560. *The Steroid Series. Part I. The Ozonolysis of Fucosterol and Some of its Derivatives.*

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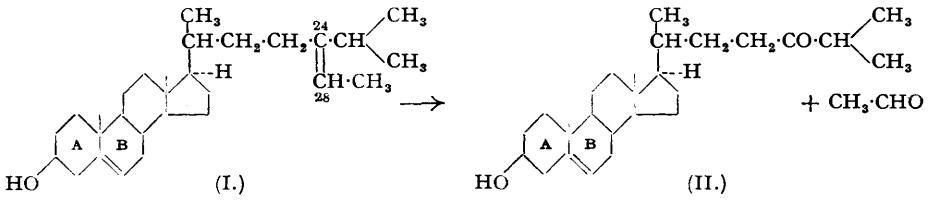
The constitution and stereochemical configuration of fucosterol (I) has been confirmed by the isolation of acetaldehyde and 24-ketocholesterol (II) on ozonolysis. The 24-ketocholesterol has been converted into cholesterol by reduction. Similar ozonolyses of fucosteryl acetate, fucosteryl methyl ether, and fucostadienone are also described.

The first evidence for the existence of a sterol in algæ was obtained by Willstätter and Page (*Annalen*, 1914, **404**, 237), who detected a new sterol in *Fucus vesiculosus*. Heilbron, Phipers, and Wright (*Nature*, 1934, **133**, 419; *J.*, 1934, 1572) isolated the pure sterol, to which they gave the name fucosterol, from this sea-weed, and its presence in many other algæ was later demonstrated by Carter, Heilbron, and Lythgoe (*Proc. Roy. Soc.*, *B*, 1939, **128**, 82). Oxidation of fucosterol by perbenzoic acid and bromination showed the presence of two double bonds in the molecule. Further, since fucosterol was readily reduced to fucostanol, which is identical with stigmastanol, it is clear that fucosterol can differ from stigmasterol only in the positions of the double bonds. Because no volatile product was obtained on the ozonolysis of fucosterol it was considered that both of the double bonds were located in the nucleus (Heilbron, Phipers, and Wright, *loc. cit.*), but further oxidation studies to elucidate the positions occupied by the double

bonds were unsuccessful (Coffey, Heilbron, Spring, and Wright, *J.*, 1935, 1205). The claim was made by Bengtsson (*Z. physiol. Chem.*, 1935, **237**, 46) that fucostanol was not identical with stigmastanol, but the preparation of further derivatives confirmed their identity beyond all doubt (Coffey, Heilbron, and Spring, *J.*, 1936, 738; Larsen, *J. Amer. Chem. Soc.*, 1938, **60**, 2431). One of the two double bonds of fucosterol was found to be more readily hydrogenated than the other, and in this way " α "-dihydrofucosterol was obtained and was shown to be identical with " β "-sitosterol having its double bond at the 5 : 6 position in ring B. The corresponding ketone,



" α "-fucostenone, was shown to have a conjugated system. " α "-Dihydrofucosterol thus resembles cholesterol in having a 5 : 6 double bond, which moves into the 4 : 5 position when the secondary hydroxyl group at position 3 is oxidised. The position of the second double bond in fucosterol remained in doubt until MacPhillamy (*J. Amer. Chem. Soc.*, 1942, **64**, 1732) showed that acetaldehyde was obtained after the sterol had been treated with ozone for a much shorter time than had been used by the earlier workers (Heilbron, Phipers, and Wright, *loc. cit.*). This indicated that the second double bond is located in the side chain between carbon atoms 24 and 28, as indicated in (I).

