[Contribution from the Sterling Chemistry Laboratory, the Bingham Oceanographic Laboratory, and the Bermuda Biological Station for Research]

CONTRIBUTIONS TO THE STUDY OF MARINE PRODUCTS. XXI. CHONDRILLASTEROL

WERNER BERGMANN and FRANK H. McTIGUE[1]

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Chondrilla nucula Schmidt is probably the most ubiquitous sponge of the shallow waters of the Bermuda Archipelago. Unlike most other common sponges it is very dense, and in shape, size, and color reminiscent of a chicken liver. The contents of fatty materials of this sponge are shown in Table I.

The crude sterol obtained from *Chondrilla* was dextrorotatory and gave a positive Tortelli-Jaffé (1) reaction. It was at once benzoylated, and the benzoates were subjected to a series of fractional recrystallizations. There were eventually obtained two fractions, of which the least soluble, m.p. 195°, represented the benzoate of a sterol different from all other sterols which have so far been described. Since the sterol was first isolated from *Chondrilla,* it is proposed to name it chondrillasterol. The properties of this sterol and of some of its derivatives are shown in Table II.

Titration with perbenzoic acid proved the sterol to be diunsaturated. Its strongly positive Tortelli-Jaffé reaction, its rotation and those of its derivatives (2) coupled with the fact that the melting point of the acetate is higher than that of the sterol (3) at once suggested the absence of a $\Delta^{5,6}$ and the presence of a $\Delta^{7,8}$ double bond. Since the data also indicated unsaturation in the side chain, it was assumed that chondrillasterol possessed a structure analogous to that of α-spinasterol, stellasterol, and dihydroergosterol (2).

In accordance with these views, chondrillasteryl acetate readily took up one mole of hydrogen upon catalytic hydrogenation in an acidic medium to give the expected α-chrondrillastenyl acetate (Table II). Rearrangement of the α-acetate by hydrogen chloride to the β-acetate and catalytic hydrogenation of the latter gave a stanyl acetate which was converted into the stanol. The properties of chondrillastanol (Table II) and its acetate were so similar to those of poriferastanol and its acetate (4) as to suggest the identity of the two stanols.

Further evidence indicating the relationship between chondrillasterol and poriferasterol was obtained from the results of the ozonolysis of chondrillasteryl acetate. It gave a volatile aldehyde which was isolated in the form of its 2,4-dinitrophenylhydrazone. This slightly dextrorotatory compound was identical with the corresponding derivative of the ethylisopropyl acetaldehyde which had previously been obtained by ozonolysis of poriferasteryl acetate (5).

The observations outlined above prove that the structural formula of chondrillasterol is that of $\Delta^{7,8;\,22,23}$ poriferastadienol-3 (I). Since Fernholz (6) and

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TABLE I

Composition of Dried *Chondrilla nucula*[a]

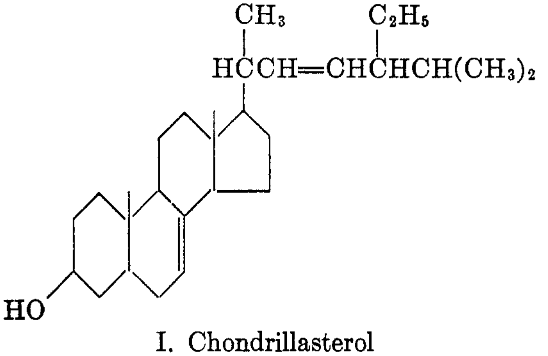
FRACTION	TOTAL SPONGE	ORGANIC MATTER, %	FAT	UNSAPON. MATTER
Spicules	0.5			
Organic	99.5	100		
Fat	3.5	3.5	100	
Unsap. Matter	1.45	1.45	40.5	100
Sterol	1.25	1.25	35.5	86

[a] See explanation in experimental section.

