Marine Sterols. XXI.[1] Isolation of (24*S*)-3β-Hydroxyergost-5-en-21-oic Acid from a *Sclerophytum* sp. of Soft Coral

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The lipid extract of the *Sclerophytum* sp. of soft coral, collected off the coast of the Andaman and Nicobar Islands, afforded a new sterol 1a. The structure of 1a was shown to be (24*S*)-3β-hydroxyergost-5-en-21-oic acid, the first member of a class of marine sterols having a C-21 carboxylic acid, by spectral analyses and conversion to (24*S*)-ergostane.

Keywords Coelenterate; soft coral; *Sclerophytum* sp.; (20*R*,24*S*)-3β-hydroxyergost-5-en-21-oic acid

In the preceding paper, we described the structures of andamansterol and nicobarsterol obtained from a *Sclerophytum* sp. soft coral (Coelenterate).[1] Both compounds are oxygenated at C-21, which is rare in the marine sterols except for those found in brittle stars (Echinoderms). Examination of another *Sclerophytum* sp. soft coral, collected off the coast of Neil Island, Andaman and Nicobar Seas, resulted in the isolation of a new steroid **1a**, which is the first example to have a carboxylated C-21, together with a known compound, 3β,4α-dihydroxypregn-20-ene 4-*O*-β-D-arabinopyranoside **4**.[2]

Compound **1a**, $C_{28}H_{46}O_3$, is a monohydroxy C_{28} sterol and showed proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra (in $CDCl_3$) similar to those reported for (24*R*)- or (24*S*)-3β-hydroxyergost-5-enes (Δ^5-ergostenols).[3,4] In the mass spectrum (MS), three distinct fragment ions, which are known to be characteristic of Δ^5-sterols with a saturated side chain,[5] appeared at *m/z* 345 (M^+-H_2O and C_5H_7), *m/z* 319 (M^+-H_2O and C_7H_9), and at *m/z* 291 (M^+-H_2O and C_9H_{13}). The difference between **1a** and Δ^5-ergostenols was that one of the four secondary methyl signals of Δ^5-ergostenols was replaced by that of a carboxyl group (^{13}C-NMR, δ 178.5) in **1a**. The ^{1}H- and ^{13}C-NMR chemical shifts of the three secondary methyl signals corresponded to those of the C-26, 27, 28 of Δ^5-ergostenols,[3,4] but those due to C-17 (^{13}C-NMR, δ 53.2) and C-22 (δ 32.3) were shifted *ca.* 3 and 1.5 ppm upfield, respectively. The signal of 18-Me (^{1}H-NMR, δ 0.75) was shifted *ca.* 0.07 ppm down-field. These facts indicated that **1a** is a derivative of Δ^5-ergostenol whose C-21 has been converted to carboxylic acid. This simple structure, however, has not previously been found in natural steroids from marine or terrestrial sources. The occurrence of such steroidal carboxylic acid derivatives, oxygenated at various sites, could be expected in soft corals and gorgonians (another class of coelenterates known to produce polyhydroxysterols). If the C-21 oxidation process involves an intermediate having a Δ^{20} double bond, the C-20 of **1a** could take both the biogenetically conventional (20*R*)-configuration and its diastereoisomer. In the two precedent examples,[1] the C-21 hydroxy derivatives andamansterol and nicobarsterol, the C-20 configuration was established as (20*R*), the biogenetically normal one, by X-ray crystallography and chemical conversion. Although the biogenetic analogy suggested the same C-20 configuration for **1a**, this should be proved rigorously by correlation to known compounds. The configuration at C-24 was also uncertain, though in our experience the C_{28} sterols isolated from soft corals have invariably been derivatives of (24*S*)-ergostane and its Δ^{22} compound.[6]

