943. *The Steroid Series. Part II.* Further Reactions of Fucosterol and 24-Ketocholesterol.*

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Evidence is presented confirming the structure of fucosterol set out in Part I. Differences in the reactions of cholesterol and fucosterol and their derivatives with thionyl chloride and hydrochloric acid, due to the double bond in the side-chain of fucosterol, are noted.

As reported in Part I,* the isolation of 24-ketocholesterol and acetaldehyde after ozonolysis of fucosterol completed the elucidation of the structure of this compound. Bergmann and Klosty (*J. Amer. Chem. Soc.*, 1951, **73**, 2935) report that they have independently verified our results. Further confirmatory evidence has now been obtained and the full results are summarised in Table 1, which shows clearly that 24-ketocholesterol and its derivatives prepared by us from fucosterol are identical with the compounds prepared by Kaye and Riegel (*J. Amer. Chem. Soc.*, 1944, **66**, 723) from 3-hydroxycholenic acid.

TABLE 1.

	Prepared by Kaye and Riegel		Prepared by direct ozonolysis of the fucosterol derivative		Prepared from 24-ketocholesterol obtained by ozonolysis	
Compound	M. p.	$[\alpha]_D^{20}$	M. p.	$[\alpha]_D^{15}$	M. p.	$[\alpha]_D$
24-Ketocholesterol	137—138·5°	−37°	137—138°	−39°	—	—
acetate	127·5—128	−41	127—128	−43	—	—
toluene-*p*-sulphonate	119—120	−35	—	—	118—119·5°	−33°
24-Keto*cyclo*cholesteryl methyl ether	90·5—91·5	+52	90·5—91	—	90—91	+52
24-Hydroxycholesterol	168—169	—	—	—	166—168	−40
diacetate	95—96	—	—	—	93—94	−43

One method available for protecting the nuclear double bond of fucosterol during ozonisation involved the preparation of *cyclo*fucosteryl methyl ether. The general process for making derivatives of this type (Beynon, Heilbron, and Spring, *J.*, 1936, 907) gave a mixture, chromatographic separation of which yielded an oil (40%), $[\alpha]_D^{20}$ +51°, fucosteryl methyl ether (10%), and fucosterol (40%). The oil was shown to be *cyclo*fucosteryl methyl ether by conversion into 24-ketocholesteryl acetate, and by ozonolysis to 24-keto-*cyclo*cholesteryl methyl ether, identical with a specimen prepared from 24-ketocholesterol and having physical constants similar to those of Kaye and Riegel's compound (*loc. cit.*). Bergmann and Klosty (*loc. cit.*), by the same reactions, obtained *cyclo*fucosteryl methyl ether as an oil, $[\alpha]_D^{23}$ +36°, which they converted into 24-ketocholesteryl acetate.

TABLE 2.

		Sterol		*cyclo*Ether		
Compound	Ref.	$[\alpha]_D$	$[M]_D$	$[\alpha]_D$	$[M]_D$	Molecular rotation difference
Brassicasterol	1	−64°	−255°	+20°	+ 82°	337°
Campesterol	2	−33	−132	+62	+257	389
Cholesterol	3, 4	−39	−151	+55	+220	371
Stigmasterol	1, 3	−49	−202	+35	+149	351
24-Ketocholesterol	—	−39	−156	+52	+215	371
					Mean	364

Refs.: 1, Fernholz and Ruigh, *J. Amer. Chem. Soc.*, 1940, **62**, 3346. 2, Fernholz and Ruigh, *ibid.*, 1941, **63**, 1157. 3, Fieser and Fieser, "Natural Products Related to Phenanthrene," Reinhold, 3rd Edn., 1949, p. 94. 4, Fieser and Fieser, *op. cit.*, p. 252.

$[\alpha]_D^{20}$ of fucosterol = −42°; $[M]_D$ of fucosterol = −173°.
Calc. $[M]_D$ of *cyclo*fucosteryl methyl ether = +191°.
∴ Calc. $[\alpha]_D$ of *cyclo*fucosteryl methyl ether = +45°.

The specific rotation of the oil is in fair agreement with that (+45°) calculated for *cyclo*fucosteryl methyl ether from molecular rotation difference figures ($[M]_D$ of methyl *cyclo*ether − $[M]_D$ of sterol) for a number of sterols (Table 2).

* Part I, *J.*, 1950, 2881.

