[CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY AND THE BINGHAM OCEANOGRAPHIC LABORATORY, YALE UNIVERSITY]

CONTRIBUTIONS TO THE STUDY OF MARINE PRODUCTS. XXIV THE OCCURRENCE OF BRASSICASTEROL IN MOLLUSKS

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In the Vth communication of this series (1) the statement was made that the sterol mixture obtained from the oyster, *Ostrea virginica*, contains small amounts of stigmasterol. The identification of this sterol was based primarily on the properties of the steryl acetate tetrabromide. It has been suggested, however, in a later communication (2) that the sterol in question was brassicasterol rather than stigmasterol, and that shakosterol, first isolated by Tsujimoto (3) from *Tridacne gigas*, is also identical with brassicasterol. Lack of adequate amounts of starting material has so far delayed the isolation of pure brassicasterol from the oyster. No difficulties, however, were encountered in isolating substantial quantities of this sterol from another bivalve, the mussel, *Modiolus demissus*. The starting material was a mussel sterol mixture, a large amount of which had been obtained through the courtesy of the Silmo Laboratories. This mixture contained only insignificant quantities of 7-dehydrosterols, the bulk of them having first been removed by fractional crystallization. The isolation of brassicasterol was carried out by way of the difficultly-soluble acetate tetrabromide, obtained by fractionation of the acetate bromide mixture. Fractionation of the lower-melting, difficultly-soluble, acetate bromides eventually led to the isolation of cholesteryl acetate dibromide.

While these investigations were nearing completion, the authors received a series of reprints of papers by Toyama and collaborators which had been published in Japan during the war years. In the VIIth communication of their series (4a), the Japanese authors have indicated the presence of brassicasterol in the sterol mixture obtained from the bivalve, *Corbicula leana*, and in a subsequent paper (4b), they have convincingly demonstrated its occurrence in the oyster, *Ostrea gigas*. In addition the dissertation of van der Vliet (5) contains a reference to some unpublished work by Stevens, according to which the presence in the mussel, *Mytilus edulis*, of brassicasterol and cholesterol has been ascertained. The occurrence of brassicasterol in bivalves has therefore been demonstrated by three independent groups, and the identity of shakosterol and brassicasterol has been made more probable if not certain.

The more recent Japanese literature contains the description of several other new sterols from bivalves. The physical properties of these sterols and their known derivatives are shown in Table I. The most unique and interesting sterol of this group is corbisterol, which Matsumoto and Toyama (4c) have isolated from the sterol mixture of *Corbicula leana*. On the basis of iodine numbers and saponification values, the Japanese authors have concluded that corbisterol is a tetra-unsaturated sterol of the empirical formula $C_{29}H_{44}O$ or $C_{28}H_{42}O$. An evaluation of the differences between the molecular rotations of the sterol and its acetate, and of corresponding saturated and unsaturated derivatives [Barton (6)],

however, strongly indicates that corbisterol is a $\Delta^{5,7}$-di-unsaturated sterol. Such a suggestion is supported, rather than contradicted by the high iodine number reported by the Japanese authors, for it is well known that 7-dehydrosterols are prone to give iodine numbers substantially higher than the theoretical values.

It is well established that the sterol mixtures of certain mollusks contain such substantial quantities of 7-dehydrosterols, as to have made them a commercial source for provitamin-D. The isolation of 7-dehydrosterol from a gastropod has been claimed in a patent by Boer, *et al.* (7), and Kind and Herman (8) have recently intimated its identity with 7-dehydroclionasterol (9). Van der Vliet (5, 10) has concluded on the basis of indirect evidence, derived from a systematic study of irradiation products, that the "mussel provitamin-D" of *Mytilus edulis*, consists of ergosterol, 7-dehydrocholesterol, $\Delta^{5,7,22}$-cholestatrien-3-ol, and an unknown sterol. It appears quite unlikely that corbisterol is identical with any one of the named 7-dehydrosterols. The probability, however, is indicated that it is essentially 22,23-dihydroergosterol, or more likely a mixture of this sterol with its 24-methyl epimer, the as yet unknown 7-dehydrocampesterol.

TABLE I

NEW MOLLUSK STEROLS

STEROL	STEROL		ACETATE		BENZOATE		STANOL		STANYL ACETATE	
	M.p., °C	$[\alpha]_D^\circ$	M.p., °C	$[\alpha]_D^\circ$	M.p., °C	$[\alpha]_D^\circ$	M.p., °C	$[\alpha]_D^\circ$	M.p., °C	$[\alpha]_D^\circ$
Corbisterol (4c)	149	−93.5	151	−70.4	141–144	−45	142.5	+26	140	+13
Conchasterol (12)	133–134		144–145							
Meretristerol (11)	134.5	−44	144–145	−50.2	141–145	−20	146.5		140	+13
Pectosterol (13).....	134–137	−35	137							
Magakisterol (14)....	152.8	−47	149.5	−52						

