343. *The Chemistry of the Algæ. Part I. The Algal Sterol Fucosterol.*

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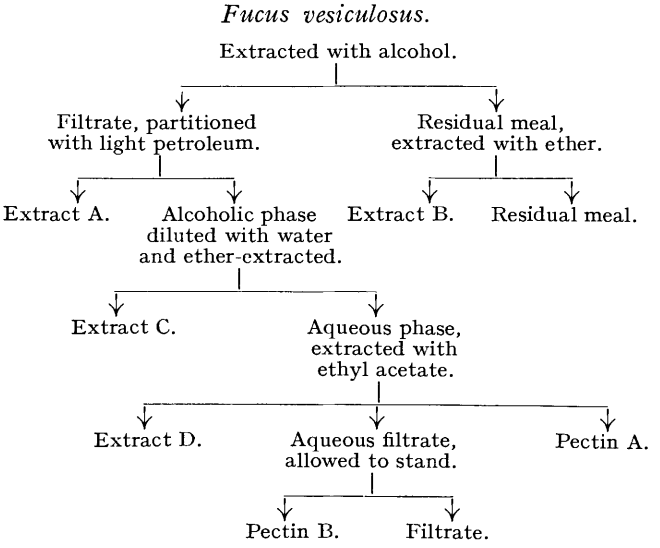
Although innumerable investigations have been carried out on the algæ, no complete systematic chemical survey of this group has so far been made. Work in this field has been confined largely to a study of (a) the polysaccharides and certain rather ill-defined but probably related substances, *e.g.*, fucosan, phycophain, laminarin, alginic acid, fucin, fucoidin (Kylin, *Z. physiol. Chem.*, 1911, **74**, 105; 1912, **76**, 396; 1913, **83**, 171; 1915, **94**, 337; *Ber. deut. bot. Ges.*, 1918, **36**, 1. See also Leroux, *Rev. gén. Sci. pures appliquées*, 1926, **37**, 471, Bibl.), (b) the pigments (Kylin, *loc. cit.*; *Z. physiol. Chem.*, 1927, **166**, 39; Willstätter and Page, *Annalen*, 1914, **404**, 237; Lemberg, *Annalen*, 1928, **461**, 46; 1930, **477**, 195; 1933, **505**, 151), and (c) in a lesser degree, the fatty constituents of certain species (Haas and Hill, *Ann. Bot.*, 1933, **47**, 55; Takahashi, Shirahama, and Tase, *J. Chem. Soc. Japan*, 1933, **54**, 619).

It is our intention to investigate the algæ in detail and in the present paper the results of an examination of the non-saponifiable material of *Fucus vesiculosus* (the bladder wrack), a member of the *Phæophyceæ* or brown algæ, will be discussed.

The dried and powdered alga was extracted in the cold with 95% alcohol and further resolved as in the annexed scheme.



Extract A. This fraction, which contains all the chlorophyll and a portion of the carotenoid pigments, was concentrated and saponified. Crystallisation of the unsaponifiable matter from methyl alcohol furnished a sterol, m. p. 124°, for which we have suggested the name *fucosterol* (Heilbron, Phipers, and Wright, *Nature*, 1934, **133**, 419). The final mother-liquors of this fraction deposited on concentration a waxy solid, m. p. 67°, identical with hentriacontane, a common constituent of the higher plants (Kuhn and Grundmann, *Ber.*, 1932, **65**, 898; Collison and Smedley-Maclean, *Biochem. J.*, 1931, **25**, 606) but not hitherto recorded in the algæ. After its removal a powerful-smelling thick oil was left which distilled in steam, giving a pale yellow, mobile liquid which solidified in the ice-chest. This portion, which is almost certainly a mixture of terpenes, has been resolved by vacuum distillation into three main fractions which await detailed investigation.

To determine whether the sterol exists in the free state or combined as a wax, a small portion of the extract A, after the removal of solvent, was treated with warm alcohol. The resultant deep green solution was separated from a tarry residue and deposited fucosterol on cooling.

