167. *Studies in the Sterol Group. Part XXIII. The Location of the Ethenoid Linkage of α-Dihydrofucosterol and the Identity of Sitostanol, Fucostanol, Stigmastanol, and Ostreastanol.*

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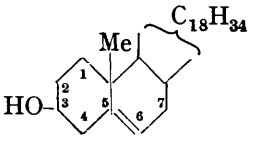
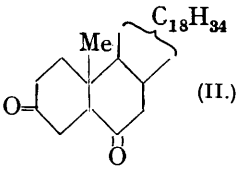
It was recorded in Part XX that the hydrogenation of fucosteryl acetate in presence of a palladium catalyst gives a mixture of α- and β-dihydrofucosteryl acetates (Coffey, Heilbron, Spring, and Wright, J., 1935, 1205).

The oxidation of α-dihydrofucosterol with perbenzoic acid has now been found to give *α-dihydrofucosterol oxide*, also obtained by similar oxidation of α-dihydrofucosteryl acetate, followed by hydrolysis with sodium methoxide. Treatment of the α-oxide with sulphuric acid gives *α-fucostanetriol*, which is also formed by the action of hydrogen peroxide upon α-dihydrofucosterol. Chromic acid oxidation of α-fucostanetriol yields *α-fucostanedionol*, demonstrating that an ethenoid linkage of the type $>C{=}\overset{|}{C}H$ is present in α-dihydrofucosterol. This dionol is readily dehydrated by means of hydrogen chloride in chloroform to *α-fucostenedione*, which on reduction is converted into *α-fucostanedione* giving a pyridazine derivative, the formation of which proves the diketone to be fucostane-3 : 6-dione (II). It therefore follows that, as in the case of cholesterol (Windaus, *Z. physiol. Chem.*, 1933, **213**, 147) and stigmasterol (Fernholz, *Annalen*, 1934, **508**, 215), α-dihydrofucosterol (I) contains an ethylenic linkage between C_5 and C_6.

(I.)





(II.)

Confirmation of the relative positions of the hydroxyl group and the ethenoid linkage in α-dihydrofucosterol (I) has been obtained from an examination of α-fucostenone,* m. p. 81—82°, prepared by oxidation of dibromo-α-dihydrofucosterol, followed by debromination.

* The "α-fucostenone," m. p. 158°, previously described by Coffey, Heilbron, Spring, and Wright (*loc. cit.*) and prepared by the *direct* chromic acid oxidation of α-dihydrofucosterol, is a mixture containing α-fucostenedione together with unidentified products.

α-Fucostenone exhibits the typical absorption spectrum of an αβ-unsaturated ketone (Menschick, Page, and Bossert, *Annalen*, 1932, **495**, 225), from which it follows that during the oxidation of α-dihydrofucosterol the $\Delta^{5:6}$-ethylenic linkage has migrated to the $\Delta^{4:5}$-position, a transformation which has its exact parallel in the oxidation of cholesterol to cholestenone (Windaus, *loc. cit.*).

A comparison of stigmastanol and sitostanol (both natural dihydrositosterol and the hydrogenated sterol from tallöl) has led Bengtsson (*Z. physiol. Chem.*, 1935, **237**, 46) to postulate their identity. The conclusion of Heilbron, Phipers, and Wright (J., 1934, 1572) that stigmastanol and fucostanol are identical is criticised by this author on the grounds of the fallibility of the mixed melting-point test and also because of the low rotation previously recorded for fucostanol. We have again compared the physical characteristics of a variety of derivatives of fucostanol with those of corresponding derivatives of stigmastanol. This comparison clearly confirms the identity of fucostanol and stigmastanol. Furthermore, Bergmann (*J. Biol. Chem.*, 1934, **104**, 317, 553) records that the

	Fuco *		Stigma *		
	M. p.	$[\alpha]_D$.	M. p.	$[\alpha]_D$.	
-stanol	134·5—135—136° †	+24·7°	135·4—136·4—137°	+24·8°	(1)
-stanyl acetate	128—129—130	+15·1	128—129·8—131·2	+15·3	(1)
-stanyl 3 : 5-dinitrobenzoate	214—215	+13·8 (in C_6H_6)	208—214—215	+13·1 (in C_6H_6)	(1)
-stanyl benzoate	135—136	—	133—135·4—137·2	—	(1)
-stanone	156·5—157	+40·2	151—157—157·4	+40·6	(1)
-stanone oxime	217—218	+30·1	219	+30	(2)
-stanedicarboxylic acid	227—229	—	229—230	—	(3)
epi-stanol	200	+25·5	200	+25	(2)
epi-stanyl acetate	87	+27·4	85	+27	(2)

* All rotations, except where otherwise stated, are for chloroformic solution.