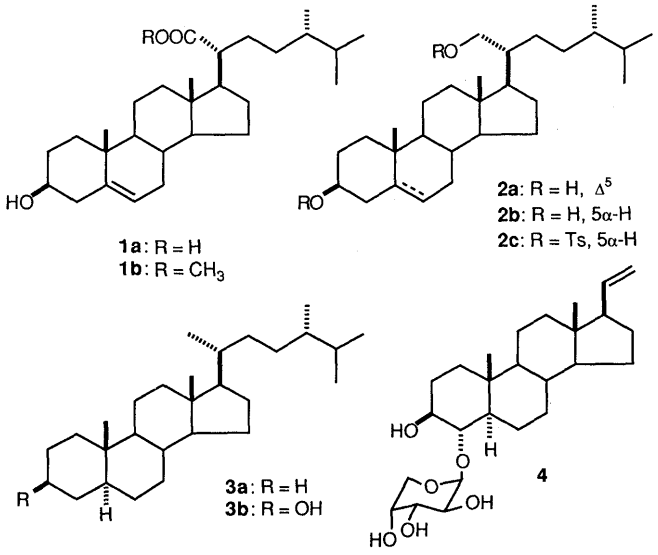

Chart 1

The carboxylic acid **1a** was converted to the methyl ester **1b**. This was converted to the diol **2a** by lithium aluminium hydride (LAH) reduction. The ^{13}C-NMR spectrum of **2a** showed the signals due to C-17, C-20, C-21 and C-22 at δ 50.2, 42.9, 63.0 and 27.2, respectively. The deviations of the chemical shifts between **2a** and Δ^5-ergosterols[4] at C-17, C-20, C-21, and C-22 were −6.0, +7.3, +44.3, −6.5 ppm, respectively. Such differences are in accord with the well-known α- (on C-21), β- (on C-20), and γ-hydroxy substituent effects (on C-17 and C-22). The C-21 hydroxyl group was reductively cleaved through the 3,21-di-*p*-toluenesulfonate (tosylate). The displacement of the tosylate group in this homoallylic alcohol system (C-3 to C-6) is known to follow a 3,5-cyclocation route and gives a 3,5-cyclo derivative.[7] For this reason, the alcohol **2a** was converted to the stanol **2b** by catalytic hydrogenation. The ^{1}H- and ^{13}C-NMR spectra of **2b** were identical with those of cholestanol,[8] as regards the steroid nucleus. The LAH treatment of the ditosylate **2c** gave three products, the hydrocarbon **3a**, 3β-hydroxyergostane **3b**, and the stanol **2b**, the latter two compounds being formed by cleavage of the S–O bond of the 3-or 3,21-ditosyl group. The C-21 methyl group of the unnatural (20*S*)-

TABLE I. ^{1}H-NMR Data (δ) for **3a** and **3b** and the Reported Values[3] of (24*R*)- and (24*S*)-Ergostanes (400 MHz, in $CDCl_3$)

Compound	18-H_3	21-H_3	26-H_3	27-H_3	28-H_3
3a	0.641	0.902	0.851	0.779	0.772
3b	0.646	0.902	0.852	0.780	0.771
(24*R*)-Ergostane	0.644	0.891	0.848	0.801	0.768
(24*S*)-Ergostane	0.641	0.900	0.850	0.777	0.770

sterols, unlike that of **3a** (Table I), is shifted 0.1 ppm upfield in the ^{1}H-NMR spectrum relative to the conventional (20*R*)-counterpart.[9] The chemical shifts of the side chain signals of **3a**, especially 27-H_3, were identical with those of (24*S*)-ergostane and different from those of the (24*R*) diastereomer, thus establishing compound **1a** to be (20*R*,24*S*)-3β-hydroxyergost-5-ene-21-oic acid.

Experimental

Melting points were determined on a Kofler hot stage and are uncorrected. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. NMR spectra were determined on a JEOL JNM GX-400 spectrometer at 400 MHz (^{1}H) and on a JEOL JNM FX-90Q spectrometer at 22.5 MHz (^{13}C) with tetramethylsilane (δ 0.00), $CDCl_3$ (center peak δ 77.1), and pyridine-d_5 (center peak δ 135.5) as internal standards. MS were determined on a JEOL JMS D 300 mass spectrometer. Flash column chromatography[10] was performed on silica gel (Wako gel C-300, 200—300 mesh, Wako Pure Chemical Industries).

Material The soft coral, code name MF-VA-02 (1.5 kg after dehydration), was collected in March 1989 on the coasts of the Andaman and Nicobar Islands (Neil Island, 93°43′E, 11°41′N). The organism was washed with fresh water, cut into thin slices and preserved in EtOH. The extraction was carried out using EtOH by percolation every 4 d. The process was repeated 7 times. The solvent was evaporated off by distillation under reduced pressure, and the dark-colored residue was extracted with ethyl acetate several times. The ethyl acetate-soluble portion was passed over anhydrous $MgSO_4$. The extract (30 g) was chromatographed over silica gel (500 g, Acme 100—200 mesh) using solvent mixtures of petroleum ether–ethyl acetate with increasing polarities. The two fractions eluted with ethyl acetate–petroleum ether (1:4 and 2:3), on repeated chromatography with the same solvent mixtures followed by recrystallization from MeOH–$CHCl_3$, gave **1a** (120 mg) and **4** (30 mg), respectively.