Extract B. This portion, which contains the bulk of the true fat, was saponified as above, and yielded a further and approximately equal quantity of fucosterol, together with a larger amount of hentriacontane and traces of the odoriferous constituents. In addition to the above substances, various lipoid pigments have been isolated (notably zeaxanthin) and will be dealt with in a forthcoming publication. In contrast to the previous extract, no free sterol could be isolated from this fraction.

Freshly gathered *Fucus vesiculosus* has also been examined and has yielded the above-mentioned constituents.

Fucosterol.—Like the majority of the sterol group, the algal sterol forms an alcohol-insoluble *digitonide*, m. p. 223—225°. The only recorded sterols melting in the region of 124° are spongosterol (Henze, *Z. physiol. Chem.*, 1903, **41**, 109; 1908, **55**, 427) and microcionasterol (Bergmann and Johnson, *ibid.*, 1933, **222**, 220). When, however, the rotation and the melting points of the esters of these three sterols are compared (see table, p. 1574), the entity of fucosterol is definitely established.

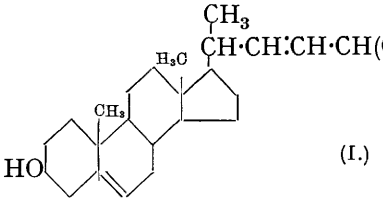
Sterol.	M. p.	$[\alpha]_D$.	M. p. of Acetate.	M. p. of Propionate.	M. p. of Benzoate.
Fucosterol	124°	−38·42°	119°	105—106°	120°
Spongosterol	124	−19·59	124	135—136	128
Microcionasterol	126—127	−19·76	125—126	128	143

The presence of two ethenoid linkages is clearly demonstrated by (a) the preparation of *fucosteryl acetate tetrabromide*, m.p. 133°, (b) perbenzoic acid titrations, and (c) the formation of a tetrahydro-derivative (fucostanol), m. p. 130—131°. The diethenoid character of fucosterol further distinguishes it from either spongosterol or microcionasterol, the former of which is saturated and the latter contains only one double bond. Analysis of the above-mentioned esters leads to a formula $C_{29}H_{48}O$ or $C_{30}H_{50}O$. Fucostanol yields an acetate, m. p. 127—129°, and is oxidised to a ketone, m. p. 153—155° (oxime, m. p. 211—213°); these compounds, as shown below, present a striking parallel to the results obtained by Windaus and Brunken (*Z. physiol. Chem.*, 1924, **140**, 47) for the corresponding derivatives from stigmastanol.

Fucosterol derivative.	M. p.	Stigmasterol derivative.	M. p.
Fucostanol	130—131°	Stigmastanol	134°
Fucostanyl acetate	127—129	Stigmastanyl acetate	128
Fucostanone	153—155	Stigmastanone	155·5
Fucostanone oxime	211—213	Stigmastanone oxime	215—216

That the fully saturated algal sterol is actually identical with stigmastanol has been demonstrated by mixed melting points of the two sterols and their acetates, no depressions being observed.

Fucosterol must thus have the formula $C_{29}H_{48}O\overline{|}_2$ and is consequently isomeric with stigmasterol (Windaus and Hauth, *Ber.*, 1906, **39**, 4378); the latter has the structural formula (I) (Fernholz, *Annalen*, 1934, **508**, 215), but fucosterol contains both its double bonds in the nucleus, for treatment with ozone fails to give ethyl*iso*propylacetaldehyde (Guiteras, *Z. physiol. Chem.*, 1933, **214**, 89). Although the exact positions of the ethenoid linkages have not so far been elucidated, the fact that fucosterol is not reduced by sodium and ethyl alcohol and does not react with maleic anhydride provides evidence of the absence of a conjugated system. Further, the ease with which it is hydrogenated to fucostanol indicates the absence of the "inert" double bond characteristic of ergosterol (Heilbron, Simpson, and Spring, J., 1933, 626).



(I.)