† Where three temperatures are recorded, they represent preliminary softening, melting to a cloudy liquid, and clearing.

(1) Bengtsson, *loc. cit.* (2) Dalmer, Werder, Honigmann, and Heyns, *Ber.*, 1935, **68**, 1814. (3) Windaus and Brunken, *Z. physiol. Chem.*, 1924, **140**, 47.

fully hydrogenated ostreastanol is identical with sitostanol; the interesting fact thus emerges that the characteristic sterols of molluscs, algae, and of the vegetable kingdom have a common carbon skeleton. In this connection, however, it is to be observed that γ-sitostanol differs from stigmastanol (Bonstedt, *Z. physiol. Chem.*, 1928, **176**, 269).

Sitosterol of wheat-germ oil has been shown to contain its ethylenic linkage between C_5 and C_6 (Pickard and Yates, J., 1908, **93**, 1928; Fernholz, *Annalen*, 1934, **508**, 215) and must therefore be identical with α-dihydrofucosterol. In view of the heterogeneity of sitosterol and the great variation in the literature regarding its constants and those of its acetate (Anderson *et alia*, *J. Amer. Chem. Soc.*, 1924, **46**, 1450, 1717; 1926, **48**, 2972; Bonstedt, *loc. cit.*; Ichiba, *Inst. Phys. Chem. Res., Tokyo*, 1935, **28**, 112) a comparison is difficult. The table below, however, shows the striking similarity in the melting points of corresponding derivatives of the two series and leads us to the opinion that α-dihydrofucosterol is identical with $\Delta^{5:6}$-sitosterol, which has not hitherto been isolated free from isomeric contaminants.

	α-Fuco	Sito
-stenol (1)	136—137°	138°
-stenyl acetate (1)	133—134	134
-stanetriol (1)	248—250	252
-stanedionol (1)	256	256
-stenedione (1)	166	166 (129°)

	α-Fuco	Sito
-stanedione (1)	192—194°	196°
,, pyridazine deriv. (1)	200	197—200
-stenone (2)	81—82	81—82
,, -semicarbazone (2)	250	254

(1) Fernholz, *loc. cit.*; Pickard and Yates, *loc. cit.* (2) Heiduschka and Gloth, *Arch. Pharm.*, 1915, **253**, 415.

Experimental.

α-Dihydrofucosterol Oxide.—α-Dihydrofucosterol (1·0 g.) in chloroform (30 c.c.) was treated at −5° during 30 minutes with a chloroformic solution of perbenzoic acid (1·1 atom O). After

remaining at 0° for 24 hours, the solvent was removed, and the crude oxide dissolved in ether and washed with aqueous sodium carbonate. The *oxide* recovered from the ether separated from methyl alcohol in needles of constant m. p. 134°, $[\alpha]_D^{20°}$ − 35·6° (l = 1, c = 6·94 in chloroform) (Found: C, 80·5; H, 11·6. $C_{29}H_{50}O_2$ requires C, 80·8; H, 11·6%).

α-Dihydrofucosteryl acetate oxide, prepared by acetylation of the oxide or by oxidation of α-dihydrofucosteryl acetate with perbenzoic acid, separated from methyl alcohol in clusters of needles, m. p. 141° (Found: C, 78·7; H, 11·3. $C_{31}H_{52}O_3$ requires C, 78·8; H, 11·0%).

α-Fucostanetriol.—(a) α-Dihydrofucosterol oxide (1·0 g.) in glacial acetic acid (17·5 c.c.) was treated with two drops of concentrated sulphuric acid, the whole being maintained at 16° for 16 hours. The solution was diluted with water and extracted with ether, and the extract washed free from acetic acid. After removal of the ether the residue was hydrolysed by refluxing for 2 hours with alcoholic potash (100 c.c.; 5%), and the product isolated by water precipitation. α-Fucostanetriol separated from methyl alcohol in needles, m. p. 248—250°.

