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STEROLS OF MARINE MOLLUSKS. II. THE STEROLS OF THE PERIWINKLE, *LITTORINA LITTOREA*[1]

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Bergmann and Low have recently called attention to the constancy in fat content and nonsaponifiable matter exhibited by marine mollusks (1). On the basis of available data it appears that the gastropods invariably contain cholesterol as the principal sterol although sterols of other orders (C_{28}, C_{29}) may be present as minor components of the mixtures which are so frequently obtained.

The first paper in the present series (2) has described the isolation of cholesterol from two marine gastropods. In the case of the *Nassa obsoleta* a second component of the mixture was isolated but not identified due to a lack of material. It was suggested that this substance might be clionasterol, first isolated from sponges (3, 4), and later reported as present in a species of gorgonia (5). Clionasterol has now been definitely characterized as a minor component of the sterol fraction of the *Littorina littorea*, and it therefore seems likely that it is also present in the closely related *Nassa*.

The marine snail, *Littorina littorea*, is one of the common mollusks of the New England coast and is readily obtainable in large quantities. The material used in the present work was obtained from the Marine Biological Laboratory at Woods Hole, Massachusetts. The nonsaponifiable matter melted at 130–135° and consisted of 61% sterol determined by precipitation with digitonin. Acetylation and subsequent bromination yielded insoluble acetate bromides which decomposed on standing. Attempts to prepare the steryl bromides likewise led to the formation of unstable products. Benzoylation of the crude sterol however yielded a product which was only partially soluble in hot ethanol, a behavior which had previously been observed in the case of the benzoate prepared from the nonsaponifiable matter of the *Nassa obsoleta*. A series of fractional crystallizations from absolute alcohol led to the separation of cholesteryl benzoate as the least soluble component, constituting about 85% of the mixture. This was further identified by conversion to the free sterol and the acetate. The occurrence of cholesterol in such a high proportion lends further support to the contention of Bergmann and Low regarding the significance of this sterol in gastropods. Concentration of the mother liquors yielded a material which after purification melted at 133–135°. The behavior of this benzoate during cooling from the melt was similar to that described for clionasteryl benzoate (4). The free sterol melted at 137–138° and exhibited a specific rotation in agreement with that reported for clionasterol.

The occurrence of clionasterol in the *Littorina* is significant because of the bearing it may have on the structure of the "periwinkle" provitamin D reported

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by previous workers. Bock and Wetter have determined the provitamin D content to be 9.6% based on spectroscopic evidence (6). Boer *et al.* (7) have isolated the crystalline provitamin and determined the potency of the irradiation product. On the basis of the present findings, the provitamin would be expected to be related either to cholesterol or to clionasterol. The data in Table I show clearly that the provitamin is not 7-dehydrocholesterol. Comparison with 7-dehydroclionasterol, prepared by Bergmann, Lyon, and McLean (8), shows close agreement in melting points of both the sterol and the acetate. Although the optical rotations do not appear to be in agreement, the fact that Boer *et al.* used benzene instead of chloroform as the solvent may account for the discrepancy. We were unable to obtain a sample of 7-dehydroclionasterol to determine the solvent effect but it is of interest to note that 7-dehydrocholesterol obtained by Boer *et al.* (9), gave $(\alpha)_D$ −127° (benzene), as compared with −113.6°

TABLE I

COMPARISON OF THE LITTORINA PROVITAMIN D WITH 7-DEHYDROCHOLESTEROL AND 7-DEHYDROCLIONASTEROL

	STEROL		ACETATE	
	M.P., °C.	$(\alpha)_D$	M.P., °C.	$(\alpha)_D$
7-Dehydrocholesterol....	143	−114	129	−85
7-Dehydroclionasterol....	138	−98	139	−72
Periwinkle provitamin...	137	−124[a]	136	−85[a]

[a] Rotation taken in benzene.

(chloroform) obtained by Windaus, Lettré, and Schenck (10) for the synthetic compound. The data in Table I therefore suggest to us that the provitamin of the *Littorina* is identical with 7-dehydroclionasterol.

EXPERIMENTAL

Isolation of the sterol mixture. Thirty-six kilograms of meat and shells was pulverized and air-dried for a period of several weeks. Batches of about four kilograms were further dehydrated with acetone in a Soxhlet apparatus and then exhaustively extracted with ether. The solvents were removed and the extracts were saponified in the usual manner. The nonsaponifiable matter consisted of 8.1 g. of a light yellow crystalline solid which melted at 130–135° and gave a positive Liebermann-Burchard reaction.

To a solution of 204 mg. of crude sterol in ethanol was added 60 cc. of a one per cent solution of digitonin in ethanol. The digitonide was cleaved by Bergmann's procedure (11) yielding 125 mg. of sterol which melted at 133–137°.

Preparation of the benzoates. To a solution of 2.5 g. of crude sterol in dry pyridine was added 4 cc. of benzoyl chloride and the mixture allowed to stand for 48 hours. The benzoates were precipitated with water, filtered, and washed with cold ethanol. A series of ten fractional crystallizations from absolute alcohol yielded 0.86 g. of benzoate which melted to a turbid liquid at 146° and cleared at 174°. When mixed with cholesteryl benzoate there was no depression of the melting point. $(\alpha)_D^{24}$ −16.6° (34.3 mg. in 3 cc. of chloroform gave an α reading of −0.19°).

Saponification of the benzoate yielded cholesterol, m.p. 147–148°. $(\alpha)_D^{25}$ −38.0° (36.4 mg. in 3 cc. of chloroform gave an α reading of −0.46°).

Anal. Calc'd for, $C_{27}H_{46}O$: C, 83.87; H, 11.99.
Found: C, 83.80; H, 11.75.

Acetylation with acetic anhydride gave cholesteryl acetate, m.p. 114°. The acetate gave no depression in melting point when mixed with authentic material.

Clionasterol. Concentration of the mother liquors from the separation of cholesteryl benzoate yielded 0.162 g. of material. This was crystallized eleven times from 95% ethanol, and melted at 133–135°. On cooling, the benzoate momentarily changed to a green-blue at 115° and solidified at 106°. $(\alpha)_D^{25}$ −17.8° (40.2 mg. in 3 cc. of chloroform gave an α reading of −0.24°). When mixed with clionasteryl benzoate[2] the melting point was 133–134°.

Anal. Calc'd for $C_{36}H_{54}O_2$: C, 83.33; H, 10.49.
Found: C, 83.09; H, 10.54.

Saponification of the benzoate yielded clionasterol, m.p. 137–138°. $(\alpha)_D^{25}$ −37.7° (21.5 mg. in 3 cc. of chloroform gave an α reading of −0.26°). When mixed with authentic clionasterol[2] the melting point was 137–138°.

Anal. Calc'd for $C_{29}H_{50}O$: C, 83.99; H, 12.15.
Found: C, 84.37; H, 12.08.

SUMMARY

An examination of the sterol mixture obtained from the periwinkle, *Littorina littorea* has revealed the presence of cholesterol and clionasterol. These components were separated by fractional crystallization of the mixed benzoates from absolute alcohol.

This report describes the first observation of the presence of clionasterol in mollusks. It has been suggested that the isolation of this sterol may shed light on the nature of the "periwinkle provitamin D". Evidence for the identity of the provitamin with 7-dehydroclionasterol has been presented.

The results also confirm the suggestion that gastropod mollusks contain cholesterol as the principal sterol.

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