MARINE STEROLS. XIII.* ISOLATION AND SYNTHESIS OF 1β,3β,5,6β-TETRAHYDROXY-5α-ANDROSTAN-17-ONE FROM THE SOFT CORAL SARCOPHYTON GLAUCUM

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ABSTRACT

A new polyhydroxysterol 1β,3β,5,6β-tetrahydroxy-5α-androstan-17-one (1) was isolated from the soft coral Sarcophyton glaucum. The structure of 1 was deduced from comparison of the spectral data with those of known 1β,3β,5α,6β-tetrahydroxysterols and confirmed by the synthesis starting from 1β,3β-dihydroxy-5,16-pregnadien-20-one (6a)

INDTRODUCTION

The soft coral Sarcophyton glaucum is one of the common species found in Indian and Pacific coastal waters. A variety of monohydroxysterols (1) and polyhydroxysterols (2) have been characterized from the lipid extract of S. glaucum collected from Ishigaki Island in southern Japan. Further examinations of the polyhydroxysterol fractions showed that still more unidentified compounds were present in small amounts. In the present study we report on the isolation, structure and synthesis of a novel steroid 1β,3β,5,6β-tetrahydroxy-5α-androstan-17-one (1). To our knowledge, this is the first report on the isolation of a polyoxygenated androstane derivative from marine invertebrates.

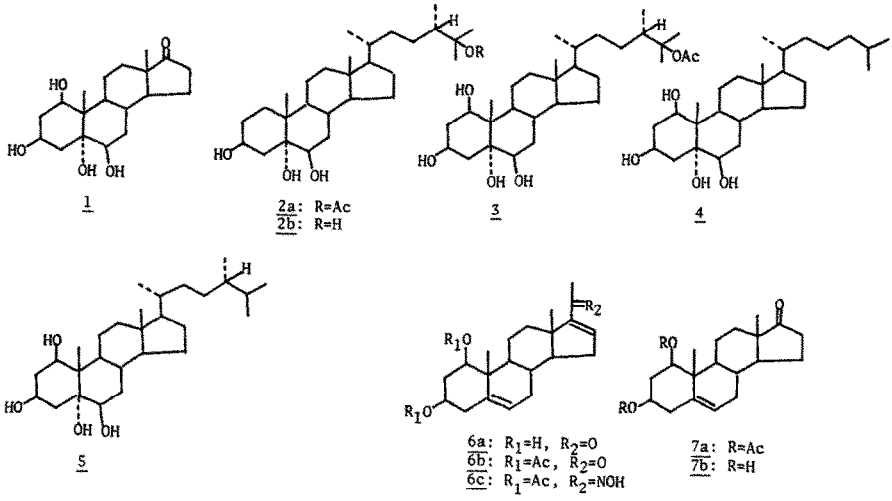
RESULTS AND DISCUSSION

All polyhydroxysterols known to occur in soft corals are cholestane and 24-methylcholestane derivatives. From S. glaucum we have hitherto characterized five polyhydroxysterols (2 to 5) (2,4). The unknown component 1 was found from the more polar fraction than those of 2 to 5 suggesting a further oxygenated structure.

Repetitive flash chromatography (3) of the polyhydroxysterol fraction from the crude lipid extract (840 g) of S. glaucum gave 95 mg of compound 1, mp 249-251°, $[\alpha]_D$ +37.2°. The elemental analysis and mass spectrum showed an unusually low molecular formula $C_{19}H_{30}O_5$. It showed a molecular ion (M^+) at m/z 338 and dehydration ions at m/z 320, 302 and 284. The ^{1}H-NMR spectrum (in pyridine-d_5) showed signals of methyl groups only for C-18 (δ 0.878) and C-19 (1.874). These and other signals were strikingly similar to those of compounds 3 to 5 and suggested the presence of the 1β,3β,5α,6β-tetrahydroxy A/B ring system. Three hydroxy-methine signals were observed at δ 4.18 (1H, broad s, 6-H), 4.88 (1H, dd, J=5, 11Hz, 1α-H), and at 4.85-5.1 (1H, m, 3α-H). The 4β-axial proton signal occurred at δ 3.06 as a triplet (J=12 Hz). The proton signal was observed at δ 2.95 in the major compound 2a (4) and at 3.05-3.08 in the 1β-hydroxyderivatives 3 to 5 (2). The signals of the 1α- and 3α-methines, 4β-proton, and 19-methyl occurred, as in 3 to 5, at considerably deshielded positions due to the 1,3-diaxial interaction with the hydroxyl group. These signals were further influenced by pyridine-induced deshielding (5).

