[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CONNECTICUT AND THE BERMUDA BIOLOGICAL STATION FOR RESEARCH]

STEROLS OF MARINE MOLLUSKS. IV. Δ^7-CHOLESTENOL, THE PRINCIPAL STEROL OF *Chiton tuberculatus L.*[1]

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The occurrence of Δ^7-cholestenol (*lathosterol*) as a natural product was first reported by Fieser (1) who found this substance to accompany cholesterol in a variety of animal tissues. Idler and Baumann have demonstrated that relatively large amounts occur as components of skin sterols (2). These observations acquire an added significance in view of the report of Langdon and Bloch who have shown that feeding Δ^7-cholestenol to the rat causes an increase in liver cholesterol and, in addition, depresses the rate of the acetate-cholesterol conversion (3). The role of the Δ^7-stenol as an intermediate in the biosynthesis of cholesterol is therefore suggested.

In view of the remarkable variety of sterol structures obtainable from marine invertebrate animals, the presence of Δ^7-cholestenol is to be expected. Toyama and Tanaka have recently isolated this substance from the chiton, *Loliophura japonica* (4), and Matsumoto has reported its occurrence as a component of the unsaponifiable matter of the starfish, *Asterias amurensis* (5). The occurrence in chitons is of particular interest since this sterol has not previously been found in mollusks and, in addition, it occurs as the principal sterol in *Loliophura*.

The present communication describes the isolation of Δ^7-cholestenol from *Chiton tuberculatus*, the chiton common in Bermuda waters. The contents of the fatty material obtained from sun-dried tissue are shown in Table I and are compared with the data of Toyama and Tanaka. The sterol may be conveniently obtained by recrystallization of the unsaponifiable matter from methanol. The relative ease of isolation of the pure sterol and the fact that material isolated *via* the digitonide was of equal purity indicates that there is virtually no contamination with cholesterol or sterols of order $C_{28,\ 29}$. Both the sterol and its esters were slightly dextrorotatory. Inconsistent results were obtained on titration with perbenzoic acid and with iodine, a behavior not uncommon to Δ^7-stenols. The sterol gave a rapid Liebermann-Burchard reaction, a positive test with the selenious acid reagent of Fieser (1), and developed a green color (Tortelli-Jaffe reaction) when bromination experiments were attempted. Confirmation of the location of the double bond and identification of the carbon skeleton were carried out using the following isomerization series: Δ^7-stenol $\rightarrow$ $\Delta^{8(14)}$-stenol $\rightarrow$ Δ^{14}-stenol $\rightarrow$ cholestanol. Comparison of the properties of these products is shown in Table II.

The presence of Δ^7-cholestenol as a major component sterol in chitons affords another example of the marked differences in sterols to be found among the various classes of mollusks. These differences have been pointed out by Bergmann

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TABLE I

LIPID CONTENT OF CHITONS

	C. tuberculatus	*L. japonica*
% Lipid	1.1	1.05
% Unsap. matter	11	19
% Sterol	30	44.8

TABLE II

STEROL AND DERIVATIVES FROM CHITONS

	C. tuberculatus		*L. japonica*		Δ⁷-Cholestenol[a]	
	m.p., °C.	$(\alpha)_D$	m.p., °C.	$(\alpha)_D$	m.p., °C.	$(\alpha)_D$
Sterol	121–123	+4.3	122	0	122–123	0
Acetate	116–118	+0.92	118–119	0	118–119	—
Benzoate	153–155	+9.1	158–159	+6.9	157–158	—
$\Delta^{8(14)}$-Stenol	118–119	—	118–119	—	119–120	
Δ^{14}-Stenol benzoate	163–165	—	168–169		168	
Stanol	140–141	+28.9	—	—	141–142	+34.2

[a] Data from Elsevier's Encyclopedia of Organic Chemistry, Vol. 14, Elsevier Publishing Co. 1940, p. 57.

(6) and emphasized in previous papers in this series (7). The class Amphineura (chitons) occupies a key position in the phylum Mollusca since members of this class are presumed to be only slightly differentiated from the prototype mollusk. The occurrence of a possible precursor in cholesterol biosynthesis as the major component sterol may be related to the biological "precursor" position occupied by the Amphineura in the phylogenetic classification of the mollusks.[4]

EXPERIMENTAL

Melting points were taken with Anschütz total immersion thermometers. Optical rotations were taken in a 2-decimeter tube, the sample being dissolved in 5 ml. of chloroform.