Beynon, Heilbron, and Spring (*loc. cit.*) found that *cyclo*cholesteryl methyl ether was converted into cholesteryl chloride by hydrochloric acid in acetic acid. This reaction, applied in the present investigation to *cyclo*fucosteryl methyl ether, yielded a crystalline solid (m. p. 77—78°), containing more chlorine (11·6%) than that required (8·1%) for fucosteryl chloride. 24-Keto*cyclo*cholesteryl methyl ether was converted in this way into 24-ketocholesteryl chloride without complication. Now, treatment of cholesterol with thionyl chloride alone gives pure cholesteryl chloride (Fieser and Fieser, *op. cit.*, p. 258), whereas fucosterol with the same reagent has now been shown to give a mixture which, after repeated recrystallisation, yielded 24-chlorofucost-5-enyl chloride. Since fucosterol differs from cholesterol in having an ethylidene group in place of two hydrogen atoms at $C_{(24)}$ this further reaction of fucosterol with thionyl chloride is assumed to be due to the addition of hydrogen chloride there. This accounts for the high chlorine content of the crude fucosteryl chloride obtained above from the *cyclo*ether. Both fucosterol and cholesterol react with thionyl chloride in light petroleum to give the disteryl sulphites.

It was also found in the present work that, whereas hydrochloric acid in acetic acid does not react with cholesteryl acetate, it does add to fucosteryl acetate. However, in all experiments, even after repeated recrystallisation, the product had a low chlorine content. A purer product resulted from treating the acetate with hydrogen chloride in thionyl chloride. The only product from dehydrochlorination of this was fucosteryl acetate, showing that addition had not been at $C_{(5)}$–$C_{(6)}$, otherwise, by analogy with the behaviour of cholesterol (Windaus, *Annalen*, 1927, **453**, 101), fucost-4-enol would have been obtained.

Experimental

Solutions in organic solvents which are described as having been washed and dried, were washed with aqueous sodium carbonate (5%) or dilute sulphuric acid, then with water, and dried over sodium sulphate. When not specified, the light petroleum used had b. p. 60—80°.

Fucosteryl Methyl Ether.—Fucosteryl toluene-*p*-sulphonate (0·15 g.), prepared as described in Part I, was boiled under reflux in methanol (5 ml.) for 10 minutes. Recrystallisation from acetone of the solid which separated on cooling gave fucosteryl methyl ether, m. p. 68—69° $[\alpha]_D^{20}$ −48·5° (*c*, 0·8 in $CHCl_3$).

cyclo*Fucosteryl Methyl Ether.*—Fucosteryl toluene-*p*-sulphonate (0·5 g.) was boiled under reflux for 3 hours in anhydrous methanol (75 ml.) containing freshly fused potassium acetate (0·5 g.). After removal of methanol (50 ml.) by distillation, the solution was diluted with water and extracted with ether. The washed and dried extracts on concentration gave a residue which did not crystallise. Chromatography of a light petroleum solution on alumina and elution with the same solvent gave *cyclo*fucosteryl methyl ether (0·19 g.), $[\alpha]_D^{20}$ +51° (*c*, 1·1 in $CHCl_3$). Further elution with light petroleum–benzene (4 : 1) yielded fucosteryl methyl ether (0·05 g.), m. p. and mixed m. p. with the authentic specimen prepared above, 67—68°. The alumina was finally extracted with methanol for 6 hours in a Soxhlet apparatus. Concentration of the extracts left fucosterol (0·2 g.), m. p. 120—121°, undepressed after mixing with an authentic specimen.

Action of Hydrochloric Acid on cyclo*Fucosteryl Methyl Ether.*—A solution of *cyclo*fucosteryl methyl ether (0·1 g.) in acetic acid (5 ml.) and concentrated hydrochloric acid (0·2 ml.) was left overnight at room temperature, after which the solution was diluted with water and extracted with ether. The extracts were washed, dried, and evaporated. The residue, after three recrystallisations from acetone, gave impure fucosteryl chloride, m. p. 76—77° (Found : C, 77·9; H, 10·3; Cl, 11·6. Calc. for $C_{29}H_{47}Cl$: C, 80·9; H, 10·9; Cl, 8·1%).

Ozonolysis of cyclo*Fucosteryl Methyl Ether.*—A solution of *cyclo*fucosteryl methyl ether (0·7 g., 1 mol.) in chloroform (40 ml.) was ozonised for 25 minutes at room temperature (0·25 g. ozone per hour, 1·2 mols.). After addition of hydrogen peroxide (20 vols.; 40 ml.), the chloroform solution was steam-distilled for an hour. The chloroform solution of the non-volatile gum was washed, dried, and concentrated. The residue (0·6 g.), which did not crystallise, was chromatographed from a light petroleum solution on alumina. On elution with light petroleum–benzene (4 : 1) crude 24-keto*cyclo*cholesteryl methyl ether (0·27 g.) was obtained which, after two recrystallisations from acetone, had m. p. 90·5—91°.

