A New Epoxy Sterol from the Sponge *Ircinia fasciculata*†

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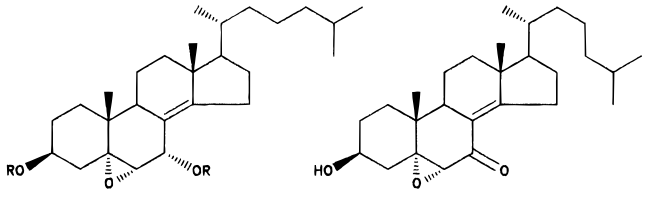
A new epoxy sterol (**1**) and four known steroids have been isolated from the sponge *Ircinia fasciculata* and characterized by spectral studies.

Marine organisms have been found to be storehouses of sterols, particularly in terms of unique side-chain structures and unusual functionalization.[1] In continuation of our search for biologically active secondary metabolites from marine organisms, we have investigated the sponge *Ircinia fasciculata* Pallas (Spongillidae), collected off the coast near Tuticorin, Tamilnadu, India, at a depth of 40 ft during April 1993. In our earlier communication, we reported the presence of three new heptaprenylhydroquinone derivatives from this species.[2] Further examination of the sterol-containing lipid fraction of this species afforded the new epoxy sterol **1** and four known sterols. Compound **1** was obtained as part of a mixture with other sterols and was purified as its acetate **1a** on a $AgNO_3$-impregnated Si gel column.

Compound **1a** was obtained as a solid, mp 138−140 °C, $[\alpha]_D$ −73.4° (*c* = 0.4, $CHCl_3$), and analyzed for $C_{31}H_{48}O_5$ by EIMS (*m*/*z* 440 [M^+ − AcOH]) and combustion analysis. It was transparent in the UV and showed absorption in its IR spectrum at 1735, 1601, and 1220 cm^{-1}. The ^{1}H NMR spectrum contained signals for five methyl groups at δ 0.92 (6H, s, H_3-18 and H_3-19), 0.94 (3H, d, J = 7 Hz, H_3-21), and 0.88 (6H, d, J = 7 Hz, H_3-26 and H_3-27) characteristic of a sterol, although the C-18 methyl generally resonates at higher fields. Further, the ^{1}H-NMR spectrum showed signals corresponding to two acetate methyls at δ 2.02 (3H, s) and 2.12 (3H, s), an acetoxy methine at δ 4.93 (1H, tt, J = 11, 3 Hz), an allylic acetoxy methine at δ 5.62 (1H, br s) and a trisubstituted epoxy methine at δ 3.22 (1H, d, J = 3.5 Hz). The ^{1}H-NMR decoupling experiment indicated that the epoxide proton was coupled with the allylic acetoxy methine at δ 5.63 (1H, br s). These findings were corroborated by ^{13}C-NMR signals at δ 170.01 (2c), 135.9, 129.8, 77.36, 77.01, 68.38, and 65.89. The signals at129.85 and 135.95 indicated the presence of a fully substituted double bond as there were no olefinic protons in the ^{1}H-NMR spectrum.

The foregoing spectral data and a literature survey[1] indicated that compound **1** was a obiquitously available 3β-sterol having both an acetoxy and an epoxy group and a fully substituted double bond. Mild alkaline hydrolysis of **1a** afforded compound **1**. The ^{1}H-NMR spectrum of the free sterol **1** revealed the presence of two secondary hydroxy groups at δ 3.95 (1H, br m) and 4.22 (1H, br s) and the trisubstituted epoxide proton at δ 3.08 (1H, d, J = 3.0 Hz). The signal at δ 3.95, higher than the normal chemical shift of 3β-hydroxy methine sterols, was consistent with the presence of a 5α,6α epoxy group. The position of the tetrasubstituted double bond was determined by comparing the ^{1}H-NMR chemical shifts of the H_3-18 and H_3-19 methyls with those of Δ^8 and $\Delta^{8(14)}$ sterols; the observed shifts were found to be in good agreement with those of $\Delta^{8(14)}$ sterols (Table 1). The foregoing spectral data led to two possible isomers 3β,7α(or 7β)-dihydroxy-5α,6α-epoxycholest-8(14)-ene. From molecular mechanics calculations, the coupling constant between 6−βH and 7−αH and between 6−βH and 7−βH were calculated as 4.33 Hz and 2.52 Hz, respectively, from energy-minimized molecular structures. The experimentally observed coupling constant (3.0 Hz) between 6H and 7H is close to the calculated value for 6−βH and 7−βH, indicating that 7-OH is in the α-configuration. Thus, the structure of compound **1** was established as 5α,6α-epoxy-3β,7α-dihydroxycholest-8(14)-ene. The structure was confirmed by converting compound **1** to the known ketosterol, 5α,6α-epoxy 3β-hydroxycholest-8(14)-en-7-one (**2**)[3] by selective oxidation of **1** using MnO_2. The UV, ^{1}H-NMR, and MS data of the synthetic compound were found to be identical with those of the reported ketone.