The experiments of MacPhillamy have now been repeated and his conclusions confirmed. In one experiment when the ozonisation was deliberately restricted to ten minutes, the yield of acetaldehyde (as the dimedon derivative) corresponded to 76% of the theoretical quantity, calculated on the weight of fucosterol not recovered. After the acetaldehyde had been removed from the ozonolysis products, there remained a waxy solid or oil, the nature of which depended on the time of ozonisation. Under certain experimental conditions it has been possible to isolate from this residue pure 24-ketocholesterol (II), a compound which had already been described by Riegel and Kaye (*J. Amer. Chem. Soc.*, 1944, **66**, 723), who prepared it by the action of cadmium di*iso*propyl on 3-acetoxychol-5-enyl chloride. Since the 24-ketocholesterol thus obtained has been converted into cholesterol by reduction both by Clemmensen's and by the Wolff–Kishner method (Riegel and Kaye), the complete conversion of fucosterol into cholesterol is made possible. In similar manner the ozonolysis of fucosteryl acetate gave 24-ketocholesteryl acetate, which has been hydrolysed to 24-ketocholesterol, and fucosteryl methyl ether has been converted into 24-ketocholesteryl methyl ether, and fucostadienone into 24-ketocholestenone. It was further found that if the time in the ozonisation of fucosterol was prolonged (*e.g.*, 3 hours with 4% ozone) no acetaldehyde was obtained. This probably provides the reason for the failure of Heilbron, Phipers, and Wright (*loc. cit.*) to isolate acetaldehyde in their experiments.

The isolation and identification of both the fragments resulting from the ozonolysis of fucosterol and its derivatives now place the constitution and configuration of fucosterol beyond all doubt.

Experimental.

Fucosterol.—The fucosterol used in this work was in part extracted directly from *Fucus vesiculosus* by the methods described by Heilbron, Phipers, and Wright (*loc. cit.*). Supplies of *Fucus vesiculosus* were received from Mr. A. H. Fennall, British Schering Manufacturing Laboratories Ltd., and also from Dr. J. Wilkinson of the University College of the South-West, Exeter. Extracts containing crude fucosterol were obtained from Dr. L. N. Owen, Imperial College of Science and Technology, London, S.W.7, from material supplied by the Scottish Seaweed Research Association, and also from Mr. A. H. Fennall. Samples of crude fucosterol were also supplied by Professor E. R. H. Jones. Specimens of crude fucosterol were purified by converting them into the acetate as described below. After recrystallisation the acetate was stirred into boiling methanol containing about 0·2% of sodium methoxide, and pure fucosterol crystallised on cooling. The pure sterol formed soft, feathery crystals, m. p. 124°, $[\alpha]_D^{20}$ −41·8° (*c*, 5 in chloroform). Heilbron *et al.* record m. p. 124°, $[\alpha]_D^{20}$ −38·42°, and MacPhillamy (*loc. cit.*) gives m. p. 121°, $[\alpha]_D^{20}$ −41°.

Fucosteryl Acetate.—Fucosterol (1 g.) was heated on a boiling water-bath with acetic anhydride (5 c.c.) and anhydrous sodium acetate (0·5 g.) for 1 hour. The solution was poured on ice and, after being left overnight, the crystalline product was collected. Recrystallisation from ethanol gave pure fucosteryl acetate (1 g.), m. p. 118°, $[\alpha]_D^{20}$ −45·9° (*c*, 5·1 in chloroform). MacPhillamy records m. p. 118°, $[\alpha]_D$ −45°; Heilbron, Phipers, and Wright record m. p. 118—119°, $[\alpha]_D$ −43·8° (*c*, 5 in chloroform).

Fucosteryl Toluene-p-*sulphonate*.—A solution of fucosterol (2 g.) and toluene-*p*-sulphonyl chloride (1·5 g.) in pyridine (5 c.c.) was kept at room temperature for 4 days. Crystallisation from acetone of the solid which separated on the addition of water gave the *toluene*-p-*sulphonate* (2·2 g.) in fine needles, m. p. 109—110°, $[\alpha]_D^{20}$ −38·4° (*c*, 1·0 in chloroform) (Found: C, 76·4; H, 9·4; S, 5·7. $C_{36}H_{54}O_3S$ requires C, 76·3; H, 9·4; S, 5·6%) {Coffey, Ph.D. Thesis, Univ. Manchester, 1937, records m. p. 108—109°, $[\alpha]_D^{20}$ −38·9° (*c*, 1·4 in chloroform)}.