Reaction of 24-*Keto*cyclo*cholesteryl Methyl Ether with Hydrochloric Acid.*—A solution of 24-keto*cyclo*cholesteryl methyl ether (0·18 g.) in acetic acid (5 ml.) containing concentrated hydrochloric acid (0·5 ml.) was left overnight at room temperature. The solid which separated

on dilution with water was recrystallised from methanol and proved to be 24-*ketocholesteryl chloride*, m. p. 97—98°, $[\alpha]_D^{20}$ −28·7° (*c*, 0·8 in $CHCl_3$) (Found: C, 77·1; H, 10·2. $C_{27}H_{43}OCl$ requires C, 77·5; H, 10·3%).

Conversion of 24-*Keto*cyclo*cholesteryl Methyl Ether into* 24-*Ketocholesteryl Acetate.*—A solution of 24-keto*cyclo*cholesteryl methyl ether (0·11 g.) in acetic acid (5 ml.) containing freshly fused zinc acetate (0·3 g.) was boiled under reflux for 6 hours. The cold solution was diluted with water and extracted with chloroform. The chloroform extracts were washed, dried, and evaporated. The residue (0·08 g.) failed to crystallise from acetone, but after purification on an alumina column and recrystallisation from methanol was found to be 24-ketocholesteryl acetate, m. p. 124—125°, undepressed on admixture with authentic compound.

24-*Keto*cyclo*cholesteryl Methyl Ether.*—A solution of 24-ketocholesterol (0·41 g.) and toluene-*p*-sulphonyl chloride (0·05 g.) in dry pyridine (5 ml.) was left overnight at room temperature and then poured on ice. The solid was filtered off and washed with dilute hydrochloric acid, then with water, and dried in a vacuum-desiccator. Recrystallisation from acetone gave 24-keto-cholesteryl toluene-*p*-sulphonate (0·4 g.), softening at 115°, m. p. 118—119·5°, $[\alpha]_D^{20}$ −33° (*c*, 1·1 in $CHCl_3$). Kaye and Riegel (*loc. cit.*) give softening 115°, m. p. 119—120°, $[\alpha]_D^{20}$ −35°, for this compound. The toluene-*p*-sulphonate (0·4 g.) was boiled under reflux for 3 hours in dry methanol (50 ml.) containing freshly fused potassium acetate (0·5 g.). After concentration to half its volume the solution was poured into water and extracted with ether. The ethereal extract was washed, dried, and evaporated, and the residue (0·3 g.) was chromatographed in light petroleum (b. p. 40—60°) on alumina. Elution with light petroleum (b. p. 40—60°)–benzene (10 : 1 ⟶ 1 : 1) gave, after recrystallisation from acetone, 24-keto*cyclo*cholesteryl methyl ether (0·2 g.) in needles, m. p. 90—91° (undepressed after mixture with the compound prepared above), $[\alpha]_D^{20}$ +52° (*c*, 0·73 in $CHCl_3$). Kaye and Riegel (*loc. cit.*) record m. p. 90·5—91°, $[\alpha]_D^{20}$ +52°.

Preparation of 24-*Hydroxycholesterol.*—A solution of 24-ketocholesteryl acetate (0·69 g.) in dry ether (10 ml.) was added gradually to lithium aluminium hydride (0·5 g.) in dry ether (50 ml.). After 15 minutes' boiling under reflux, the complex was hydrolysed with ice-cold dilute hydrochloric acid, and the ethereal layer washed, dried, and evaporated. The residue, recrystallised twice from ethyl acetate, gave 24-hydroxycholesterol (0·6 g.), m. p. 166—168°, $[\alpha]_D^{20}$ −40° (*c*, 2·0 in $CHCl_3$) (Found: C, 80·1; H, 11·4. Calc. for $C_{27}H_{46}O_2$: C, 80·6; H, 11·4%).