Isolation of the sterol. The sun-dried specimens (25.8 kg.) were pulverized and extracted with acetone for 48 hours in modified 5-liter extractors (6). The acetone-soluble fat (289 g.) was saponified by refluxing for two hours with 2.9 liters of 10% KOH in 75% ethanol. Ether extraction of the saponification mixture yielded 33 g. of viscous red-brown unsaponifiable matter.

The unsaponifiable matter was treated with hot methanol from which, on cooling, there was deposited 9.79 g. of crude sterol. The methanol was removed and the residue was dissolved in ethanol and treated with 1% digitonin in ethanol. The insoluble digitonide (7.72 g.) was split by the method of Bergmann (8) and yielded an additional 2.0 g. of sterol. Three recrystallizations from absolute methanol gave a product which melted at 121–123°: $[\alpha]_D^{22}$ +4.3° (40.4 mg.; α +0.07°).

Anal. Calc'd for $C_{27}H_{46}O$: C, 83.87; H, 11.99.

Found: C, 83.62; H, 12.01.

The sterol gave an intense and rapid Liebermann-Burchard reaction. The Tortelli-Jaffe reaction was positive and a red precipitate of selenium deposited in 15 minutes with the Fieser reagent.

[4] A similar relationship involving arginine and creatine was suggested by O. Meyerhof in 1941, quoted by G. Wald (9).

Steryl acetate. A sample of sterol was acetylated by refluxing with acetic anhydride. The acetate was recrystallized from methanol and melted at 116–118°; $[\alpha]_D^{27}$ +0.92° (49 mg.; α +0.018°).

Steryl benzoate. A sample of sterol was treated with benzoyl chloride in pyridine. The benzoate was recrystallized from absolute ethanol and melted to an opalescent liquid at 153–155°, clear at 170°. $[\alpha]_D^{27}$ +9.1° (47.7 mg.; α +0.174°).

Conversion to the $\Delta^{8(14)}$-stenol. To a sample of sterol (110 mg.) in 30 ml. of a 1:1 mixture of glacial acetic acid and ethyl acetate was added 50 mg. of platinum oxide catalyst and the mixture was hydrogenated at 30 p.s.i. for four hours. After removal of catalyst and solvent the residue was saponified by refluxing with 4 ml. of 8% KOH in ethanol. The saponification mixture was diluted with water, extracted with benzene, and dried over sodium sulfate. The stenol was recrystallized from absolute methanol; m.p. 118–119°.

Treatment of the steryl acetate with hydrogen and platinum oxide gave the $\Delta^{8(14)}$-acetate; m.p. 73–74°.

Treatment of the steryl benzoate with hydrogen and platinum oxide gave the $\Delta^{8(14)}$-benzoate; m.p. 118°.

Preparation of cholestanol. The chiton sterol (2 g.) was benzoylated and isomerized by treatment with hydrogen and platinum oxide. The $\Delta^{8(14)}$-benzoate was dissolved in dry chloroform and anhydrous hydrogen chloride was bubbled through the solution for five hours at 0°. The solution was washed with saturated sodium bicarbonate and water, and dried over sodium sulfate. The chloroform was removed and the product was recrystallized six times from ethyl acetate; m.p. 163–165°.

The Δ^{14}-stenyl benzoate was dissolved in 45 ml. of 1:1 glacial acetic acid-ethyl acetate, containing 150 mg. of platinum oxide catalyst, and was hydrogenated at 30 p.s.i. for five hours. The catalyst and solvents were removed and, since the residue gave a positive Liebermann-Burchard test, the treatment with HCl and subsequent hydrogenation was repeated. The product (0.94 g.) gave a faint Liebermann-Burchard reaction and was therefore subjected to the treatment of Anderson and Nabenhauer (9) to remove unsaturated material. The stanyl benzoate was saponified with 8% KOH in ethanol and the stanol was recrystallized from methanol: m.p. 140–141.5° $[\alpha]_D^{22}$ +28.9° (31.2 mg.; α +0.36°). The stanol gave no depression in melting point when mixed with cholestanol.

Anal. Calc'd for $C_{27}H_{48}O$: C, 83.43; H, 12.45.

Found: C, 83.63; H, 12.40.

The acetate was prepared by the usual method and it melted at 109–110°; $[\alpha]_D^{22}$ +18.3° (32.3 mg.; α +0.237°). A mixture melting point with cholestanyl acetate gave no depression.

SUMMARY

Δ^7-Cholestenol has been identified as the principal sterol of the mollusk, *Chiton tuberculatus*. Identification is based on the properties of the sterol and its derivatives, and by conversion to cholestanol.

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