(24*S*)-3β-Hydroxyergost-5-en-21-oic Acid (1a) Needles, mp 269—271 °C; $[\alpha]_D^{21}$ −30° (c=0.30, pyridine). ^{1}H-NMR (pyridine-d_5) δ: 0.78 (3H, d, J=7.0 Hz), 0.83 (3H, d, J=6.5 Hz), 0.84 (3H, d, J=7.0 Hz), 0.96, 1.04 (each 3H, s), 3.84 (1H, br, $W_{1/2}$=20 Hz, 3α-H), 5.43 (1H, m, 6-H); ($CDCl_3$) δ: 0.75 (3H, s), 0.76 (3H, d, J=7.0 Hz), 0.78 (3H, d, J=7.0 Hz), 0.83 (3H, d, J=7.0 Hz), 0.99 (3H, s), 3.51 (1H, m, $W_{1/2}$=20 Hz, 3α-H), 5.34 (1H, m, 6-H). ^{13}C-NMR (pyridine-d_5) δ: C-1, 12 (37.8, 38.1), C-2, 7, 22 (32.3, 32.6, 32.7), C-3 (71.3), C-4 (43.5), C-5 (142.0), C-6 (121.1), C-8 (32.4), C-9 (50.7), C-10 (36.9), C-11 (21.3), C-13 (42.5), C-14 (56.6), C-15 (24.1), C-16 (27.6), C-17 (53.2), C-18 (12.2), C-19 (19.6), C-20 (48.9), C-21 (178.5), C-23 (30.6), C-24 (39.0), C-25 (31.6), C-26, 27 (17.5, 20.7), C-28 (15.5). MS m/z: 430 (M^+), 412, 397, 384, 370, 345, 319, 291, 273, 271, 255, 239, 213, 161; High-resolution MS: 430.3455. Calcd for $C_{28}H_{46}O_3$: 430.3447.

(24*S*)-3β-Hydroxyergost-5-en-21-oic Acid Methyl Ester (1b) Compound **1a** (30 mg) was dissolved in Et_2O and treated with ethereal diazomethane solution until N_2 formation ceased. The excess reagent was decomposed with AcOH. The evaporation residue was recrystallized from MeOH to give **1b** (29.6 mg). Needles, mp 115—116 °C, $[\alpha]_D^{23}$ −33° (c=1.48, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.70 (3H, s, H-18), 0.75, 0.76, 0.84 (each 3H, d, J=7.0 Hz), 1.02 (3H, s, 19-H_3), 3.53 (1H, m, $W_{1/2}$=20 Hz, 3α-H), 3.65 (3H, s, OMe), 5.34 (1H, m, 6-H). MS m/z: 444 (M^+), 429, 426, 411, 384, 359, 333, 305, 273, 255, 239, 213. High-resolution MS: 444.3609. Calcd for $C_{29}H_{48}O_3$: 444.3604.