1 R=H
1a R=Ac

2

In addition to the sterol **1**, the known ketosterols (22*E*)3β-hydroxycholesta-5,8,22-trien-7-one, (22*E*,24ξ)-3β-hydroxy-24-methylcholesta-5,8,22-trien-7-one, and (22E,24ξ)3β-hydroxy 24-ethylcholesta-5,8,22-trien-7-one[6] were obtained as a mixture of sterols that could not be separated even after acetylation. The sterols were identified as their acetates by their UV and ^{1}H-NMR spectra in comparison with literature data.[6] The known sterol (22*Z*)-stigmasta-5,7,24(28)-trien-3β-ol was also isolated and characterized by spectroscopic data.[7]

Experimental Section

General Experimental Procedures. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (50 MHz) spectra were recorded on Varian 400 MHz and Varian Gemini 200 MHz spectrometers, respectively, using TMS as internal standard. Chemical shifts are reported in ppm and coupling constants (*J*) are expressed in Hz. Elemental analysis was carried out on a Perkin-Elmer 240c instrument. Optical rotations were measured with a JASCO DIP-370 polarimeter. UV and IR spectra were recorded on Shimadzu and Perkin-Elmer 1310 spectrophotometers, respectively. Mass spectra were recorded on a Finni-

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Table 1. Comparison of Selected ^{1}H-NMR Chemical Shifts of Compound **1a** with Δ^8 and $\Delta^{8(14)}$ sterols

proton position	compd **1a**	5,6α-epoxy-3b-hydroxycholest-8(14)-en-7-one[a]	5,6α-epoxy-3β,7α-diacetoxycholestan-8-one[b]	5,6β-epoxy-3β,7α,11α-triacetoxycholest-8-ene[c]
Me-18	0.92 0.79[d]	0.92 0.79[d]	0.58	0.52
Me-19	0.92 0.61[d]	0.92 0.68[d]	1.17	1.13
3-H	4.93 (tt, J = 11,3 Hz) 5.2 (m)[d]	3.9 (m) 3.28 (m)[d]	4.95 (m)	4.89 (tt, J = 5, 11 Hz)
6-H	3.22 (d, J = 3.5 Hz) 3.28 (d, J = 3 Hz)[d]	3.16 (br s)	3.37 (d, J = 2.5 Hz)	3.31 (d, J = 2.5 Hz)
7-H	5.63 (br s) 5.72 (br s)[d]		5.53 (br s)	5.53 (br s)

[a] Data are from Aiello *et al.*[3] [b] Data are from Kobayashi and Kanada.[4] [c] Data are from Isaacs *et al.*[5] [d] Chemical shifts are recorded in C_6D_6 solvent.

gan-MAT 1020 instrument. The molecular mechanics calculations were done using PCMODEL (Version 2.0), Serena software, Bloomington, IN 47402-3076. The coupling constants were calculated by substituting bond angles (for 6α and 7βH ϕ = 61.80° and 6α and 7α-H, ϕ = 65.11°) obtained from energy-minimized structures in Karplus's equation.[8]

Animal Material. The sponge *Ircinia fasciculata* was collected by scuba at a depth of 40 ft from the Tuticorin coast [N 8°48′, E 78°10′] in the Gulf of Mannar, Tamilnadu, India, during April 1993. A voucher specimen (IIC-124) is on deposit at the NIO Museum, Goa, India.