Preparation of 24-*Acetoxycholesteryl Acetate.*—(*a*) 24-Hydroxycholesterol (0·25 g.) was refluxed for 3 hours in acetic acid (3 ml.) and acetic anhydride (1 ml.). The product was isolated in ether and gave, after two recrystallisations from methanol, 24-acetoxycholesteryl acetate, m. p. 93—94°, $[\alpha]_D^{20}$ −43° (*c*, 1·1 in $CHCl_3$) (Found: C, 76·2; H, 10·5. Calc. for $C_{31}H_{50}O_4$: C, 76·4; H, 10·6%). (*b*) The same product was obtained by reaction of 24-hydroxycholesterol with acetic anhydride in pyridine. The diacetate (0·2 g.) was converted into 24-hydroxy-cholesterol (m. p. 166—168°) by boiling methanol (10 ml.) containing sodium (0·1 g.).

Reaction of Fucosterol with Thionyl Chloride.—(*a*) A mixture of thionyl chloride (1·5 g.) and fucosterol (0·5 g.) was left at room temperature for 3 hours after the vigorous initial reaction had subsided. The residue left after removal of the thionyl chloride under reduced pressure was dissolved in ether and the solution washed and dried. Evaporation left a gum (0·4 g.) which, after three recrystallisations from acetone, gave 3 : 24-*dichlorofucost-5-ene*, needles, m. p. 108—110° (Found: C, 74·8; H, 10·1; Cl, 15·1. $C_{29}H_{48}Cl_2$ requires C, 74·7; H, 10·3; Cl, 15·0%). (*b*) Fucosterol (0·5 g.) in light petroleum (2 ml.) was added to thionyl chloride (0·25 g.) in light petroleum (1·5 ml.), and after the vigorous initial reaction the mixture was left for an hour before evaporation under reduced pressure. Two recrystallisations of the residue from benzene–acetone gave *difucosteryl sulphite*, m. p. 181—183° (Found: C, 80·6; H, 11·0. $C_{58}H_{94}O_3S$ requires C, 80·3; H, 10·7%).

Dicholesteryl Sulphite.—Cholesterol and thionyl chloride, under similar conditions, gave di-cholesteryl sulphite, m. p. 185—186°. This compound was prepared by a slightly different method by Daughenbaugh and Allison (*J. Amer. Chem. Soc.*, 1929, **51**, 3665), who record m. p. 186—187°.

Addition of Hydrogen Chloride to Fucosteryl Acetate.—(*a*) A solution of fucosteryl acetate (0·3 g.) in acetic acid (80 ml.) containing concentrated hydrochloric acid (3 ml.) was left over-night at room temperature. The solid obtained on dilution with water was dissolved in ether, and the ethereal solution was washed and dried. Evaporation yielded a residue which, after recrystallisation from methanol, had m. p. 115—117°, mixed m. p. with fucosteryl acetate, 108—112°. Two further recrystallisations gave 24-*chlorofucost-5-enyl acetate*, m. p. 117—118°, $[\alpha]_D^{20}$ −42° (*c*, 0·7 in $CHCl_3$) (Found: C, 76·3; H, 10·1; Cl, 5·8. $C_{31}H_{51}O_2Cl$ requires C, 75·9;

H, 10·4; Cl, 7·1%). (*b*) The solution obtained by passing dry hydrogen chloride for 20 minutes through fucosteryl acetate (0·5 g.) in acetic acid (50 ml.) was poured into water and then extracted with ether. Purification of the residue obtained by evaporation of the washed and dried extracts was achieved by chromatography of its solution in light petroleum (b. p. 40—60°) on alumina. The only product obtained was eluted with light petroleum (b. p. 40—60°)–benzene (5 : 1) and was fucosteryl acetate (0·4 g.), m. p. and mixed m. p. 117—118°. (*c*) Dry hydrogen chloride was passed for 30 minutes through a solution of fucosteryl acetate (0·5 g.) in thionyl chloride (10 ml.). The residue left after removal of the thionyl chloride by distillation under reduced pressure gave, after three recrystallisations from ethyl alcohol–ethyl acetate, 24-chlorofucost-5-enyl acetate, m. p. 116—118°, mixed m. p. with fucosteryl acetate, 110—112°. The m. p. was not depressed on admixture with the product obtained by method (*a*) (Found : C, 75·1; H, 10·2; Cl, 6·6%).

Dehydrochlorination of 24-Chlorofucost-5-enyl Acetate.—A solution of the crude acetate (0·3 g.) in dimethylaniline (3 ml.) was boiled under reflux for 10 minutes. The cooled solution was poured into water and extracted with ether. The extracts were washed and dried. Recrystallisation of the residue left after evaporation gave fucosteryl acetate (0·16 g.), m. p. and mixed m. p. 117—118°, mixed m. p. with the chloro-compound 113—115°.

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