

**310.** *Studies in the Sterol Group. Part XXVII. The Oxidation of Cholesteryl Hydrogen Phthalate with Potassium Permanganate:  $\beta$ -7-Hydroxycholesterol and Tetrahydroxycholestane.*

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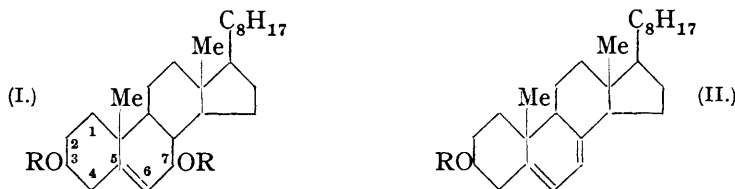
ALTHOUGH potassium permanganate has been successfully employed in the stepwise degradation of many bile acid derivatives (see, for example, Wieland and Kulenkampff, *Z. physiol. Chem.*, 1919, **108**, 295; Wieland and Schulenberg, *ibid.*, 1921, **114**, 167) and of acidic sterol derivatives such as Diels's acid (Windaus, *Ber.*, 1908, **41**, 614), cholesterol itself

is remarkably stable to this reagent in neutral solution, and shaking of a benzene solution of the sterol with alkaline permanganate produces only a small amount of a cholestanetriol (Windaus, *Ber.*, 1907, **40**, 257).

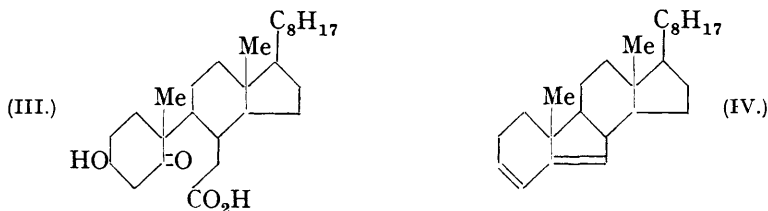
The oxidation of cholesteryl hydrogen phthalate has now been studied with the object of investigating whether such an acid ester is more prone to attack by alkaline potassium permanganate, and three products have been isolated. The least soluble is the acid phthalate of an unsaturated compound  $C_{27}H_{46}O_2$ , m. p. 184—185°. That this is monoethenoid was demonstrated both by quantitative perbenzoic acid titration and by the preparation of a saturated *monoxide*,  $C_{27}H_{46}O_3$ ; catalytic hydrogenation gives a mixture of cholestanol and cholestane. That the compound is a dissecondary alcohol has been shown by the Zerewitinoff method and the preparation of a *diacetate*, m. p. 121—122°, and a *di-benzoate*, m. p. 150—151°.

The new diol is not identical with 3:6-dihydroxy- $\psi$ -cholestene (Westphalen, *Ber.*, 1915, **48**, 1064). A clue to its constitution was afforded by the observation that the diol and its esters give an intense blue coloration with the antimony trichloride reagent, a behaviour which we have observed is common to all derivatives of  $\Delta^5$ -cholestene hydroxylated at  $C_7$ , such as 7-hydroxycholestene (Dimroth and Trautmann, *Ber.*, 1936, **69**, 669), 7-hydroxystigmasterol (Linsert, *Z. physiol. Chem.*, 1936, **241**, 125), and 7-hydroxy-7-methylcholesterol (Bann, Heilbron, and Spring, this vol., p. 1274). Again, we have observed that the crude 7-hydroxycholesterol described by Windaus, Lettré, and Schenck (*Annalen*, 1935, **520**, 98) gives the same intense blue coloration.

A comparison of the constants of corresponding derivatives of the diol, m. p. 184—185°, and 7-hydroxycholesterol (I, R = H) clearly shows that the two are not identical. That they are stereoisomerides, differing from each other solely in the orientation of the hydroxyl group attached to  $C_7$ , was proved by the thermal degradation of the dibenzoate of the former (I, R = C<sub>6</sub>H<sub>5</sub>) to 7-dehydrocholesteryl benzoate (II, R = C<sub>6</sub>H<sub>5</sub>). As no information is available concerning the relative orientation of the  $C_7$  hydroxyl group and the hydrogen atom associated with  $C_8$  in the two 7-hydroxycholesterols, we name the new isomeride  $\beta$ -7-hydroxycholesterol and the diol of Windaus, Lettré, and Schenck,  $\alpha$ -7-hydroxycholesterol.