TABLE II

DERIVATIVE	M.P. °C	$[\alpha]_D^\circ$	$[M]_D^\circ$
Sterol	168–169	−1.1	−4
Acetate	175–176	−1	−5
Benzoate	194–195	+4	+21
α-Stenyl acetate	111–112	+8.9	+41
Stanol (Poriferastanol)	142–143	+22.5	+93
Stanyl acetate	139–140	+15	+69

Barton (2) have shown that α-spinasterol possesses a similar structure, and since it has been demonstrated in a previous study that stigmasterol and poriferasterol are C_{24}-epimers, it follows that chondrillasterol is the C_{24}-epimer of α-spinasterol.



I. Chondrillasterol

The more soluble fraction obtained by the fractional recrystallization of the original benzoate mixture melted at 135° and 155° and showed $[\alpha]_D$ +23.5°. It was shown to be identical with cholestanyl benzoate by its conversion to cholestanol and cholestanyl acetate. It is estimated that the original sterol mixture contained about seventy per cent cholestanol and thirty per cent chondrillasterol.

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.

Preparation of the sterol. The freshly collected sponges were first placed for several hours in sea water containing 5% of a commercial formalin solution and then air dried. The hard material was passed through a coarse meat grinder and thoroughly extracted with acetone in a large Soxhlet apparatus. After evaporation of the acetone, the extract, which contained considerable amount of water, was mixed with benzene, and the water was removed by co-distillation. Some smeary, brown, water-soluble material remained undissolved in benzene. The benzene extract was then evaporated to dryness, and the residue dried to constant weight *in vacuo* at 80°. It represented the acetone-benzene soluble material (Table I).

The sponge residue contained considerable quantities of inorganic impurities consisting essentially of calcium carbonate, such as coral sand shell fragments. In order to obtain reasonably accurate data on the fat content of the true sponge material and its organic fraction, a representative sample of the sponge, 3–10 g. was burnt and the ash determined. Another sample was boiled with concentrated nitric acid until all organic matter and the inorganic impurities had dissolved. The undissolved material consisted of the siliceous spicules of the sponge. They were washed with water, acetone, and ether, dried, and weighed. The difference between the percentages of ash and spicules represents the percentage of non-spicular ash. This ash may be regarded as of essentially extraneous origin. The figures shown in Table I are based on weights of crude sponge material from which the non-spicular ash has been subtracted.

The acetone-benzene soluble fraction of the sponge was a dark green, wax-like material. It was saponified, and the unsaponifiable matter isolated as previously described. The sterol content of an aliquot part of the unsaponifiable fraction was determined by the digitonin method. The bulk of the material was extracted with boiling methanol until all but a small amount of brown, resinous matter had dissolved. Upon cooling, the methanol solution deposited the sterol.

Chondrillasteryl benzoate. To a solution of one part by weight of the crude sterol in ten parts by volume of anhydrous pyridine was added about one part by weight of benzoyl chloride. The mixture was kept at 70° for twenty hours and then poured into a large volume of hot methanol. After cooling, the benzoate was collected and recrystallized from a mixture of ethanol and benzene; m.p. 135–170°, $[\alpha]_D^{24}$ +15°. Twelve recrystallizations of the crude benzoate from ethyl acetate, dioxane, and benzene-ethanol gave a product of constant melting point; m.p. 194–195°, $[\alpha]_D^{24}$ +4.0°, $[M]_D$ +21° (53.6 mg., α +0.07°).

Anal. Calc'd for $C_{36}H_{52}O_2$: C, 83.66; H, 10.14.
Found: C, 83.57; H, 10.13.

Chondrillasterol. The benzoate described above was saponified with alcoholic potassium hydroxide in the presence of some ether. The sterol was recrystallized four times from ethanol, m.p. 168–169°, $[\alpha]_D^{24}$ −1.1°, $[M]_D$ −4° (55.6 mg., α −0.02°). The sterol contains solvent of crystallization which is very difficult to remove.

Anal. Calc'd for $C_{29}H_{48}O$: C, 83.92; H, 12.08.
Found: C, 83.10; H, 12.10.

Chondrillasteryl acetate. The acetate was prepared by refluxing the sterol with acetic anhydride. It was recrystallized several times from absolute ethanol, a chloroform-methanol mixture, and ether, m.p. 175–176°, $[\alpha]_D^{24}$ −1°, $[M]_D$ −5° (56.6 mg., α −0.01 to −0.02°).