$LiAlH_4$ Reduction of 1b Compound **1b** (28 mg) was dissolved in Et_2O (1 ml) and the solution was stirred with 10 mg of LAH at room temperature for 30 min. The mixture was diluted with moist Et_2O and washed with 5% HCl, water, and saturated brine, then the solvent was evaporated off, giving nearly pure **2a** (27.4 mg). Needles from MeOH, mp 145—147 °C, $[\alpha]_D^{23}$ −35° (c=1.37, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.70 (3H, s), 0.79, 0.80, 0.86 (each 3H, d, J=7.0 Hz), 1.01 (3H, s), 3.52 (1H, m, $W_{1/2}$=15 Hz, 3α-H), 3.66 (1H, dd, J=11.0, 3.0 Hz), 3.72 (1H, dd, J=11.0, 4.5 Hz), 5.35 (1H, m, 6-H). ^{1}H-NMR (pyridine-d_5) δ: 0.78 (3H, s), 0.83, 0.88, 0.89 (each 3H, d, J=7.0 Hz), 1.07 (3H, s), 3.83 (1H, m, $W_{1/2}$=20 Hz, 3α-H), 3.89 (1H, dd, J=10.5, 3.5 Hz), 4.07 (1H, dd, J=10.0, 5.5 Hz). ^{13}C-NMR (pyridine-d_5) δ: C-1 (37.3), C-2, 7 (31.7, 32.0), C-3 (71.8), C-4 (42.2), C-5 (140.9), C-6 (121.6), C-8 (32.0), C-9 (50.6), C-10 (36.6), C-11 (21.1), C-12 (39.2), C-13 (42.4), C-14 (56.7), C-15 (24.2), C-16 (27.6), C-17 (50.2), C-18 (12.2), C-19 (19.5), C-20 (42.9), C-21 (63.0), C-22 (27.2), C-23 (31.0), C-24 (39.2), C-25 (31.7), C-26, 27 (17.8, 20.5), C-28 (15.5). MS m/z: 416 (M^+), 401, 398, 383, 365, 331, 305, 277, 273, 271, 255, 231, 213; High-resolution MS: 416.3657. Calcd for $C_{28}H_{48}O_2$: 416.3655.

Catalytic Hydrogenation of 2a A solution of **2a** (24 mg) in ethyl acetate–AcOH (2:1, 1 ml) was hydrogenated with 10 mg of PtO_2 for 2 h, and the mixture was filtered. Evaporation of the solvent gave **2b** (24 mg). Needles from MeOH, mp 163—165 °C, $[\alpha]_D^{21}$ +10° (c=1.20, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.67, 0.80 (each 3H, s), 0.78, 0.79, 0.85 (each 3H, d, J=7.0 Hz), 3.58 (1H, m, $W_{1/2}$=15 Hz, 3α-H), 3.64 (1H, dd, J=11.0, 4.5 Hz, 21-H), 3.71 (1H, dd, J=11.0, 2.5 Hz, 21-H). ^{13}C-NMR (pyridine-d_5) δ: C-1 (37.6), C-2 (32.5), C-3 (70.7), C-4 (39.3), C-5 (45.3), C-6 (29.3), C-7 (32.5), C-8 (35.9), C-9 (54.8), C-10 (35.9), C-11 (21.6), C-12 (39.8), C-13 (42.9), C-14 (56.8), C-15 (24.6), C-16 (27.9), C-17 (51.4), C-18, 19 (12.6, 12.7), C-20 (43.7), C-21 (62.6), C-22 (27.9), C-23 (31.3), C-24 (39.7), C-25 (32.0), C-26, 27 (17.9, 20.8), C-28 (15.7). MS m/z: 418 (M^+), 403, 400, 385, 248, 233, 215; High-resolution MS: 418.3797. Calcd for $C_{28}H_{50}O_2$: 418.3810.

$LiAlH_4$ Treatment of the 3,21-Di-*O*-*p*-toluenesulfonate of 2b The diol **2b** (2.0 mg) was converted to the di-*O*-*p*-toluenesulfonate quantitatively under usual conditions (tosylchloride–pyridine). It was dissolved in Et_2O (0.3 ml) and stirred with 10 mg of LAH at room temperature for 4 h. The mixture was diluted with moist Et_2O and washed with 5% HCl, water, and saturated brine. Column chromatography of the mixture with hexane gave **3a** (0.15 mg). Further elution with $CHCl_3$ gave **3b** (0.50 mg) and **2b** (0.30 mg).

3a: Plates from MeOH, mp 83—84 °C (lit.,[11] 85 °C). MS m/z: 386 (M^+), 371, 218, 217, 149; ^{1}H-NMR see Table I.

3b: Plates from MeOH, mp 140—141 °C (lit.,[12] 144—145 °C). MS m/z: 402 (M^+), 387, 369, 234, 233, 215; ^{1}H-NMR, see Table I.

3β,4α-Dihydroxypregn-20-ene 4-*O*-β-D-Arabinopyranoside (4) mp 282—283 °C (lit.,[2] 279 °C), $[\alpha]_D^{21}$ −70° (lit.,[2] −92°) (c=0.20, pyridine). The identification was made by comparison of the ^{1}H- and ^{13}C-NMR data (pyridine-d_5) with those reported in the literature.[2]

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