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5α-CHOLESTANE-3,6-DIONE FROM THE RED ALGA *ACANTOPHORA SPICIFERA*

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Key Word Index—*Acantophora spicifera*; Rhodophyceae; keto-steroid; 5α-cholestane-3,6-dione.

Abstract—Analysis of the chloroform extract of the marine red alga *Acantophora spicifera* led to the isolation of a keto-steroid, 5α-cholestane-3,6-dione, which was characterized on the basis of spectroscopic characteristics. The identity was confirmed by comparison with the synthetic sample prepared from cholesterol. This is the first report of the natural occurrence of this keto-steroid.

INTRODUCTION

The marine sterols of the majority of red algae are C_{27} compounds, cholesterol being the major component of most of them, although in a few species desmosterol has been reported to be present in substantial amounts and may even be the major sterol [1–3].

Red algae also contain, though in minor amounts, C_{26}, C_{28} and C_{29} sterols and in some species the presence of a Δ^4-3-keto steroid has been reported [4, 5]. In this communication we report the isolation of a keto-steroid from *Acantophora spicifera*, a red alga from the west coast of India. This is a major steroid present in the alga and, to the knowledge of the authors, it is the first report of the natural occurrence of 5α-cholestane-3,6-dione.

RESULTS AND DISCUSSION

The chloroform extract of air-dried seaweed was chromatographed over silica gel and the column eluted successively with solvents of increasing polarity. From the EtOAc–petrol (10:90) eluate a crystalline compound was isolated which gave a golden yellow colour on thin-layer chromatography with 2,4-DNP as the detecting agent. Its ketonic nature was evident from the strong IR absorption at ν_{max} 1700 cm^{-1}, there was no ^1H NMR evidence for an aldehyde proton and no IR band for an hydroxyl group. It also showed in the IR spectrum the presence of $-CH(CH_3)_2$ functionality (ν_{max} 1360, 1370 and 1160 cm^{-1}).

The ^1H NMR spectrum of the compound revealed that it was a steroid but it gave no reaction with the Lieberman–Burchard test thus indicating the absence of a 3β-OH group. It displayed signals at δ0.64(3H, H_{18}) and 0.9 (3H, H_{19}) for the two tertiary methyls, a signal at 0.79 was attributed to the secondary methyl at C_{21} and a signal for six protons at 0.85 was assigned to the isopropyl group situated in the side chain. Moreover, the spectrum demonstrated signals for seven protons resonating between δ2.1–2.6 probably alpha to the $\geq C=O$ group. The presence of two ketonic groups was evident from its ^{13}C NMR spectrum which displayed signals for this functionality at δ208 and 210. In view of this data it was concluded that the compound is a saturated keto-steroid. The mass spectrum exhibited a base peak molecular ion at *m/z* 400 $[M]^+$ corresponding to molecular formula $C_{27}H_{44}O_2$ further confirming the saturated nature of the compound and the tetracyclic carbon skeleton with two ketonic groups. The mass spectrum also showed prominent peaks at *m/z* 385, 371, 287, 260, 246, 244, 231, 149, 137, 123, 109, 94, 79, 69, 55. The peaks at *m/z* 287 (loss of side chain); 260, 246 (ring D cleavage); 109 (ring C cleavage); the peaks at 149 associated with fragments at 137 and 123 and 55 were indicative of a steroid belonging to the chloestane series [6] with the two carbonyl groups in rings A and B. Since cholesterol is a precursor of this type of compound [7] one of the carbonyl groups was placed at C-3 and the other would be either at C-6 or C-7. As the physical constants of the natural products agreed well with those reported for the 3,6-dione [8] and since the mass spectrum did not show peaks at *m/z* 135 and 178 which are characteristic of 7-ketones [9] the second carbonyl was placed at C-6. The stereochemistry at C-5 was inferred as α from the presence of a prominent peak at *m/z* 371 $[M-29]^+$ [10].

A definite proof of the assigned structure came finally from its synthesis by a known procedure [11]. Cholesterol was subjected to hydroboration oxidation to yield 5α-cholestane-3β,6α-diol which on Sarett's oxidation gave 5α-cholestane 3,6-dione identical in all respects (co TLC, IR, mp and ^1H NMR) with the natural diketone isolated from the red alga *Acantophora spicifera*.

An interesting feature which has been observed here is the relatively low concentrations of cholesterol present in the alga. This can be explained by it being utilized in the biosynthesis of the keto steroid. Also, the presence of oxygenated sterols is known to inhibit cholesterol synthesis [12].

EXPERIMENTAL

Mps are uncorr. TLC was accomplished on silica gel G doubly developed with 10% EtOAc in petrol and spots visualized with 2,4-DNP. IR spectra were recorded as KBr discs. Chemical shifts are reported relative to TMS; ^{13}C NMR (100.57 MHz) and ^1H NMR (90 MHz) were measured in $CDCl_3$. EIMS were obtained at 70 eV.

Collection, extraction and isolation. A. spicifera (7 kg) was collected at low tides during premonsoon periods, air-dried and extracted ×3 with $CHCl_3$. The combined extracts were evapd under red. pres. to leave a dark green viscous semisolid residue (130 g, 1.9% dry wt) which was chromatographed over silica gel eluted with mixtures of increasing polarity of petrol–EtOAc; 100 ml fractions were collected. Fractions eluted with petrol–EtOAc (90:10) gave a crystalline solid (0.007% dry wt.), mp 168–170° (lit [8] 171–172°). IR ν_{max}^{KBr} cm^{-1}: 1700 (>C=O) 1360, 1370 and 1160 [$CH(CH_3)_2$] MS *m/z* (rel. int.): 400 $[C_{27}H_{44}O_2]^+$ (100), 385 $[M-CH_3]^+$ (12.5), 371 $[M-C_2H_5]^+$ (6.2), 287 $[M-SC]^+$ (27.5), 260 $[M-C_{10}H_{20}]^+$ (7.5), 246 $[M-C_{11}H_{22}]^+$ (30), 244 $[M-C_{11}H_{24}]^+$ (62.5), 231 $[M-C_{12}H_{25}]^+$ (17.5).

Hydroporation–oxidation of cholesterol. A soln of 1.2 g of BF_3–Et_2O in 10 ml of THF was added dropwise to a mixture of 20 ml THF, 0.25 g $NaBH_4$ and 0.5 g cholesterol maintained at 25° under N_2. The mixture was allowed to stand for 1 hr and the excess hydride decomposed by dropwise addition of 10 ml of H_2O. This was followed by the addition of 3.2 ml of 15% NaOH and 3.2 ml of 30% H_2O_2. The mixture was warmed to 45°. After 15 min, 4 g of NaCl was added to sat. the H_2O phase so that the two layers separated out, cooled and extracted with Et_2O, washed with H_2O, dried over Na_2SO_4 and concd. The residue chromatographed to yield 5α-cholestane-3β,6α-diol mp 213° (lit [8] 213–215°).

Sarett's oxidation of diol. CrO_3 was added slowly to pyridine and with constant stirring till a solid complex was obtained. Subsequently the diol, 5α-cholestane-3β,6α-diol, in pyridine was added dropwise and the mixture left overnight. The mixture was poured into cold H_2O and extracted with Et_2O, dried and concd to yield 5α-cholestane-3,6-dione (0.12 g, mp 170–171°) identical in all respects (IR, ^1H NMR, mmp, coTLC) with the natural product.

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