Fucosteryl Methyl Ether.—A solution of fucosteryl toluene-*p*-sulphonate (2 g.) in methanol (10 c.c.) was boiled under reflux for 1 hour. Recrystallisation from methanol of the crystalline solid which separated on cooling gave the *methyl ether* (1·4 g.), m. p. 70—71°, $[\alpha]_D^{18}$ −47·2° (*c*, 2·2 in chloroform) (Found: C, 84·4; H, 11·6; OMe, 7·2. $C_{30}H_{50}O$ requires C, 84·4; H, 11·7; OMe, 7·3%). Coffey (*loc. cit.*) records m. p. 70—71°, $[\alpha]_D^{20}$ −45·7° (*c*, 2·1 in chloroform).

Fucostadienone.—Fucosterol was oxidised to fucostadienone by the method described by Jones, Wilkinson, and Kerlogue (*J.*, 1942, 391).

Ozonolysis Experiments.—(i) Ozonised oxygen containing 4% by wt. of ozone was passed through a cooled suspension of fucosterol (1 g.) in acetic acid (25 c.c.) and then through a wash-bottle containing water (*ca.* 50 c.c.). After 20 minutes the acetic acid solution and the water were united and diluted with water to 100 c.c. This solution was fractionated through a short column and a solution of *p*-nitrophenylhydrazine (0·5 g.) in acetic acid (20 c.c.; 50%) was added to the first 20 c.c. of the distillate. Recrystallisation of the yellow crystalline precipitate several times from alcohol gave acetaldehyde *p*-nitrophenylhydrazone, m. p. 125—126°, both alone and on admixture with an authentic specimen.

(ii) Ozonolysis of fucosterol (1·25 g.) was carried out for 10 minutes under the conditions described above and a solution of dimedone (0·5 g.) in water (100 c.c.) was added to the first 20 c.c. of the distillate. Recrystallisation of the precipitate from aqueous methanol gave the acetaldehyde–dimedone compound (0·34 g.), m. p. 140—141°, both alone and on admixture with an authentic specimen. After the acetaldehyde had been distilled off from the ozonolysis products, there remained a waxy solid which was collected. Several recrystallisations from methanol gave fucosterol (0·65 g.), m. p. and mixed m. p. 124°.

(iii) Fucosterol (1 g.) was ozonised for 20 minutes as described above. The acetic acid solution so obtained was diluted to 100 c.c. with water and then boiled in an open vessel until free from acetaldeyde. The aqueous suspension was extracted with chloroform, and the chloroform solution washed with aqueous sodium carbonate and dried. Evaporation of the solvent left crude 24-ketocholesterol (0·4 g.). A solution of the latter in methanol deposited a small quantity of fucosterol, and the purified 24-ketocholesterol was obtained by adding water to the methanol solution. Recrystallisation from aqueous methanol gave pure 24-ketocholesterol, m. p. 137—138°, $[\alpha]_D^{15}$ −39° (*c*, 0·89 in chloroform) [semicarbazone, m. p. 167—168°; 2 : 4-*dinitrophenylhydrazone* (recrystallised from ethanol), m. p. 147—148° (Found: C, 68·2; H, 8·2; N, 9·5. $C_{33}H_{48}O_5N_4$ requires C, 68·3; H, 8·3; N, 9·7%)]. Riegel and Kaye (*loc. cit.*) record m. p. 137—138·5°, $[\alpha]_D^{26}$ −37° (*c*, 1 in chloroform), for 24-ketocholesterol, and m. p. 166—168° for the semicarbazone.

(iv) Fucosterol (1 g.) was ozonised for 3 hours as described above. No acetaldehyde was detected in the distillate and no insoluble material separated from the residual solution when these were treated as before.

