (C-24).

HRESIMS: *m/z* [M + H^+] calcd for $C_{23}H_{33}O_3$: 357.2430; found: 357.2426.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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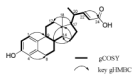

Figure 1.
gCOSY and key gHMBC correlations of **1**.

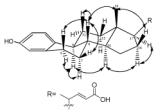

Figure 2.
Key NOESY correlations for **1**.

Table 1

NMR data for **1** (^{1}H 500 MHz, ^{13}C 125 MHz δ ppm, *J* in Hz) in CD_3OD.

position	δ_C	δ_H (*J* in Hz)	COSY	HMBC	NOESY
1	127.0, CH	7.06, d (8.5)	H-2	C-3, C-5, C-9	H-2, H-9, H-11$_\alpha$, H-11$_\beta$, H-12$_\beta$
2	113.5, CH	6.53, dd (8.5,1.9)	H-1	C-3, C-4, C-10	H-1
3	156.0, C				
4	115.8, CH	6.46, d (1.9)	H-6	C-2, C-3, C-6, C-10	H-6$_\alpha$, H-6$_\beta$
5	138.9, C				
6$_\alpha$	30.6, CH_2	2.76, m	H-4, H-7	C-4, C-5, C-7, C-8, C-10	H-4, H-6$_\beta$, H-7$_\alpha$, H-8
6$_\beta$		1.30, m	H-4		H-4, H-6$_\alpha$, H-9
7$_\alpha$	28.9, CH_2	1.86, m	H-6, H-8	C-8, C-9	H-6$_\alpha$, H-7$_\beta$
7$_\beta$		1.32, m	H-6, H-8		H-7$_\alpha$, H-9
8	40.4, CH	1.33, m	H-7, H-9, H-14	C-13, C-15	H-6$_\alpha$, H-11$_\alpha$, H-18
9	45.1, CH	2.14, m	H-8, H-11	C-7, C-11, C-14	H-1, H-6$_\beta$, H-7$_\beta$, H-11$_\beta$, H-12$_\beta$, H-14
10	132.9, C				
11$_\alpha$	27.9, CH_2	2.26, m	H-9, H-12	C-8, C-13	H-1, H-8, H-11$_\beta$, H-12$_\alpha$, H-18
11$_\beta$		1.44, m	H-9, H-12		H-1, H-9, H-11$_\alpha$
12$_\alpha$	41.1, CH_2	2.12, m	H-11	C-9, C-11, C-18	H-11$_\alpha$, H-12$_\beta$, H-18, H-21
12$_\beta$		1.44, m	H-11		H-1, H-9, H-14, H-12$_\alpha$, H-18
13	44.1, C				
14	56.7, CH	1.28, m	H-8		H-9, H-12$_\beta$, H-16$_\beta$
15$_\alpha$	29.3, CH_2	1.34, m	H-16	C-13, C-14, C-16	H-15$_\beta$, H-18
15$_\beta$		1.79, m	H-16		H-15$_\alpha$
16$_\alpha$	24.9, CH_2	1.19, m	H-15, H-17	C-13, C-15, C-17	H-16$_\beta$, H-18
16$_\beta$		1.68, m	H-15, H-17		H-14, H-16$_\alpha$, H-17
17	56.9, CH	1.34, m	H-16, H-20	C-14, C-15	H-16$_\beta$, H-20, H-22, H-23
18	12.6, CH_3	0.77, s		C-12, C-13, C-17	H-8, H-11$_\alpha$, H-12, H-15$_\alpha$, H-16$_\alpha$, H-20, H-21
20	40.7, CH	2.24, m	H-17, H-21, H-22		H-17, H-18, H-21, H-22, H-23
21	20.2, CH_3	1.12, d (6.6)	H-20	C-17, C-20, C-22	H-12$_\alpha$, H-18, H-20, H-22, H-23
22	150.1, CH	6.50, d (15.3)	H-20, H-23	C-17, C-20, C-21, C-24	H-17, H-20, H-21, H-23

position	δ_C	δ_H (*J* in Hz)	COSY	HMBC	NOESY
23	126.3, CH	5.75, d (15.3)	H-22	C-20, C-24	H-17, H-20, H-21, H-22
24	175.9, C				