Extraction and Isolation. The freshly collected specimens were cut into thin slices and soaked in MeOH at the site collection until workup. After the removal of MeOH, the sponge (500 g dry wt) was freeze dried and extracted with MeOH−CH_2Cl_2 (1:1) (2 L). After evaporation of solvent under reduced pressure, the crude extract was partitioned between EtOAc and H_2O. Concentration of the organic layer resulted in a gummy crude extract (16 g), which was subjected to Si gel (100−200 mesh) column chromatography, eluting with hexane, hexane−EtOAc mixtures and C_6H_6−Me_2CO mixtures to Me_2CO. The fraction eluting with 10% EtOAc in hexane resulted a compound that on further purification on Si gel column chromatography eluted with hexane−EtOAc mixtures and yielded (22Z)-stigmasta-5,7,24(28)-trien-3β-ol (30 mg), and the fraction eluting with 40% EtOAc in hexane resulted amixture of polar sterols, which was acetylated with Ac_2O−pyridine at room temperature for 24 h and then chromatographed on a $AgNO_3$ impregnated Si gel column eluted with hexane−EtOAc mixtures to give a mixture of ketosterols (40 mg) and compound **1a** (15 mg).

Compound 1a: obtained as a colorless crystalline solid, mp 138−40 °C; $[\alpha]_D$ −73.4 (*c* 0.4, $CHCl_3$). IR λ_{max} (KBr) 1735, 1602, 1220 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 5.63 (1H, br s), 4.93 (1H, tt, J = 11, 3 Hz), 3.22 (1H, d, J = 3.5 Hz), 2.12 (3H, s), 2.02 (3H, s), 0.94 (3H, d, J = 7 Hz, H_3-21), 0.92 (6H, s, H_3-18 and H_3-19), and 0.88 (6H, d, J = 7 Hz, H_3-26 and H_3-27); ^{1}H NMR (C_6D_6, 400 MHz), δ 5.72 (1H, br s), 5.2 (1H, m), 3.28 (1H, d, J = 3 Hz), 1.76 (3H, s), 1.70 (3H, s), 0.94 (6H, d, J = 7 Hz), 0.79 (3H, s) and 0.61 (3H, s); ^{13}C NMR ($CDCl_3$, 50 MHz), δ 170.01 (2c), 135.5, 129.8, 77.36, 71.01, 68.38, 65.89, 58.04, 56.63, 56.45, 39.51 (2c), 36.31, 35.95, 35.67 (2c), 31.84 (2c), 27.99, 27.28, 26.63, 25.23, 22.79, 22.53, 21.25, 20.99, 19.01 (2c), 18.16, 16.42; EIMS(70 ev), *m*/*z* 440 (M^+ − AcOH, 2%), 380 (M^+ − 2AcOH, 60), 365 (35), 267 (40), 249 (35), 213 (35). Anal. Calcd for $C_{31}H_{48}O_5$: C, 74.36; H, 9.66. Found: C, 74.26; H, 9.78.

Hydrolysis of 1a to obtain 1. A solution of **1a** (3 mg) in 5% ethanolic KOH (0.5 mL) was heated in a H_2O bath for 5 min. After acidification and removal of the EtOH *in vacuo*, the solution was extracted with EtOAc to yield compound **1**.^{1}H NMR ($CDCl_3$, 200 MHz), δ 4.36 (1H, br s, 7-H), 3.91 (1H, m, 3-H), 3.05 (1H, d, J = 3 Hz, H-6), 0.94 (3H, d, J = 7 Hz, Me-21), 0.88 (6H, d, J = 6 Hz), Me-26 and Me-27) and 0.87 (6H, s, Me-18 and Me-19).

Oxidation of 1 to 5α,6α-epoxy cholest 8(14) en-7 one (2). A solution of compound **1** (2 mg) in *n*-hexane (5 mL) was treatd with MnO_2 (5 mg) and stirred for 12 h. After filtration of the reaction mixture through Celite, the hexane was removed *in vacuo*, and the residue was chromatographed over Si gel to yield 5α,6α-Epoxy-3β-hydroxycholest-8(14)-en-7-one (**2**). The UV, ^{1}H-NMR and MS data of the synthetic ketosterol were found to be identical with those reported.[3]

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