(v) Ozone (4%) was passed for 30 minutes through a suspension of fucosteryl acetate (1 g.) in cooled glacial acetic acid (50 c.c.). By following the method described above for the isolation of 24-ketocholesterol, there was obtained 24-ketocholesteryl acetate (0·6 g.), which after recrystallisation from alcohol, had m. p. 127—128°, $[\alpha]_D^{15}$ −43° (*c*, 2·38 in chloroform). Riegel and Kaye (*loc. cit.*) record m. p. 127·5—128°, $[\alpha]_D^{26}$ −41° (*c*, 1·9 in chloroform). 24-*Ketocholesteryl acetate* 2 : 4-*dinitrophenylhydrazone*, recrystallised from methanol–ethyl acetate, had m. p. 169—170° (Found: C, 67·1; H, 8·2; N, 9·4. $C_{35}H_{50}O_6N_4$ requires C, 67·5; H, 8·1; N, 9·0%). A solution of 24-ketocholesteryl acetate (0·34 g.) in methanol (25 c.c.) was mixed with methanol (25 c.c.) to which sodium (0·1 g.) had been added. The solution was boiled and then concentrated to half the volume. After addition of water (5 c.c.) and acetic acid (0·2 c.c.) the crystalline precipitate was collected. Recrystallisation from aqueous methanol gave 24-ketocholesterol (0·25 g.), m. p. and mixed m. p. 137—138°. This method for preparing 24-ketocholesterol is preferable to the direct ozonolysis of fucosterol as regards both yield and purity of product.

(vi) Fucosteryl methyl ether (0·9 g.) was ozonised under the conditions described for the acetate. The acetic acid solution was diluted with water and, after it had been boiled to remove acetaldehyde, a solid separated. Several recrystallisations from methanol gave 24-*ketocholesteryl methyl ether* (0·5 g.), m. p. 129—130°, $[\alpha]_D^{20}$ −45·4° (*c*, 1·2 in chloroform) (Found: C, 81·1; H, 11·0. $C_{28}H_{46}O_2$ requires C, 81·2; H, 11·1%) [2 : 4-*dinitrophenylhydrazone*, m. p. 151—152° (Found: C, 68·5; H, 8·3; N, 9·6. $C_{34}H_{50}O_5N_4$ requires C, 68·7; H, 8·4; N, 9·6%)].

(vii) Ozone (3%) was passed for 40 minutes through a suspension of fucostadienone (1 g.) in glacial acetic acid (25 c.c.). After dilution with water and boiling to remove acetaldehyde the precipitated solid was collected. Repeated recrystallisation from methanol gave 24-*ketocholestenone* (0·4 g.), m. p. 90—91° (Found: C, 81·0; H, 10·6. $C_{27}H_{42}O_2$ requires C, 81·2; H, 10·8%) [*bis*-2 : 4-*dinitrophenylhydrazone*, m. p. 228—229° (Found: C, 61·3; H, 6·6; N, 14·6. $C_{39}H_{50}O_8N_8$ requires C, 61·7; H, 6·7; N, 14·8%)].

Reduction of 24-Ketocholesterol to Cholesterol.—Concentrated hydrochloric acid (300 c.c.) was added gradually (10-c.c. portions) during 48 hours to boiling acetic acid (300 c.c.) containing 24-ketocholesterol (1 g.) and amalgamated zinc (20 g.). After cooling, the aqueous liquid was extracted with ether. After the ethereal solution had been washed free from acid with saturated aqueous sodium hydrogen carbonate and dried ($CaCl_2$), the ether was evaporated off under reduced pressure. The residual syrup was boiled with acetic anhydride (3 c.c.). On pouring of the solution on ice a syrup was obtained which, on trituration, followed by recrystallisation from methanol, gave cholesteryl acetate (0·1 g.), m. p. and mixed m. p. with an authentic specimen 113—114°, $[\alpha]_D^{20}$ −45° (*c*, 0·6 in chloroform). Deacetylation

with sodium in methanol, followed by recrystallisation from methanol, gave cholesterol, m. p. and mixed m. p. with an authentic specimen 149—150°. When 24-ketocholesterol (0·5 g.) was reduced by the Wolff–Kishner method under the conditions described by Riegel and Kaye (*loc. cit.*) there was obtained cholesterol (0·15 g.), m. p. and mixed m. p. with an authentic specimen 147—149°.

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