Anal. Calc'd for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08.
Found: C, 81.43; H, 11.07.

α-Chondrillastenyl acetate. A solution of chondrillasteryl acetate in glacial acetic acid was hydrogenated at room temperature with a platinum black catalyst. Absorption of hydrogen ceased after one mole had been consumed. The filtered solution was concentrated to a small volume and the acetate precipitated by the addition of methanol, m.p. 111–112°, $[\alpha]_D^{19}$ +8.9, $[M]_D$ +41° (44.5 mg., α +0.13°).

Anal. Calc'd for $C_{13}H_{52}O_2$: C, 81.52; H, 11.48.
Found: C, 81.55; H, 11.29.

Chondrillastanyl (poriferastanyl) acetate. A stream of dry hydrogen chloride was passed for five hours through a cooled solution of 290 mg. of α-chondrillastenyl acetate in 10 cc. of chloroform. The volume was kept fairly constant by occasional additions of chloroform. The solution was then washed with dilute sodium carbonate solution and water, and dried over anhydrous sodium carbonate. It was evaporated to dryness, and the residue dissolved in acetic acid and hydrogenated at room temperature with a platinum black catalyst. The absorption of hydrogen ceased after somewhat less than one mole had been consumed. The filtered solution was then concentrated, the acetate collected and once more treated with hydrogen chloride and hydrogenated as described above. The final product, 150 mg., was recrystallized from methanol-chloroform, m.p. 139–140°, $[\alpha]_D^{23}$ +15°, $[M]_D$ +69° (45.1 mg., α +0.22°). The acetate did not give a melting point depression when mixed with authentic poriferastanyl acetate.

Anal. Calc'd for $C_{31}H_{54}O_2$: C, 81.16; H, 11.87.
Found: C, 81.28; H, 12.05.

Chondrillastanol (poriferastanol). Saponification of the acetate described above gave the stanol, m.p. 142–143°, $[\alpha]_D^{24}$ +22.4° (45.1 mg., α +0.33°). It did not give a melting point depression when mixed with authentic poriferastanol.

Ozonization of chondrillasteryl acetate. A sample was used which contained some cholestanyl acetate as an impurity. The ozonization was carried out as previously described (7) and the volatile aldehyde was isolated as the 2,4-dinitrophenylhydrazone. Several recrystallizations were required to raise the melting point to 115–116°, $[\alpha]_D^{23}$ +5.7° (16.1 mg., α +0.03°). When mixed with the corresponding hydrazone from ethylisopropylacetaldehyde obtained from poriferasterol (m.p. 116–117°, $[\alpha]_D^{25}$ +4°) it melted at 115–117°.

Anal. Calc'd for $C_{13}H_{18}N_4O_4$: C, 53.05; H, 6.16.
$C_{12}H_{16}N_4O_4$: C, 51.42; H, 5.76.
Found: C, 52.71; H, 6.12.

Cholestanyl benzoate. After several days there appeared in the first mother liquor from the recrystallization of chondrillasteryl benzoate some nicely crystalline material of uniform appearance. It was recrystallized several times from dioxane and benzene-ethanol, m.p. 135° (turbid liquid), 155° (clear); $[\alpha]_D^{23}$ +23.5° (31.1 mg., α +0.23°). Hydrolysis of the benzoate gave cholestanol, m.p. 142°; $[\alpha]_D^{23}$ +23° (21.3 mg., α +0.16°), and acetylation of the latter gave cholestanyl acetate, m.p. 111°; $[\alpha]_D^{24}$ +13° (31.2 mg., α +0.13°). None of these compounds gave melting point depressions when mixed with authentic material.

SUMMARY

The sterols from the sponge, *Chondrilla nucula* Schmidt, have been isolated and investigated.

One of the sterols, the major component of the mixture, has been identified as cholestanol.

The second sterol, chondrillasterol, has been shown to be a new di-unsaturated compound, $C_{29}H_{48}O$. It has been demonstrated that it is the C_{24}-epimer of α-spinasterol.

NEW HAVEN, CONN.

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