A preliminary examination has been made of another member of the *Phæophyceæ*, namely, *Pelvetia canaliculata*, and the presence of fucosterol again demonstrated (E. G. Parry, unpublished work). On the other hand, we have found that the green freshwater alga, *Nitella opaca Agh*, which approximates most closely to the land plants, contains both fucosterol and sitosterol (forthcoming publication). It would thus appear that the former is the characteristic sterol of the algæ, just as cholesterol is the common sterol of the animal kingdom, and sitosterol of the phanerogams.

Bergmann (*J. Biol. Chem.*, 1934, **104**, 317, 553) has recently isolated from oysters and other bivalves a sterol, ostreasterol, which also is isomeric with stigmasterol but on hydrogenation yields sitostanol. Dealing with the biological aspect, he states that "it seems possible that certain molluscs are unable to synthesise cholesterol but that they use directly or in dehydrogenated form the phytosterols of their food, which consists mainly of algæ or diatoms." In this connection the fact that ostreasterol is isomeric with fucosterol is highly significant.

Experimental.

Dried, powdered *Fucus vesiculosus*, collected during the summer months from the north coast of Scotland, was shaken (1500 g.) with ethyl alcohol (2000 c.c. of 95%) for 18 hours at

room temperature. After filtration the olive-green alcoholic solution was diluted with water (500 c.c.) and extracted with light petroleum (b. p. 40—60°). The light petroleum extract was concentrated to 150 c.c. under a stream of nitrogen, giving extract A. Similarly the alcoholic solution was concentrated to 1500 c.c. and diluted with water (3000 c.c.), and the whole ether-extracted. The ethereal layer was repeatedly washed with water (there are substances of high molecular weight present which, though insoluble in ether itself, are soluble to a great extent in alcohol containing ether), dried, and concentrated under nitrogen, yielding extract C. The remaining aqueous layer was extracted with ethyl acetate, giving extract D. During this operation large amounts of a pectin-like substance separated (pectin A), and the filtrate on long standing (6 weeks) deposited a second substance (pectin B). The residual meal was shaken with ether (2500 c.c.) for 12 hours at room temperature, and the greenish-red solution concentrated to 1500 c.c. (extract B). In this way a total of 340 kg. of the alga was extracted. We have at present confined ourselves to an examination of extracts A and B, but it is our intention to examine the other products as time permits.

Fucosterol.—Extracts A and B were each saponified with an equal volume of 10% methyl-alcoholic potash at 40° for 3 hours. The solution was cooled under nitrogen and poured into water, and the whole thoroughly extracted with ether. After removal of solvent from the dried solution, the residue was dissolved in methyl alcohol, and the fucosterol recrystallised from methyl alcohol, forming inch-long feathery needles, m. p. 124°, b. p. 220—230°/0·2 mm., $[\alpha]_D^{20°}$ −38·42° (c = 5 in chloroform). It gave a red acid layer in the Salkowski test, a violet-purple colour in the Liebermann–Burchard reaction, but was negative to the Tortelli–Jaffe reagent. A slight purplish colour was developed with antimony trichloride in chloroform during 12 hours. The total yield was 0·2% of the dry weight of sea-weed. Analytical figures indicate the presence of two molecules of water of crystallisation, which, however, cannot be completely removed without affecting the sterol itself.

Fucosteryl acetate. Fucosterol (1 g.) in pyridine (4 c.c.) was heated on a steam-bath for 1 hour with acetic anhydride (2·5 c.c.). Methyl alcohol (40 c.c.) was added to the cooled solution, which was boiled for 2 minutes. The deposited *acetate* was recrystallised from methyl alcohol–ethyl acetate, giving plates, m. p. 118—119°, $[\alpha]_D^{20°}$ −43·8° (c=5 in chloroform). Hydrolysis of the acetate furnished unchanged sterol (Found: C, 81·8; H, 10·9; *M*, 461,470. $C_{31}H_{50}O_2$ requires C, 81·9; H, 11·0%; *M*, 454).

Fucosteryl propionate, prepared as above, crystallised from ether–methyl alcohol in shining laminæ, m.p. 105—106° (Found: C, 82·2; H, 11·1. $C_{32}H_{52}O_2$ requires C, 82·1; H, 11·1%).