Toyama and Yajima believe that the meretristerol of the bivalve, *Meretrix meretrix* (11), is identical with Tsujimoto's conchasterol (12). They nevertheless prefer continuing the use of the new name until the question of the identity of the two sterols has been definitely demonstrated. Tsujimoto had originally regarded conchasterol as an isomer of cholesterol. Toyama and Yajima have concluded, however, on the basis of iodine numbers and saponification values, that meretristerol and hence conchasterol are di-unsaturated compounds of the probable formula $C_{28}H_{46}O$. An analysis of the differences between the molecular rotations of the sterol, its acetate and benzoate, and of the steryl and stanyl acetate supports this view, and in addition indicates the locations of the double bonds in the $\Delta^{5,22}$-positions. It remains as yet uncertain, however, that this sterol is a new isomer of brassicasterol and chalinasterol (ostreasterol) (2). The probability is strongly indicated that meretristerol and conchasterol represent some of the difficultly separable mixtures of these two 24-methyl epimers which are frequently encountered among the sterols of bivalves (2). Both these sterols afford rather insoluble acetate tetrabromides. The failure of the Japanese authors to obtain such bromides from meretristeryl acetate is probably due to

the fact that the bromination, as described by them, was carried out in a solution too dilute to permit ready separation of the adduct. The data furnished in support of the claims that pectosterol (13) and magakisterol (14) are new compounds are far too inadequate to warrant any comments concerning their probable structure.

EXPERIMENTAL

All melting points are corrected. All optical rotations were taken in a 1 dm. tube, the sample being dissolved in 3.06 cc. of chloroform.

Brassicasteryl acetate tetrabromide. The crude sterol mixture was acetylated by refluxing with acetic anhydride in the usual manner, and the acetate recrystallized once from a mixture of chloroform and methanol. To a solution of 220 g. of the crude acetate in 1200 cc. of ether was added, with stirring and cooling, 1100 cc. of a 10% solution of bromine in glacial acetic acid. The mixture was kept in a refrigerator overnight. The crystalline bromides were washed with acetic acid and methanol, and dried *in vacuo* over sodium hydroxide. The total, 176.5 g., was then digested with 1 liter of ether at room temperature. The undissolved material was collected, washed with small amounts of ether and dried. The tetrabromide thus obtained weighed 41 g.; upon heating it decomposed above 180°. For further purification it was extracted from a thimble with boiling ether; yield 39 g., m.p. (dec.) 205–209°. Subsequent recrystallization of the tetrabromide from chloroform-methanol raised the decomposition point to 209–216°.

Anal. Calc'd for $C_{30}H_{48}Br_4O_2$: Br, 42.0. Found: Br, 41.8.

Brassicasteryl acetate. Debromination of the tetrabromide with zinc dust and glacial acetic in the usual manner afforded a product which after two recrystallizations from a mixture of acetone and methanol gave brassicasteryl acetate, m.p. 152°; $[\alpha]_D^{26}$ −62.2° (52.7 mg., α, −1.07°).

Brassicasteryl acetate 22,23-dibromide. The partial debromination of the tetrabromide with sodium iodide in ethanol was carried out according to the directions of Fernholz and Stavely (15). The 22,23-dibromide thus obtained was recrystallized several times from a mixture of ether and methanol; m.p. 213–217°.

Anal. Calc'd for $C_{30}H_{48}Br_2O_2$: Br, 26.7. Found: Br, 27.0.

The melting point reported for brassicasteryl acetate 22,23-dibromide is 236–238° (15). In this laboratory, however, various samples of the dibromide have been found to melt with decomposition between 210 and 220°. Debromination of the above dibromide with zinc in glacial acetic acid afforded brassicasteryl acetate of m.p. 152°.

Brassicasteryl benzoate. This derivative was prepared in the usual manner by treating the sterol with benzoyl chloride in pyridine. It was recrystallized several times from ether; m.p. 163°; $[\alpha]_D^{25}$ −35° (30.0 mg., α, −0.34°).

Cholesteryl acetate. The ether extract of the original acetate bromide mixture was concentrated under a stream of nitrogen until the appearance of crystalline material. This was removed, and the concentration process was repeated. After the removal of a second crop of crystals, methanol was added to the mother liquor, until a copious precipitate of cholesteryl acetate dibromide was obtained. The dibromide was recrystallized from a mixture of ether and methanol, m.p. 111°. Debromination of the dibromide with zinc in glacial acetic acid gave cholesteryl acetate, m.p. 113–114°; $[\alpha]_D^{26}$ −45° (71.1 mg., α −1.03°). Hydrolysis of the acetate gave cholesterol, m.p. 147°; $[\alpha]_D^{25}$ −39.5° (75.2 mg., α, −0.97°).

SUMMARY

It has been demonstrated that the sterol mixture obtained from the mussel, *Modiolus demissus*, contains significant quantities of brassicasterol and cholesterol.

The suggestion has been made that corbisterol is a 7-dehydrosterol, and that it is probably identical with 22,23-dihydroergosterol.

It has also been suggested that meretristerol and conchasterol are mixtures of brassicasterol and chalinasterol.

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