62. *Studies in the Sterol Group. Part XXVIII. The Application of the Reformatsky Reaction to 7-Ketocholesteryl Acetate: Δ^5-Cholestene-3 : 7-diol-7-acetic Acid.*

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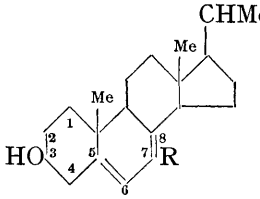
In Part XXVI an attempt was made to prepare a derivative of 7-dehydrocholesterol (I, R = H) by the action of methylmagnesium iodide upon 7-ketocholesteryl acetate. The reaction, however, gave 7-methylenecholesterol (Bann, Heilbron, and Spring, J., 1936, 1274) and not the desired 7-dehydro-7-methylcholesterol (I, R = Me). With the same object in view, the Reformatsky reaction has now been applied to 7-ketocholesteryl acetate.

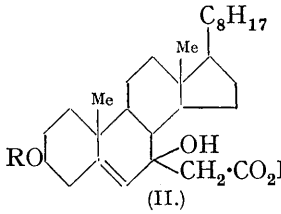
Treatment of 7-ketocholesteryl acetate with ethyl bromoacetate in the presence of zinc gives a mixture from which Δ^5-cholestene-3 : 7-diol-7-acetic acid (II, R = R_1 = H), m. p. 161° (decomp.), is isolated, characterised by its *methyl* ester (II, R = H; R_1 = Me), m. p. 161—161·5°, and by the 3-*monoacetate* (II, R = Ac; R_1 = Me), m. p. 136°, and the 3-*monobenzoate* (II, R = Bz; R_1 = Me), m. p. 158°, of the latter.

Although various methods of dehydration have been applied to (II, R = R′ = H), loss of water is invariably effected with the formation of an exocyclic ethenoid linkage. Whereas

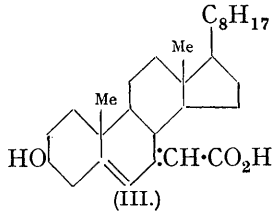
distillation of Δ^5-cholestene-3 : 7-diol-7-acetic acid in high vacuum results in severe decomposition, the 3-monobenzoate and the 3-monoacetate of the methyl ester distil unchanged, a result which is surprising in view of the facile dehydration of 7-hydroxy-7-methylcholesteryl benzoate by this method (Bann, Heilbron, and Spring, *loc. cit.*).



(I.)



(II.)



(III.)

On refluxing Δ^5-cholestene-3 : 7-diol-7-acetic acid with acetic anhydride, 7-methylenecholesterol is obtained, dehydration having been accompanied by decarboxylation. The intermediate stage in this reaction is realised by treatment of Δ^5-cholestene-3 : 7-diol-7-acetic acid with acetic anhydride in pyridine solution; *3-acetoxy-Δ^5-cholestenylidene-7-acetic acid* (III), m. p. 216—217° (decomp.), is then isolated. Its ultra-violet absorption spectrum (broad band, max. 2680A., $\log \varepsilon = 4{\cdot}16$) is in harmony with the proposed structure. If the acid had the alternative conjugated system ($C_{5:6}$–$C_{7:8}$), it would be expected to exhibit the typical absorption spectrum of ergosterol, since the carboxyl group would not be conjugated with the chromophore.

Experimental.

