A NEW STEROL FROM AN ALCYONARIAN

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ABSTRACT

In addition to cholesterol, 24α-methylcholesterol and gorgosterol, a new C_{28}-sterol, 25-hydroxy-24ξ-methylcholesterol, has been isolated from a soft coral. The structure elucidation was achieved by comparative physical methods, nuclear magnetic resonance and mass spectrometry.

INTRODUCTION

Due to the growing interest in recent years in biosynthetically interesting marine sterols [3-7] and the intensive studies [8] of the biogenesis of substituents in the sterol side chain we continued our search for new marine sterols. We now report the isolation from a "soft coral" (Alcyonarian) of a new sterol, which was assigned structure I on the basis of chemical and spectroscopic evidence. While terpenoids from sea cucumbers [9] have been encountered with oxygen functions at C-25, 25-hydroxy-24ξ-methylcholesterol (I) appears to be the first C-25 oxygenated sterol isolated from marine sources. While 25-hydroxylated compounds are quite rare amongst sterols, the parent substance, 25-hydroxycholesterol [10] has been isolated from human aorta, some have been synthesized [11-12] and their biological role, especially in the vitamin D field, emphasized [12].

EXPERIMENTAL

Melting points were measured on a Kofler hot-stage microscope and are uncorrected. Infra-red spectra were obtained using a Perkin Elmer Infracord spectrometer. All rotations were determined using chloroform as solvent. Preparative GC was carried out on a 1.5 m x 9 mm glass column packed with 3% OV-25 with a Newlett-Packard 402 gas chromatograph with injectore temperature 310°, detector 300° and column 260°.

Nuclear magnetic resonance (nmr) spectra were recorded on a Varian T-60 or HA-100 spectrometer. For all cases deuterio chloroform was employed as solvent and chemical shifts were expressed in cps and referred to tetramethylsilane (0.00 cps). Microanalyses were carried out by Messrs. E. Meier and J. Consul. Mass spectra (70 eV) were obtained with an AEI MS-9 or Atlas CH-4 spectrometer, both equipped with a direct inlet system. Column chromatography was performed using Davison 60-200 mesh silica. Preparative scale thin layer chromatography was carried out on 20 x 20 cm, 750μ, silica gel HF-254 plates.

Isolation of sterols. The sun-dried Alcyonarian (3 kg) (probably Nephtea sp.) collected in February 1970 in 15 feet of water, 3 miles south of Telukdalam, Nias Island, Indonesia, was extracted with cold 95% ethanol until a colorless extract was obtained. The resultant extract was reduced to a dry residue which was dissolved in benzene and extracted with water. Concentration of the benzene extract afforded a brown oil (26 g), a portion (2 g) of which was chromatographed on silica (100 g) with various solvents. The solvent system chloroform: methanol (50:1) yielded a crude sterol mixture (200 mg) that was purified on preparative silica gel plates using chloroform as solvent. The products obtained after extraction from the silica gel were crystallized from methanol and the crystalline mixture separated on GC to produce cholesterol, 24α-methylcholesterol, gorgosterol [7] and I. However, I was also obtained from the mother liquor by evaporation of the methanol and crystallization from ethyl acetate.

The new sterol (I), 25-hydroxy-24ξ-methylcholesterol, [$C_{28}H_{48}O_2$, M^+ 416.365234 (required 416.366943)], exhibited m.p. 189.5-190.5° (from methanol), λ_{max} (KBr) 3420 cm^{-1} and $[\alpha]_D^{21}$ = -47.1° (c = 0.150 g/100 ml in $CHCl_3$), typical of a Δ^5-sterol.

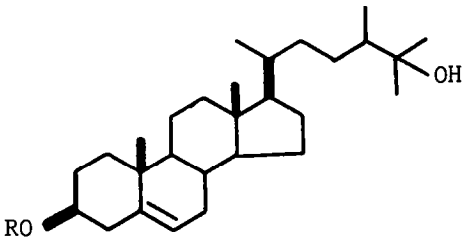
Monacetate: The diol (I) (20 mg) was kept at room temperature in acetic anhydride-dry pyridine (1:1) (2 ml) for 18 hours. The product obtained as usual was chromatographed over silica (5 g) and afforded the monacetate (II) (20 mg), m.p. 151-152° from methanol, $[\alpha]_D^{21}$ = -51.5 (c = 0.160 g/100 ml in $CHCl_3$).

Anal. Calcd. for $C_{30}H_{50}O_3 \cdot 1/2\ CH_3OH$: C, 77.21; H, 10.55. Found: C, 77.02; H, 10.92.

The monacetate (II) (15 mg) in pyridine (1 ml) was treated with pure phosphoryl chloride (0.2 ml). After 20 hours ice and then water (3 ml) was added and the mixture extracted with benzene (4 x 3 ml) to

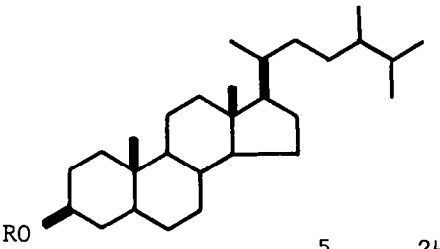
give 24-methyl-$\Delta^{5,24}$-cholestadien-3β-ol acetate (III) as colorless needles (12 mg), m.p. 142-143° from methanol, $[\alpha]_D^{21}$ = -19.9°, m/e 380 = M^+-CH_3COOH; reported [14] m.p. 145° and $[\alpha]_D$ = -21°.