The ^{13}C-NMR chemical shifts (Experimental) of the carbons 1 to 11 and 19 agreed with those of 3 (2a) while the chemical shifts of the carbons 12 to 18 were virtually the same as those of 5α-androstan-17-one (6). The largest differences from the reference compounds were 0.9 ppm (C-11) and 1.2 ppm (C-12) respectively. The IR spectrum of 1 showed an absorption band of the hydrogen-bonded five-membered ring ketone at 1720 cm^{-1}. Thus, the new C_{19} polyhydroxysterol was assigned the structure, 1β,3β,5,6β-tetrahydroxy-5α-androstan-17-one (1).

The structure of 1 was confirmed by synthesis. In a previous



study, we prepared 1β,3β-dihydroxy-5,16-pregnadien-20-one (6a) for the synthesis of 4, by oxidative degradation of the sapogenin ruscogenin ((25R)-spirost-5-ene-1β,3β-diol) (2b). The synthesis of 1 was carried out using 6a as the starting material. The diacetate 6b was converted into the oxime 6c. Beckmann rearrangement of 6c with p-toluenesulfonyl chloride follwed by the treatment with hydrochloric acid gave 1β,3β-dihydroxy-5-androsten-17-one diacetate (7a). Hydrolysis of 7a followed by 5α,6β-trans-glycolation (7) with hydrogen peroxide and formic acid and a further hydrolysis gave 1β,3β,5,6β-tetrahydroxy-5α-androstan-17-one (1) which was identical in all respects with the compound isolated from S. glaucum. Although the polyhydroxy derivatives of cholestane and 24-methylcholestane, and also pregnane in a few cases, have been found in a variety of marine invertebrates (8), this is, to our knowledge, the first report on the isolation of a polyhydroxyandrostane derivative.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded in pyridine-d_5 or $CDCl_3$ soln. on a JEOL FX 100 or FX 200 spectrometers at 100 or 200 (^{1}H-NMR) and 40.0 (^{13}C-NMR) MHz with TMS as internal standard. Mass spectra were determined on a JEOL JMS D300 spectrometer. IR spectra were taken on a Hitachi 215 spectrometer in Nujol mull. Optical rotations were determined on a JASCO DIP-4 digital polarimeter.

Isolation of 1. The lipid extract (840 g) of S. glaucum which was obtained in a previous study (1c) was partitioned with a mixture of solvents, hexane-methanol-H_2O (20:10:2), and separated into upper(690g) and lower (151 g) extracts. Monohydroxysterols and other non-polar compounds were extracted in the upper layer while the lower layer contained polyhydroxysterols and other polar compounds. The polar lipid fraction was chromatographed over a column of silica gel (1.5 kg) with a mixture of benzene-$CHCl_3$ (1:1, 40 l), $CHCl_3$ (50 l), and a gradient solvent mixture (0 to 20% methanol in $CHCl_3$, 110 l). The fraction (13.35g) which contained 1 and 3 to 5, was eluted with 18 to 20% methanol in $CHCl_3$. Flash chromatography of the mixture over a column of silica gel with 10% methanol in $CHCl_3$ gave 11.5 g of a mixture of 3 to 5, and 0.25 g of a mixture which contained 1. The mixture containing 1 was again chromatographed in the same way on a column of silica gel and gave 95 mg of 1, mp 249-251° (from acetone-hexane). For $[\alpha]_D$ and IR, mass and ^{1}H-NMR spectra, see Text. ^{13}C-NMR (pyridine-d_5):δ 73.4 (C-1), 43.9(C-2), 65.1 (C-3), 43.0(C-4), 76.8(C-5), 76.4(C-6), 35.8(C-7), 31.4(C-8), 47.2(C-9), 44.9(C-10), 24.0(C-11), 32.9(C-12), 47.6(C-13), 51.6(C-14), 22.3(C-15), 34.3(C-16), 219.9(C-17) 13.9(C-18), 10.6(C-19). Anal. Calcd. for $C_{19}H_{30}O_5 \cdot 1/4H_2O$: C, 66.54: H, 8.89. Found: C, 66.79; H, 9.15.