Δ^5-Cholestene-3 : 7-diol-7-acetic Acid.—A mixture of 7-ketocholesteryl acetate (12 g.), ethyl bromoacetate (10 g.), zinc (4 g.), and dry benzene (100 c.c.) was heated on the steam-bath. Cooling was necessary to modify the vigour of the initial reaction, after the cessation of which the mixture was heated under reflux for 6 hours. The cooled mixture was treated with dilute sulphuric acid, the benzene layer washed with water and dried, and the benzene removed. The residual gum, which would not crystallise, was hydrolysed by standing at room temperature for 5 days with a solution of potassium hydroxide (12 g.) in methyl alcohol (100 c.c.). After the addition of ice and dilute hydrochloric acid the mixture was extracted with ether, and the extract washed with water. The acid product was isolated by shaking with aqueous sodium carbonate, acidification producing a tacky solid. Crystallisation was effected from ether–petrol (b. p. 80—100°), from which Δ^5-cholestene-3 : 7-diol-7-acetic acid (3·5 g.) separated in lustrous plates, m. p. 161° (decomp.). With the antimony trichloride reagent, the acid gives an intense royal-blue coloration; $[\alpha]_D^{20°} - 30{\cdot}3°$ ($l = 1$, $c = 1{\cdot}15$ in chloroform). In spite of prolonged desiccation it was not possible to obtain the acid free from solvent. The methyl ester (II, R = H; R_1 = Me), prepared by means of diazomethane, was crystallised from acetone–petrol (b. p. 60—80°) and twice from aqueous methyl alcohol, from which *methyl Δ^5-cholestene-3 : 7-diol-7-acetate* separated in large plates, m. p. 161—161·5°. With the antimony trichloride reagent it gives a green coloration which gradually changes to blue; $[\alpha]_D^{20°} - 49{\cdot}1°$ ($l = 1$, $c = 2{\cdot}52$ in chloroform) (Found: C, 75·9; H, 10·4. $C_{30}H_{50}O_4$ requires C, 75·9; H, 10·6%). *Methyl 3-acetoxy-Δ^5-cholesten-7-ol-7-acetate* (II, R = Ac; R_1 = Me) was prepared by refluxing the methyl ester (0·1 g.) with acetic anhydride (1·5 c.c.) and anhydrous sodium acetate (0·05 g.) for 10 minutes. The solid isolated by water precipitation was crystallised twice from methyl alcohol, from which the acetate separated in fine needles, m. p. 136°; with the antimony trichloride reagent it gives a greenish-blue coloration; $[\alpha]_D^{20°} - 62{\cdot}3°$ ($l = 1$, $c = 0{\cdot}69$ in chloroform) (Found: C, 74·4; H, 9·9. $C_{32}H_{52}O_5$ requires C, 74·4; H, 10·1%). *Methyl 3-benzoyloxy-Δ^5-cholesten-7-ol-7-acetate* (II, R = Bz; R_1 = Me). A solution of the methyl ester (0·1 g.) in pyridine (1 c.c.) and benzoyl chloride (0·4 g.) was set aside at room temperature for 18 hours. The oily mixture was washed with water, and the residue triturated with cold methyl alcohol and crystallised from acetone–methyl alcohol, from which the benzoate separated in fine needles, m. p. 158°. With the antimony trichloride reagent it gives a green coloration; $[\alpha]_D^{20°} - 14°$ ($l = 1$; $c = 0{\cdot}43$ in chloroform) (Found: C, 76·7; H, 9·4. $C_{37}H_{54}O_5$ requires C, 76·8; H, 9·4%).

7-Methylenecholesterol.—A solution of Δ^5-cholestene-3 : 7-diol-7-acetic acid (0·3 g.) in acetic anhydride (4 c.c.) was heated under reflux for 4 hours; the product obtained by addition of

water was isolated by means of ether, and the extract washed with aqueous sodium carbonate. Removal of the solvent gave a gum, which could not be crystallised. It was hydrolysed by refluxing for 30 minutes on the steam-bath with methyl-alcoholic potassium hydroxide (5 c.c., 5%); treatment with water and ether extraction then yielded a semi-solid mass. This was benzoylated with pyridine (0·8 c.c.) and benzoyl chloride (0·1 g.) by standing at room temperature for 24 hours. The reaction mixture was lixiviated with water and the solid produced on grinding the oily residue with methyl alcohol was crystallised twice from acetone, giving 7-methylenecholesteryl benzoate, m. p. 141°, either alone or in admixture with an authentic specimen.

3-*Acetoxy*-Δ^5-*cholestenylidene*-7-*acetic Acid.*—A mixtue of Δ^5-cholestene-3 : 7-diol-7-acetic acid (0·3 g.), pyridine (3 g.), and acetic anhydride (3 g.) was heated on the steam-bath for 30 minutes. On pouring into water an oil separated which solidified on standing at 0° overnight. Crystallisation first from petrol (b. p. 60—80°) and then from methyl alcohol gave 3-*acetoxy*-Δ^5-*cholestenylidene*-7-*acetic acid* (0·1 g.) in aggregates of small prisms, m. p. 216—217° (decomp.). The acid can be extracted from an ethereal solution with aqueous sodium carbonate. It sublimes unchanged in a high vacuum and with the antimony trichloride reagent produces a green coloration which gradually turns to blue; $[\alpha]_D^{20°} - 334·6°$ ($l = 1$, $c = 1·93$ in chloroform) (Found : C, 76·7; H, 10·1. $C_{31}H_{48}O_4$ requires C, 76·8; H, 10·0%).

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