Hydrogenation: The monacetate (III), (5 mg) in acetic acid (2 ml) was hydrogenated over platinum oxide (4 mg). The product, freed from catalyst and solvent, was chromatographed over silica (3 g). Elution with benzene afforded 24α-methylcholestanol acetate (IV), m.p. 145° and $[\alpha]_D^{21}$ = +6° (lit [15] m.p. 145° and $[\alpha]_D$ = +6°).



I. R = H

II. R = $COCH_3$



III. R = $COCH_3$; Δ^5 and Δ^{24}

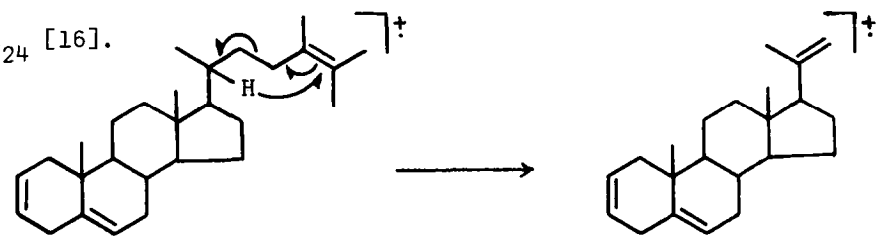
IV. R = $COCH_3$

RESULTS AND DISCUSSIONS

Mass spectrometry. The mass spectrum of I had a molecular ion peak at m/e 416 and significant peaks at m/e 401 (M^+-CH_3), 398 (M^+-H_2O), 383 [M^+-(H_2O+CH_3)], 380 (M^+-2H_2O), 365 [M^+-(2H_2O+CH_3)], 340 [M^+-(H_2O+C_3H_6O)] and two peaks of great abundance at m/e 273 (M^+-side chain) and m/e 231 [M^+-(side chain+42)] typical of ring D-fission of a steroidal nucleus [13].

The nmr signal of II at 202 cps integrated for three protons, thus showing that only one acetate function was present which in turn confirmed that one hydroxyl function was tertiary. The mass spectrum showed no peak at the expected M^+ 458 but a base peak at m/e 398 arising from M^+-CH_3COOH. The location of the tertiary hydroxyl group was immediately established from the mass spectrum of the dehydration product which displayed an in-

tense peak at m/e 296 indicating that a McLafferty rearrangement of type a → b was operating and that a double bond was therefore present at C_{24} [16].



a M^+-CH_3COOH (m/e 380) b (m/e 296)

Inspection of the literature then showed that the constants of the dehydration product (III) were completely consistent with those reported [14] for the recently prepared 24-methyl-$\Delta^{5,24}$-cholestadien-3β-ol acetate (III).

Nuclear magnetic resonance spectra: The nmr studies of the chemical shifts of methyl protons of compounds I and III (Table 1) were of the outmost importance in the structure determination.

Table 1

Chemical Shifts of Methyl Protons (cps) (100 MHz)

	C_{18}	C_{19}	C_{21}	$C_{26,27}$	C_{28}
25-Hydroxy-24ξ-methylcholesterol (I)	69.6(3H)	102.5(3H)	95.5(J=6)	117.6(6H)	90.07(J=6)
24-Methyl-$\Delta^{5,24}$-cholestadien-3β-ol acetate (III)	68.0(3H)	102.2(3H)	98.5(J=6)	162.5(6H)	162.5(3H)

Two secondary (C_{21} and C_{28}), two distinct quaternary (C_{18} and C_{19}) and two quaternary (117.6 cps) methyl groups with identical chemical environment were present in I. Therefore we concluded that this last downfield

shift was caused by deshielding, effected by the tertiary hydroxyl group at C_{25}. The dehydration product (III) gave the expected nmr spectrum (Table 1) namely five quaternary methyl groups with $C_{26,27}$ and C_{28} having the same chemical shift.

The nmr spectrum of (I) also exhibited signals at 356 cps (1H, multiplet) and 535 cps (1H, multiplet), consistent with the presence of a secondary carbinol methine and an olefinic proton. By comparing the predicted and obtained values for the chemical shifts [17] of C_{18} and C_{19} in compound III the stereochemistry of the 3-hydroxyl group and the side chain were shown to be both β.

The spectral evidence supported the plausible structure I and this was confirmed by the chemical work (see experimental section). The melting point and rotation of the dehydration product, 24-methyl-Δ^{24}-cholesterol acetate (III), were both in agreement with the literature values [14]. Final proof was provided by the fact that the hydrogenation product 24α-methylcholestanol acetate (IV) was identical in melting point, $[\alpha]_D$, m.s. and gc retention time with those of an authentic sample.

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