1β,3β-Dihydroxy-5.16-pregnadien-20-one 1,3-Diacetate Oxime (6c) 1β,3β-Dihydroxy-5,16-pregnadien-20-one (6a, mp 237-238°, 320 mg) (2b) was acetylated in a usual manner with acetic anhydride in pyridine. The oily diacetate 6b was dissolved in pyridine (4 ml). The solution was mixed with 200 mg of $HONH_2 \cdot HCl$ and refluxed for 20 min. The solution was poured into H_2O and the mixture was extracted with ether. After the usual work-up, the solvent was evaporated and the residue was recrystallized from methanol to give 6c (400 mg), mp 193-197°, $[\alpha]_D$+7° (c=1.63, $CHCl_3$). IR: 3400 cm^{-1}. Anal. Calcd. for $C_{25}H_{37}O_5N$: C, 69.90 H, 8.21; N, 3.26. Found: C, 69.78; H, 8.49; N, 3.14.

1β,3β-Dihydroxy-5-androsten-17-one (7b) The oxime (6c, 350 mg) was dissolved in pyridine (3 ml) and treated with a solution of p-toluenesulfonyl chloride (380 mg) in pyridine (1 ml) at 0° for 5 min. and then at room temperature overnight. The mixture was poured into H_2O and extracted with ether. The ether extract was worked up as usual and the solvent was evaporated. The residue was dissolved in 2 ml of methanol and treated with a mixture of H_2O and conc. HCl (1:1, 2 ml) at 0° for 3 min. The mixture was poured into H_2O and extracted with ether. The ether extract was worked up as usual and the solvent was evaporated. Hydrolysis of the residue (2.5% KOH in methanol, reflux 15 min) and crystallization from methanol gave 7b (210 mg), mp 175-180°

$[\alpha]_D$ -7° (c=0.52, $CHCl_3$). IR: 3100-3600, 1720 cm^{-1}. ^{1}H-NMR (100 MHz, in $CDCl_3$):δ 0.894 (18-Me), 1.058 (19-Me), 3.44 (1H, dd, J=5,11 Hz, 1α-H), 3.3-3.7 (1H, m, 3α-H), 5.57 (1H, m, 6-H). Mass spectrum: m/z 304 (M^+), 286 (base peak, M^+-H_2O), 268 (M^+-2H_2O), 253 (M^+-2H_2O,-Me). Anal. Calcd. for $C_{19}H_{28}O_3 \cdot 1/4\ H_2O$: C, 73.86; H, 9.30. Found: C, 73.94 H, 9.16.

1β,3β,5,6β-Tetrahydroxy-5α-androstan-17-one (1) Compound 7b (150 mg) in THF (3 ml) was cooled in an ice bath. A mixture of 88% formic acid (2 ml) and 30% H_2O_2 (0.14 ml) was added dropwise with stirring. The mixture was left at room temperature for 2 days. Excess H_2O_2 was decomposed with $NaHSO_3$ (300 mg) in H_2O (1.5 ml) and the mixture was evaporated at low temperature to dryness. A solution of 2.5% KOH in methanol (5 ml) was added and the mixture was refluxed for 2 hrs. The product (1) was readily soluble in H_2O. The mixture was evaporated at low temperature to dryness and the residue was subjected to flash chromatography over silica gel with 18% methanol in $CHCl_3$ and gave 99.7 mg of 1, mp and mixed mp with the compound 1 from S. glaucum 249-253°, $[\alpha]_D$+37° (c=0.78, methanol). The IR, ^{1}H-NMR, and mass spectra were completely identical to those of natural 1. Anal. Calcd. for $C_{19}H_{30}O_5 \cdot 1/4H_2O$: C, 66.54; H, 8.89. Found: C,66.53 H, 9.08.

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