[CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY; YALE UNIVERSITY]

STEROLS OF ALGAE. I. THE OCCURRENCE OF CHONDRILLASTEROL IN *SCENEDESMUS OBLIQUUS*

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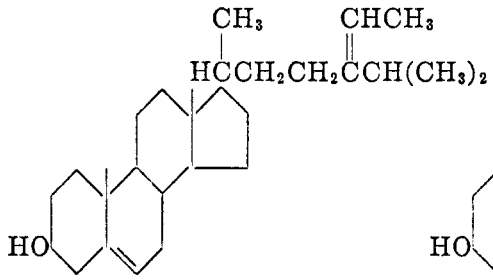
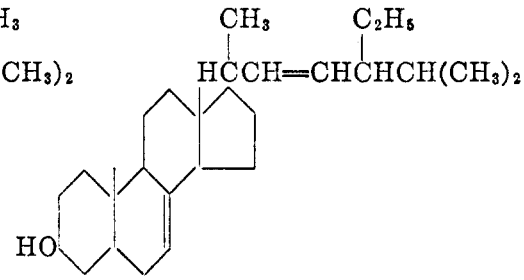
Fucosterol (I) is the only well characterized algal sterol (1) which has been described prior to the present publication. Ten years ago, in a comprehensive communication, Heilbron, *et al.* (2) reported the ubiquity of this sterol in the *Phaeophyceae* (marine brown algae), and its presence in a few species of algae belonging to other classes. The same authors also described the occurrence in *Chlorophyceae* (green algae) and some *Rhodophyceae* (red algae) of sterols of m.p. 128–137°, which afforded acetates of m.p. 128–134°. These melting points are significantly higher than those of fucosterol, m.p. 124°, and fucosteryl acetate, m.p. 119°, and reminiscent of those reported for sitosterol and its acetate. The authors therefore drew the conclusion that "*Chlorophyceae* approximate to the land plants since all members contain sitosterol".

During the past decade it has been shown that sitosterol as a rule consists of a more or less complex mixture of mono- and di-unsaturated sterols of the order C_{28} and C_{29}, and also that many impure invertebrate sterols show properties



I. Fucosterol



II. Chondrillasterol

similar to those of sitosterol. It appeared of interest therefore to reinvestigate the "sitosterols" of green algae, not only in order to establish their identity, but also their relationship to the general taxonomy of algae.

A study of the first green algae by the present authors has already led to results which were unexpected on the basis of Heilbron's earlier observations. The organism was *Scenedesmus obliquus* D^3, of which a substantial amount had been obtained through the courtesy of Dr. E. W. Fager of the University of Chicago. This alga yielded about 12% of acetone-benzene soluble material, which in turn afforded 17.5% of unsaponifiable matter. The latter contained large quantities of hydrocarbons and higher aliphatic alcohols which interfered with the smooth isolation of the sterol. It was eventually obtained by way of its benzoate. Surprisingly enough this sterol showed no resemblance to any of the sitosterol-like sterols. It was di-unsaturated and gave a strong Tortelli-Jaffe reaction. As shown in Table I, its physical properties and those of its derivatives

are those expected of a $\Delta^{7,22}$-stenol and are so much like those of chondrillasterol (II) (3) as to suggest the identity of the two sterols. Hydrogenation of the algal steryl acetate to α-chondrillastenyl acetate supports this suggestion. Chondrillasterol appears to occur in *Scenedesmus* to the practical exclusion of other sterols. It remains to be seen whether this sterol is typical for all green algae or whether it merely represents an exceptional case. Further studies along these lines are in progress in this laboratory.

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.

Chondrillasteryl benzoate. A total of 820 g. of air-dried *Scenedesmus obliquus* D_3 was extracted with acetone for two days in a large Soxhlet apparatus. After evaporation of the acetone, the extract, which contained some water, was mixed with benzene, and the water was removed by co-distillation. A small amount of brown, water-soluble material remained undissolved. Evaporation of the benzene-solution gave 97 g. of a very viscous, dark green oil, corresponding to about 12% of the starting material. The oil was saponified, and the unsaponifiable matter isolated as described previously; yield, 17.0 g., corresponding to

TABLE I

COMPARISON OF SCENEDESMUS STEROL AND CHONDRILLASTEROL

DERIVATIVE	SCENEDESMUS		CHONDRILLA	
	M.P., °C.	$[\alpha]_D^{\circ}$	M.P., °C.	$[\alpha]_D^{\circ}$
Sterol	168–169	−2	168–169	−1.1
Acetate	174.5–175.5	−0.7	175–176	−1
Benzoate	194–195	+4	194–195	+3.9
α-Stenyl acetate	115	+7.9	111–112	+8.9

17.5% of the oil. Extraction of this fraction with boiling methanol, and cooling gave 4 g. of a waxy material. It was treated at 70° for 40 hours with a mixture of 20 cc. of pyridine and 3 cc. of benzoyl chloride. The mixture was then poured into 300 cc. of hot methanol. Upon cooling, 1.9 g. of chondrillasteryl benzoate was obtained (m.p. 187–191°). After four recrystallizations from dioxane it melted at 194–195°, $[\alpha]_D^{24}$ +3.9° (31.2 mg., α +0.04°). It did not show a depression of the melting point when mixed with authentic material.

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

Found: C, 83.28; H, 10.27.

Chondrillasterol. The benzoate described above was saponified with alcoholic potassium hydroxide in the presence of some ether. The sterol was recrystallized twice from chloroform-methanol; m.p. 168–169°, $[\alpha]_D^{24}$ −2° (30.7 mg., α −0.02°). It did not show a depression of the melting point when mixed with authentic material.

Chondrillasteryl acetate. The acetate was prepared by refluxing the sterol with acetic anhydride. It was recrystallized three times from chloroform-methanol, m.p. 174.5–175.5°, $[\alpha]_D^{24}$ −0.7° (30.5 mg., α −0.007°). It did not give a depression of the melting point when mixed with authentic material.

Anal. Calc'd for $C_{31}H_{50}O_2$: C, 81.88; H, 10.14.

Found: C, 81.87; H, 10.54.

α-Chondrillastenyl acetate. The above acetate was hydrogenated at room temperature in glacial acetic acid with a platinum black catalyst. Absorption of hydrogen ceased after one mole had been rapidly consumed. The filtered solution was concentrated to a small volume

and the acetate precipitated by the addition of methanol. It was recrystallized twice from chloroform-methanol, m.p. 115°, $[\alpha]_D^{24}$ +7.9° (30.2 mg., α +0.08°).

SUMMARY

It has been shown that the sterol of the green alga, *Scenedesmus obliquus*, is identical with chondrillasterol.

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REFERENCES

(1) MACPHILLAMY, *J. Am. Chem. Soc.*, **64**, 1732 (1942).
(2) CARTER, HEILBRON, AND LYTHGOE, *Proc. Roy. Soc.* (*London*) **B128**, 82 (1939).
(3) BERGMANN AND MCTIGUE, *J. Org. Chem.*, **13**, 738 (1948).