The second product isolated from the oxidation mixture is a *tetrahydroxycholestane hydrogen phthalate*,  $C_{35}H_{52}O_7$ , m. p. 236—237°, giving a *tetrahydroxycholestane*, m. p. 230—231°, on hydrolysis. The tetrol gives a *tribenzoate*, m. p. 271—272°, and a *triacetate*, m. p. 219—220°; it does not react with the usual carbonyl reagents, from which we infer that the fourth oxygen atom is present as a tertiary hydroxyl group. It is probable that the tetrol is either 3:5:6:7- or 3:4:5:6-tetrahydroxycholestane and we hope to differentiate between these two possibilities by means of experiments which we have in progress.



The third oxidation product of cholesteryl hydrogen phthalate is the acid ester of the hydroxy-keto-acid,  $C_{27}H_{46}O_4$  (III), previously obtained by the oxidation of cholesteryl acetate with chromic acid (Windaus and Resau, *Ber.*, 1915, **48**, 851) and characterised by

its thermal degradation to the diethenoid hydrocarbon (IV) (Lettré, *Z. physiol. Chem.*, 1933, 218, 67).

## EXPERIMENTAL.

*$\beta$ -7-Hydroxycholesterol.*—A suspension of cholesteryl hydrogen phthalate (Weidemann, *Biochem. J.*, 1926, 20, 685) (10 g.) in *N*-sodium carbonate (200 c.c.) was shaken with *N*/4-potassium permanganate (1000 c.c.), added in four equal portions at intervals of 12 hours. The mixture was clarified by sulphur dioxide, and the residual solid collected and dried. Extraction of this solid with cold chloroform-ether (1 : 1) (extract A), followed by repeated crystallisation of the residue from aqueous pyridine, gave  *$\beta$ -7-hydroxycholesteryl hydrogen phthalate* in fine needles, m. p. 199—201° (Found : C, 76.1; H, 9.1.  $C_{35}H_{50}O_5$  requires C, 76.3; H, 9.15%). Hydrolysis of the ester (1 g.) with alcoholic potassium hydroxide (25 c.c.; 10%) gave  *$\beta$ -7-hydroxycholesterol*, which, after several crystallisations from methyl alcohol, separated in fine needles, m. p. 184—185°,  $[\alpha]_D^{20} - 86.4^\circ$  ( $l = 1$ ,  $c = 0.98$  in chloroform) (Found : C, 80.5; H, 12.1.  $C_{27}H_{46}O_2$  requires C, 80.5; H, 11.5%).

*Active hydrogen determination.* 9.40 Mg. of  *$\beta$ -7-hydroxycholesterol* evolved 0.98 c.c. of methane at 20°, corresponding to 1.9 atoms of active hydrogen.

The *diacetate*, prepared by means of acetic anhydride in pyridine solution, separated from aqueous alcohol in needles, m. p. 121—122°,  $[\alpha]_D^{20} - 174.6^\circ$  ( $l = 1$ ,  $c = 0.86$  in chloroform) (Found : C, 76.1; H, 10.35.  $C_{31}H_{50}O_4$  requires C, 76.5; H, 10.3%). The *dibenzoate*, prepared by means of benzoyl chloride in pyridine, separated from alcohol in fine needles, m. p. 150—151°,  $[\alpha]_D^{20} - 105.3^\circ$  ( $l = 1$ ,  $c = 1.32$  in chloroform) (Found : C, 80.6; H, 9.2.  $C_{41}H_{54}O_4$  requires C, 80.6; H, 8.9%).

*$\beta$ -7-Hydroxycholesterol Oxide.*— *$\beta$ -7-Hydroxycholesterol* (1.4 g.) in chloroform (20 c.c.) was treated with a solution of perbenzoic acid in chloroform (1.1 atoms O) and set aside at 0° for 18 hours. After removal of the solvent, the residue was taken up in ether, and the solution washed with aqueous sodium carbonate and then water. Removal of the solvent, followed by repeated crystallisation of the residue from methyl alcohol, gave  *$\beta$ -7-hydroxycholesterol oxide* in small plates, m. p. 173—175°. The oxide gave no coloration either with the antimony trichloride reagent or with tetranitromethane in chloroform (Found : C, 77.5; H, 10.4.  $C_{27}H_{46}O_3$  requires C, 77.5; H, 11.0%).  *$\beta$ -7-Acetoxycholesteryl acetate oxide*, prepared by refluxing the diol oxide with acetic anhydride in pyridine, separated from alcohol in needles, m. p. 203—204° (Found : C, 74.5; H, 9.8.  $C_{31}H_{50}O_5$  requires C, 74.0; H, 10.0%).

