Marine Natural Products: 9α,11α-Epoxycholest-7-ene-3β,5α,6β,19-tetrol 6-Acetate from a Sponge, *Dysidea* sp.

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Numerous new sterols have been isolated from marine organisms, most having novel side-chain alkylation patterns but also a few with nuclear modifications.[1] A second, as yet small class of unusual sterols are the polyhydroxy and ring-C seco sterols;[1b] however, one of us has noted earlier that future studies would be likely to uncover additional polar, hydroxylated sterols in marine organisms.[1b] In this paper we report the isolation of a new hydroxylated sterol with the unusual features of a 9,11-epoxide and a 19-hydroxyl group. It has been noted[2] that 19-hydroxylated sterols may be intermediates in the formation of 19-demethylsterols isolated from marine organisms. 9,11-Epoxy sterols are logical intermediates in the formation of 9,11-seco sterols.

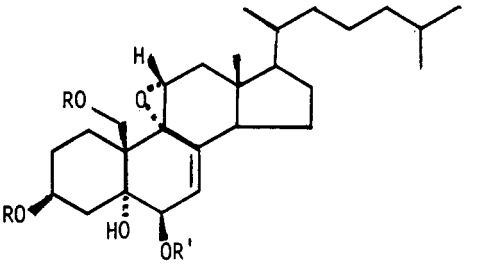
The new sterol was isolated from a *Dysidea* sp. of sponge collected in Guam. Silica gel followed by high-pressure C_{18} reversed-phase chromatography of the chloroform:methanol (1:1) and methanol extracts yielded a crystalline solid: mp 229–230 °C, $[\alpha]^{26}_D$ +42.6° (*c* 0.07, $CHCl_3$). The formula $C_{29}H_{46}O_6$ was established by mass spectrometry: low-resolution M^+ 490; high-resolution 472.31624 ($C_{29}H_{44}O_5$, calcd 472.31887, $M^+ - H_2O$). Infrared data indicated the presence of hydroxyl groups (3585, 3450 cm^{-1}) and an acetate (1736, 1240 cm^{-1}). The ^1H NMR spectrum confirmed the presence of one acetate (δ 2.15 (s)) and revealed the presence of three hydroxyl groups (exchangeable signals at δ 5.82 (s), 6.21 (d), and 6.55 (t)). The ^1H NMR spectrum also contained signals for four of the five methyl groups typical of a sterol: δ 0.60 (s, C-18), 0.86 (6 H, d, J = 7 Hz, C-26, 27), and 0.92 (d, J = 7 Hz, C-21). Although the methyl singlet typical for the C-19 protons was absent, an AB quartet centered at δ 3.90, J = 12 Hz was observed, which was ascribable[2] to a hydroxylated C-19 group. Consistent with this assignment were prominent (~20%) losses of CH_2OH + AcOH and CH_2OH + AcOH + H_2O in the mass spectrum.

In the mass spectrum, peaks were also observed at 299.16488 for $M^+ - (CH_3CO_2H + C_8H_{17})$ and 281.15433 $M^+ - (H_2O + CH_3CO_2H + C_8H_{17})$ (see Experimental Section), indicating a conventional C_8H_{17} side chain.[3] A peak was also observed at 245.11806, corresponding to loss of the side chain plus ring-D[3] [$M^+ - (CH_3CO_2 + CH_2OH + C_{11}H_{23})$], and thus it was established that all of the oxygen substituents were confined to the A, B, and/or C rings in the molecule.

The presence of a typical 3β-hydroxyl group was indicated by a broad methine multiplet at δ 4.06, the lower than normal chemical shift being consistent with deshielding from an axial 5α-hydroxyl group.[4] This methine signal was shifted downfield to δ 4.75 in pyridine-d_5, and irradiation of it collapsed a double doublet (1 H) at δ 2.77 (J = 14.2, 5.2 Hz) to a doublet (J = 14.2 Hz). This is as expected for a 4α-hydrogen next to a substituted C-5 postion in an A/B trans steroid, providing further evidence for C-5 substitution.