Fucosteryl benzoate crystallised from methyl alcohol–ethyl acetate in pearly plates, m. p. 120°. The benzoate, unlike that of cholesterol, does not melt to an anisotropic liquid (Found: C, 83·6; H, 10·0. $C_{36}H_{52}O_2$ requires C, 83·7; H, 10·1%).

Perbenzoic acid titrations. After 24 hours, 0·4244 g. of fucosterol absorbed the equivalent of 3·14 mg. of oxygen, corresponding to 1·97 double bonds, and after 48 hours, 0·5072 g. absorbed 3·72 mg., corresponding to 1·95 double bonds.

Bromine titration. Fucosterol (1 g.) in dry chloroform (15 c.c.) was treated with a solution of bromine (3 g.) in chloroform (20 c.c.) at 0° until a permanent colour was obtained (bromine absorbed = 0·825 g. $C_{29}H_{48}O|\overline{=}_2$ requires 0·725 g.). The tetrabromide thus obtained, m. p. 106—110°, was very unstable, darkening rapidly in air and decomposing on attempted recrystallisation even from cold solvents.

Fucosteryl acetate tetrabromide. Fucosteryl acetate (1 g.) in ether (10 c.c.) was treated with a 5% solution of bromine in glacial acetic acid (22 c.c.). The reaction mixture was kept over-night at 0°; a white granular powder was then deposited, m. p. 133° (decomp.), which was repeatedly washed with methyl alcohol. This compound is likewise unstable and cannot be recrystallised (Found: Br, 40·6. $C_{31}H_{50}O_2Br_4$ requires Br, 41·3%).

Fucostanol (*Stigmastanol*).—A solution of fucosterol (0·5192 g.) in glacial acetic acid (15 c.c.) and ethyl acetate (45 c.c.) was hydrogenated with Adams's platinum oxide for 12 hours (H_2 absorbed = 54·2 c.c. $C_{29}H_{52}O$ requires 56·5 c.c.). After removal of solvents under reduced pressure and precipitation with water, the product was recrystallised from methyl alcohol, giving needles, m. p. 130—131° (mixed with stigmastanol, m. p. 134°, it melts at 131—133°), $[\alpha]_D^{20°}$ + 12·75° (c = 4·75 in chloroform) (Found: C, 83·5; H, 12·3. Calc. for $C_{29}H_{52}O$: C, 83·7; H, 12·5%). The acetate (stigmastanyl acetate) crystallised from methyl alcohol in plates, m. p. 127—129° (admixture with stigmastanyl acetate, m. p. 128°, gives m. p. 127—129°), $[\alpha]_D^{20°}$ + 14·6° (c = 4·55 in chloroform).

Fucostanone (*Stigmastanone*).—A solution of fucostanol (3·5 g.) in glacial acetic acid (650 c.c.) was oxidised at room temperature with a solution of chromic anhydride (1·5 g.) in glacial

acetic acid (70 c.c.) and water (5 c.c.) during 9 hours, the whole being mechanically stirred. The reaction mixture was precipitated with water, and the crude ketone recrystallised from methyl alcohol, from which fucostanone separated in needles, m. p. 153—155°. The oxime, prepared in the usual manner, crystallised from methyl alcohol in micro-needles, m. p. 211—213°.

Isolation of Hentriacontane.—After the removal of fucosterol the mother-liquors were further concentrated, a greasy semi-solid mass being obtained. Repeated crystallisation from methyl alcohol–ethyl acetate resolved this into a more soluble fraction, m. p. 120—122°, which was shown to be fucosterol, and a less soluble portion, m. p. 63—66°, which was recrystallised from ethyl acetate, giving the hydrocarbon in small plates, m. p. 67° (Collison and Maclean, *loc. cit.*, give m. p. 68—68·5°) (Found : C, 85·2; H, 14·5. Calc. for $C_{31}H_{64}$: C, 85·3; H, 14·7%).

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