(b) A solution of α-dihydrofucosteryl acetate (10 g.) in glacial acetic acid (50 c.c.) was treated with hydrogen peroxide (Merck's perhydrol, 10 c.c.) and maintained for 1 hour at 100°. The solid separating from the cooled solution was directly hydrolysed with alcoholic sodium methoxide, and the product isolated by water precipitation, followed by crystallisation from methyl alcohol, from which *α-fucostanetriol* separated in needles, m. p. 248—250° alone or in admixture with that obtained by method (a) (Found: C, 77·5; H, 11·9. $C_{29}H_{52}O_3$ requires C, 77·6; H, 11·7%).

α-Fucostanedionol.—α-Fucostanetriol (1·0 g.) in glacial acetic acid (50 c.c.) was oxidised at room temperature with a solution of chromic anhydride (1·0 g.) in water (2 c.c.) and glacial acetic acid (8 c.c.), added during 3 hours. The solution was then stirred for 2 hours, diluted with water, and extracted with ether. The neutral portion, isolated from the extract in the usual manner, separated from methyl alcohol in needles, which after several recrystallisations from the same solvent had m. p. 256° (Found: C, 78·5; H, 10·8. $C_{29}H_{48}O_3$ requires C, 78·5; H, 10·9%).

Active-hydrogen determination (Zerewitinoff method).* 9·077 Mg. of fucostanedionol evolved 0·46 c.c. of methane at 20° and 0·48 c.c. of methane at 95°, corresponding to 1·006 and 1·05 atoms of active hydrogen per mole respectively.

α-Fucostenedione.—A solution of α-fucostanedionol (0·3 g.) in chloroform (30 c.c.) was treated for 1 hour with a rapid stream of hydrogen chloride. After washing with sodium carbonate solution, the chloroform was removed under reduced pressure, and the residue taken up in methyl alcohol, from which *α-fucostenedione* separated in needles, m. p. 166° (Found: C, 81·3; H, 10·8. $C_{29}H_{46}O_2$ requires C, 81·6; H, 10·9%).

α-Fucostanedione.—A solution of α-fucostenedione (0·3 g.) in acetic acid (10 c.c., 90%) was refluxed for 4 hours with zinc dust (0·3 g.). The excess of zinc was removed, the clear solution precipitated with water, and the solid collected. After several crystallisations from methyl alcohol *α-fucostanedione* separated in needles of constant m. p. 192—194° (Found: C, 81·1; H, 11·2. $C_{29}H_{48}O_2$ requires C, 81·2; H, 11·3%). The *pyridazine* derivative separated from chloroform–ethyl alcohol in needles, m. p. 200° (Found: C, 81·85; H, 11·3; N, 6·5. $C_{29}H_{48}N_2$ requires C, 82·0; H, 11·4; N, 6·6%).

Fucostenone.—α-Dihydrofucosterol (5 g.) in glacial acetic acid (150 c.c.) was treated first with bromine (2 g.) in acetic acid (50 c.c.) and then with a solution of chromic anhydride (1·5 g.) in acetic acid (50 c.c.; 90%), and the whole set aside for 15 hours. The solid separating after the addition of water was collected, washed with water, and debrominated by heating on a steam-bath for 10 minutes with zinc dust (20 g.) and glacial acetic acid (50 c.c.). The product, isolated by means of ether, separated from methyl alcohol in needles which after several recrystallisations from the same solvent had the constant m. p. 81—82°. α-Fucostenone separates with solvent of crystallisation which it holds tenaciously. *Light absorption in alcohol*: maxima, (a) 2405 A., log ε = 3·9; (b) 3150 A., log ε = 2·0. The *semicarbazone* separated from ethyl alcohol in needles, m. p. 250° (Found: C, 76·7; H, 10·5; N, 9·0. $C_{30}H_{51}ON_3$ requires C, 76·7; H, 10·9; N, 9·0%).