Two low-field signals with one common coupling constant, δ 5.22 (J = 3.5, 0.8 Hz) and 5.32 (J = 3.5, 1.0 Hz) were assigned to an acetoxy-deshielded allylic methine proton and a vicinal olefinic proton, respectively. Decoupling and difference decoupling confirmed that both of these protons were coupled (0.8 and 1.0 Hz, respectively) to a common allylic methine proton that resonated at δ 2.36 (br dd, J = 7.6, 6.6 Hz). These data require the fragment $CHOAcCH{=}CCHCH_2$.

The only remaining downfield signal was a doublet at δ 3.40 (J = 4.7 Hz), suitable for the proton of a trisubstituted epoxide, a ring that would account for the last of the seven degrees of unsaturation present in the new sterol. Decoupling established that the δ 3.40 proton was coupled to a proton resonating at δ 2.28 (J = 15.1, 4.7 Hz). The multiplicity of the δ 2.28 signal demonstrates that the corresponding proton is part of a methylene group next to a blocked position as in the partial structure $>C(O)-CHCH_2$. Incorporation of these last two partial structures into the sterol nucleus, keeping all oxygens in rings A, B, or C yields structure **1a**. The rather high-field postion of the C-18 methyl signal in this structure is in good agreement with that expected[5] for a Δ^7 sterol.



1a, R = H; R′ = Ac
1b, R, R′ = Ac
1c, R, R′ = H

Acetylation of **1a** yielded the triacetate **1b**, confirming the presence of two acetylatable oxygens. Hydrolysis of **1a** with KOH in aqueous ethanol afforded a product, **1c**, whose low-resolution mass spectrum showed the expected M^+ of 448, appropriate for $C_{27}H_{44}O_5$. Product **1c** lacked carbonyl absorption in the IR, thus removing any doubt that the original compound contained a second carbonyl absorption that was masked by the acetate carbonyl. In the ^1H NMR spectrum of **1c** in pyridine-d_5, only one proton appeared below δ 5 (δ 5.72), thus confirming that one of the δ >5 signals in **1a** was due to an acetoxy-deshielded allylic methine proton.

Tori and co-workers[6] have shown that for 9β,11β-epoxy steroids $J_{11\alpha,12\alpha}$ and $J_{11\alpha,12\beta}$ are ~1.5 Hz while for the 9α,11α-isomer $J_{11\beta,12\alpha} \simeq 0$, $J_{11\beta,12\beta} \simeq 4.5$ Hz. Hence the single 4.7-Hz coupling for H-11 in **1a** confirms the 9α,11α stereochemistry. The 3.5-Hz coupling of H-6 to H-7 is compatible with the near-zero dihedral angle for these protons in the 6β-acetoxy-Δ^7 moiety, as assigned in **1a**, whereas the epimeric 6α-acetoxy isomer would be expected to have a near-zero coupling for H-6/H-7.[7]

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(7) Cf. $J_{6,7}$ < 1 Hz in 24-methylenecholest-5-ene-3β,7β,19-triol; see ref 2.

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Only one other 19-hydroxylated sterol has been isolated[2] from natural sources to the best of our knowledge.

Sterol 1 was found to be slightly cytotoxic, ED_{50} in PS, 4.9 μg/mL.[8]

Experimental Section

Melting points are uncorrected. Infrared spectra were taken on a Perkin-Elmer 298 spectrophotometer. NMR spectra were taken on Varian XL-100 and Nicolet 270 MHz instruments in the solvent specified; signals are reported in parts per million (δ) downfield from internal tetramethylsilane. Mass spectra were taken on CEC 110 (Du Pont) and Hewlett-Packard 5985B spectrometers. A Perkin-Elmer 141 polarimeter was used for obtaining optical rotations. The chromatographic adsorbent used was Brinkmann silica gel 60 (230–400 mesh). An Altex 5-μm 10 mm × 25 cm preparative silica gel (LiChrosorb 60) column was used for HPLC separations.