*Thermal Decomposition of  $\beta$ -7-Benzoyloxycholesteryl Benzoate.*—The dibenzoate was heated in a retort at 200° and  $1 \times 10^{-3}$  mm. for 4 hours. The residue after trituration with acetone was crystallised from chloroform-acetone, from which 7-dehydrocholesteryl benzoate separated in plates, m. p. 134—136° and clearing completely at 170°. The compound showed no depression on admixture with an authentic specimen, prepared by the method of Windaus, Lettré, and Schenck (*loc. cit.*); it exhibited the typical ultra-violet absorption spectrum of ergosterol (Found : C, 83.6; H, 10.2. Calc. for  $C_{34}H_{48}O_2$  : C, 83.5; H, 9.9%).

*Tetrahydrocholestane.*—The chloroform-ether extract A on concentration gave a crystalline mass, which after repeated crystallisation from ethyl acetate yielded *tetrahydrocholestane hydrogen phthalate* in needles, m. p. 236—237° (Found : C, 72.3; H, 8.9.  $C_{35}H_{52}O_7$  requires C, 71.9; H, 9.0%). Hydrolysis of the ester with alcoholic potassium hydroxide (10%), followed by crystallisation of the product from aqueous alcohol, gave *tetrahydrocholestane* in fine needles, m. p. 235—236°, which contained solvent of crystallisation (Found : C, 71.0; H, 11.4.  $C_{27}H_{48}O_4 \cdot H_2O$  requires C, 71.3; H, 11.1%). The anhydrous tetrol, m. p. 230—231°, was obtained by high-vacuum sublimation of the solvated material (Found : C, 74.5; H, 10.8.  $C_{27}H_{48}O_4$  requires C, 74.3; H, 11.1%). Crystallisation of the anhydrous tetrol from aqueous alcohol gave the solvated tetrol, m. p. 235—236°.

The *triacetate*, prepared from the hydrated tetrol by refluxing with acetic anhydride, separated from aqueous alcohol in needles, m. p. 219—220°,  $[\alpha]_D^{20} + 23.1^\circ$  ( $l = 1$ ,  $c = 0.65$  in chloroform) (Found : C, 70.7; H, 9.6.  $C_{33}H_{54}O_7$  requires C, 70.4; H, 9.7%). The *tribenzoate* separated from methyl alcohol in fine needles, m. p. 271—272°,  $[\alpha]_D^{20} + 114.0^\circ$  ( $l = 1$ ,  $c = 1.77$  in chloroform) (Found : C, 76.8; H, 8.1; *M*, 746.  $C_{48}H_{60}O_7$  requires C, 76.95; H, 8.1%; *M*, 748).

*Hydroxy-keto-acid,  $C_{27}H_{46}O_4$  (III).*—The chloroform-ether mother-liquors from tetrahydrocholestane hydrogen phthalate on further concentration gave intractable resin. The solvent was completely removed, and the residue (8 g.) hydrolysed by refluxing with alcoholic potassium hydroxide (160 c.c.; 10%). After dilution with water the mixture was extracted with ether, and the aqueous solution acidified with dilute sulphuric acid. The oily acids were isolated by

means of ether and boiled repeatedly with water to remove phthalic acid. The residual acidic material could not be induced to crystallise; it was directly distilled at 1 mm., the pale yellow oil obtained partly solidifying in the receiver. The semi-solid distillate was taken up in ether and washed with dilute sodium carbonate solution. After removal of solvent, the residue was repeatedly crystallised from alcohol; the diethenoid hydrocarbon (IV) then separated in needles, m. p. 76°, showing no depression on admixture with the hydrocarbon prepared by a similar distillation of the acid fraction of the oxidation products of cholesteryl acetate with chromic acid (Found : C, 88.2; H, 12.2. Calc. for  $C_{26}H_{42}$  : C, 88.1; H, 11.9%).

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