Fucostanyl Acetate.—The acetate described by Heilbron, Phipers, and Wright (*loc. cit.*; m. p. 127—129°, $[\alpha]_D^{20°}$ + 14·6°), after several recrystallisations from ethyl alcohol, had the constant m. p. 128—129—130° and $[\alpha]_D^{20°}$ + 15·1° (l = 1, c = 1·558 in chloroform).

Fucostanol.—Hydrolysis of this acetate with alcoholic potash, followed by repeated crystallisation of the product from methyl alcohol, gave fucostanol of constant m. p. 134·5—135—136°, which after prolonged desiccation had $[\alpha]_D^{20°}$ + 24·7° (l = 1, c = 1·336 in chloroform).

Fucostanyl 3 : 5-*Dinitrobenzoate.*—A solution of fucostanol ($[\alpha]_D^{20°}$ + 24·7°; 1 g.) in pyridine

* This determination was made by Mr. R. N. Jones, to whom we express our thanks.

(6 c.c.) was heated on a steam-bath for 1 hour with 3 : 5-dinitrobenzoyl chloride (1 g.). The solution was poured into water, and the precipitated solid repeatedly crystallised from ethyl acetate, from which *fucostanyl* 3 : 5-*dinitrobenzoate* separated in colourless plates of constant m. p. 214—215°, $[\alpha]_D^{20°}$ + 13·8° (l = 1, c = 1·447 in benzene) (Found: C, 70·7; H, 8·6; N, 4·8. $C_{36}H_{54}O_6N_2$ requires C, 70·8; H, 8·9; N, 4·6%).

Fucostanyl Benzoate.—Fucostanol ($[\alpha]_D^{20°}$ +24·7°; 1 g.) in pyridine (4 c.c.) was heated on the steam-bath for 30 minutes with benzoyl chloride (3 c.c.). Methyl alcohol (50 c.c.) was added, and the mixture refluxed for 5 minutes. The *benzoate* separated on cooling and after several crystallisations from ethyl acetate it formed plates of constant m. p. 135—136° (Found: C, 82·9; H, 11·1. $C_{36}H_{56}O_2$ requires C, 83·0; H, 10·8%).

Fucostanone.—Oxidation of fucostanol ($[\alpha]_D^{20°}$ + 24·7°) by the method described by Heilbron, Phipers, and Wright (*loc. cit.*), followed by separation of the neutral product and crystallisation of this from methyl alcohol, gave fucostanone in needles, m. p. 157°, $[\alpha]_D^{20°}$ + 40·2° (l = 1, c = 1·518 in chloroform). The oxime separated from methyl alcohol in needles, m. p. 217—218°, $[\alpha]_D^{20°}$ + 30·1° (l = 1, c = 1·356 in chloroform).

Fucostanedicarboxylic Acid.—The acidic *product* obtained from the oxidation described above, after several crystallisations from methyl alcohol, separated in colourless plates of constant m. p. 227—229° (Found: C, 75·4; H, 10·5. $C_{29}H_{50}O_4$ requires C, 75·3; H, 10·9). The *dimethyl* ester, prepared by means of diazomethane, separated from methyl alcohol in plates, m. p. 89—90° (Found: C, 75·8; H, 11·0. $C_{31}H_{54}O_4$ requires C, 75·85; H, 11·1%).

Epifucostanol.—A solution of fucostanone (6 g.) in acetic acid (150 c.c.; 96%) and hydrogen bromide (2 c.c.; 48%) was shaken at 60° with hydrogen in the presence of reduced platinum oxide (0·5 g.) for 3 hours. The solid precipitated from the filtered solution with water was collected and directly hydrolysed by refluxing for 2 hours with methyl-alcoholic potash (300 c.c.; 10%). The product was crystallised from methyl alcohol, from which *epifucostanol* separated in needles, m. p. 200°, $[\alpha]_D^{20°}$ + 25·5° (l = 1, c = 1·80 in chloroform) (Found: C, 83·3; H, 12·0. $C_{29}H_{50}O$ requires C, 83·5; H, 12·4%). The *acetate* separated from methyl alcohol in plates, m. p. 86—87°, $[\alpha]_D^{20°}$ + 27·4° (l = 1, c = 1·79 in chloroform) (Found: C, 81·1; H, 12·0. $C_{31}H_{54}O_2$ requires C, 81·1; H, 11·9%).

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