Isolation of 1a. Specimens of the sponge *Dysidea sp.* were collected at ~10–15 M around Guam Is. and transported frozen to Oklahoma. Freshly thawed material (wet weight 177.4 g) was extracted at room temperature first with chloroform–methanol (1:1) for 2 days and then with methanol for 1 week. Evaporation of the extracts in vacuo afforded residues weighing 1.64 and 0.36 g, respectively. Chromatography of 1.5 g of the combined extracts on 100 g of silica gel using chloroform followed by chloroform with increasing amounts of methanol afforded 18 fractions. Crystallization of fraction 13 (34 mg, eluted with chloroform–methanol (98:2)) from 4 mL of chloroform–hexane (1:2) overnight in a refrigerator yielded 6 mg of white crystals. This crystalline material was purified further by high-pressure liquid chromatography using a reverse-phase C_{18} column with a mobile phase of methanol–water (90:10). Crystallization of the major HPLC fraction from chloroform–hexane (1:2) yielded 4.2 mg of white crystals: mp 229–230 °C; $[\alpha]^{26}_D$ +42.6° (*c* 0.07, $CHCl_3$); IR($CHCl_3$) 3585, 3650–3250 (brd), 1736, 1665 (vs), 1468, 1375, 1240 cm^{-1}; 270-MHz ^1H NMR ($CDCl_3$) (number of protons, multiplicity, *J* in Hz, assignment) δ 0.60 (3, s, H-18), 0.86 (6, d, *J* = 7 Hz, H-26, 27), 0.92 (3, d, *J* = 7 Hz, H-21), 2.07 (1, dd, *J* = 14.2, 5.2 Hz, 4α-H), 2.15 (3, s, OAc), 2.18 (1, dd, *J* = 15.0, 6.3 Hz, 12β-H), 2.36 (1, m, 14α-H), 3.40 (1, d, *J* = 4.7 Hz, 11β-H), 3.80 (1, dd, *J* = 12, 4.35 Hz, H-19 coupled also with OH), 6-H), (1, dd, *J* = 12, 4.35 Hz, H-19 coupled also with OH), 4.06 (1, m, 3α-H), 5.22 (1, dd, *J* = 3.5, 1.0 Hz, H-7), 5.32 (1, dd, *J* = 3.5, 0.8 Hz, 6α-H); mass spectrum (12 eV, low resolution), *m/e* (relative intensity) 490 (M^+ $C_{29}H_{46}O_6$, 3), 473 (5), 472 (17), 455 (2), 431 (15), 430 (42), 413 (34), 412 (100), 402 (10), 401 (18), 400 (20), 399 (16), 397 (10), 395 (14), 394 (35), 385 (12), 384 (21), 365 (24), 326 (21), 304 (63), 292 (29), 289 (24), 245 (43), 237 (46), 191 (22), 152 (21); high-resolution mass spectrum, observed *m/e* (composition, interpretation, calculated millimass) 472.316 24 ($C_{29}H_{44}O_5$, $M^+ - H_2O$, 472.318 87), 454.311 32 ($C_{29}H_{42}O_4$, $M^+ - 2H_2O$, 454.308 31), 430.305 07 ($C_{27}H_{42}O_4$, M^+ – AcOH, 430.308 31), 412.297 52 ($C_{27}H_{40}O_3$, $M^+ - H_2O$ – AcOH, 412.297 74), 400.293 95 ($C_{26}H_{40}O_3$, M^+ – AcOH – CH_2O, 400.297 74); 394.285 93 ($C_{27}H_{38}O_2$, M^+ – AcOH –$2H_2O$), 382.287 95 ($C_{26}H_{38}O_2$, M^+ – AcOH – H_2O – CH_2O, 382.287 18), 299.164 88 ($C_{19}H_{23}O_3$, M^+ – H_2O – AcOH – C_8H_{17}, 299.164 72), 281.154 33 ($C_{19}H_{21}O_2$, M^+ – $2H_2O$ – AcOH – C_8H_{17}, 281.154 15), 245.118 06 ($C_{15}H_{17}O_3$, M^+ – AcOH – CH_2O – $C_{11}H_{23}$, 245.117 77), 227.107 01 ($C_{15}H_{15}O_2$, M^+ – H_2O – AcOH – CH_2O – $C_{11}H_{23}$, 227.107 20), 155.085 75 ($C_{11}H_{23}$); 300-MHz ^1H NMR (pyridine-d_5) δ 0.82 (3, s, H-18), 0.87 (9, d, *J* = 7 Hz, H-21, -26, -27), 1.50 (m, H-20, H-25), 1.94 (1, d, *J* = 15.0 Hz, 12α-H), 2.01 (3, s, OAc), 2.29 (1, dd, *J* = 15.0, 4.7 Hz, 12β-H), 2.62 (1, m, H-14), 2.77 (1, dd, *J* = 14.2, 5.2 Hz, 4α-H), 3.92 (1, d, *J* = 4.7 Hz, 11β-H), 4.21 (1, dd, *J* = 12.6, 5 *Hz*, H-19 coupled also with OH), 4.50 (1, dd, *J* = 12.6, 5 Hz, H-19 coupled also with OH), 4.75 (1, m, 3α-H), 5.67 (1, br s, 6α-H), 5.82 (1, s, 5-OH), 5.86 (1, br s, H-7), 6.21 (1, d, 3-OH), 6.55 (1, t, 4, C-19-OH).

Acetylation of 1a. Sterol **1a** (1 mg) was reacted with 1 mL of acetic anhydride–pyridine (1:19) at room temperature overnight. Purification of the product by HPLC using silica gel with petroleum ether–chloroform (1:2) afforded **1b** which showed the following spectral properties: IR (neat) 3600 (w), 3500 (s), 1742, 1470, 1370, 1240 cm^{-1}; 100-MHz ^1H NMR ($CDCl_3$) δ 0.51 (3, s, H-18), 0.88 (6, d, *J* = 7 Hz, H-26, -27), 0.90 (3, d, H-21), 2.01, 2.08, 2.17 (3 each, s, OAc), 3.33 (1, d, 5.0, 11β-H), 4.10 (1, d, *J* = 11.5 Hz, H-19), 4.55 (1, d, *J* = 11.5 Hz, H-19), 5.16 (1, m, 3α-H), 5.31 (2, m, H-6, -7); mass spectrum (70 eV, low resolution), *m/e* (relative intensity) 514 (3% $M^+ - CH_3COOH$), 486 (0.9), 472 (0.7), 471 (1.2), 455 (1.3), 412 (2), 395 (13), 394 (32), 281 (5), 253 (8), 248 (16), 232 (21), 149 (19), 129 (11), 106 (46), 83 (100).

Hydrolysis of 1a. **1a** (1.5 mg) was heated with 5% KOH in 95% ethanol (5 mL) under reflux for 30 min, and the reaction mixture was cooled to room temperature, acidified, diluted with water, and then extracted with methylene chloride. The organic phase was dried over $MgSO_4$, the solvent evaporated in vacuo, and the residue purified by HPLC using a silica gel column and $CHCl_3$–MeOH (98:2) as eluant to yield pure **1c**: IR (neat) 3435 (sh), 3340, 1575, 1482, 1375 cm^{-1}; 270-MHz ^1H NMR (pyridine-d_5) δ 0.86 (6, d, *J* = 7 Hz, H-26, -27), 0.88 (3, s, H-18), 0.97 (3, d, *J* = 7 Hz, H-21), 3.04 (1, dd, *J* = 13.1, 2.7 Hz, 4α-H), 3.90 (1, d, *J* = 8.7 Hz, H-19), 4.16 (1, d, *J* = 8.7, H-19), 4.48 (2, m, H-11 and COH), 4.52 (1, m, 3α-H), 5.72 (1, s, H-7); low-resolution mass spectrum (70 eV), *m/e* (relative intensity) 448 ($C_{27}H_{44}O_5$, 2%), 431 (5), 430 (13), 415 (1), 413 (3), 412 (8), 401 (4), 383 (6), 317 (11), 305 (12), 299 (10), 430 (13), 227 (13), 213 (12), 211 (12), 209 (12), 205 (17), 193 (22), 182 (20), 167 (24), 163 (29), 161 (24), 159 (26), 149 (49), 147 (43), 137 (55), 121 (76), 109 (90), 95 (86), 81 (100).

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Registry No. **1a**, 84473-32-5; **1b**, 84473-33-6; **1c**, 84473-34-7.

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Total Synthesis of Ellipticine and 9-Methoxyellipticine via Benzotriazole Intermediates

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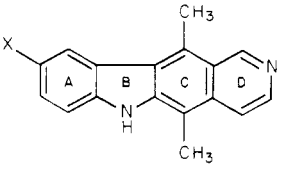
Ellipticine (**1**) and 9-methoxyellipticine (**2**), members of



1, X = H
2, X = OCH_3

the 6*H*-pyrido[4,3-*b*]carbazole class of alkaloids, have been shown to possess significant anticancer activity.[1] As a

