302. *Studies in the Sterol Group. Part XXXIII. The Constitution of the Isomeric Ethers of Cholesterol (Continued from Part XXIX).*

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The dextrorotatory ethers of cholesterol are shown to contain an unstable bridge-ring system and to be derived from *i*-cholesterol. The latter exhibits the anomalous reactions towards the halogen acids and bromine previously described as characteristic of the dextrorotatory ethers. Crystallographic data establish that *i*-cholesterol is hydroxylated at C_3; the location of the unstable bridge-ring is discussed.

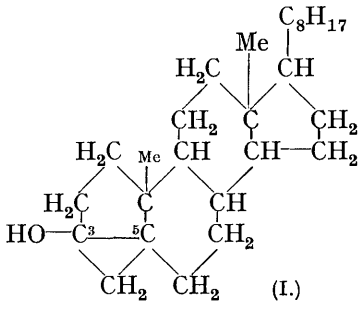
In an attempt to invert the hydrogen and the hydroxyl group attached to C_3 in the naturally occurring sterols, Stoll ascertained that, whereas cholesteryl *p*-toluenesulphonate reacts with alcohols to give the corresponding normal lævorotatory cholesteryl ethers without stereochemical inversion, yet in the presence of potassium acetate isomeric dextrorotatory ethers are formed (*Z. physiol. Chem.*, 1932, **207**, 147). Wagner-Jauregg and Werner (*ibid.*, 1932, **213**, 119) have also found that cholesteryl chloride reacts similarly, in that with methyl alcohol at 125° in a bomb-tube it is converted into the normal cholesteryl methyl ether, whereas in the presence of potassium acetate the dextrorotatory methyl ether is formed.

The two series of ethers are sharply differentiated in their reactivity; whereas the normal ethers are unaffected by the halogen acids, the dextrorotatory ethers are smoothly converted into the corresponding cholesteryl halides (Beynon, Heilbron, and Spring, J., 1936, 907). Again, with nitric acid, normal cholesteryl methyl ether gives 6-nitrocholesteryl methyl ether, whilst the dextrorotatory ether is converted into 6-nitrocholesteryl nitrate, a result which led us to conclude that both ethers contain an ethylenic linkage at C_5–C_6 (Beynon, Heilbron, and Spring, this vol., p. 406). Stoll (*Z. physiol. Chem.*, 1937, **246**, 14), from a consideration of optical rotatory power, has recently suggested that *allo*cholesterol is the parent of the dextrorotatory methyl ether, and states that the lability of the latter is in favour of this view. It must be emphasised, however, that *allo*cholesterol is not readily

rearranged to cholesterol (Schoenheimer and Evans, *J. Amer. Chem. Soc.*, 1936, **58**, 182; *J. Biol. Chem.*, 1936, **114**, 567) but tends to undergo dehydration to $\Delta^{2:4}$-cholestadiene. In the same connection we find that, in contradistinction to *allo*cholesterol and its epimer, the dextrorotatory methyl ether fails to give a coloration with the Rosenheim reagent.

A new clue to the nature of the dextrorotatory cholesteryl methyl ether was forthcoming with the observation that it is recovered unchanged after treatment with ozone; again, it does not absorb oxygen from a solution of perbenzoic acid, nor does it give a coloration with tetranitromethane in chloroform solution. These results not only show that this methyl ether is a saturated compound and hence pentacyclic, but lend support to the view expressed by Wallis, Fernholz, and Gephart that the dextrorotatory cholesteryl ethers are related to the recently described *i*-cholesterol, the acetate of which is formed by the action of acetic anhydride upon cholesteryl *p*-toluenesulphonate in the presence of potassium acetate (*J. Amer. Chem. Soc.*, 1937, **59**, 137).

Since Stoll (*loc. cit.*, 1932) has shown that cholesterol is formed by warming cholesteryl *p*-toluenesulphonate with *aqueous* acetone, it is to be expected by analogy that, if the hydrolysis be effected in the presence of an excess of potassium acetate, the parent alcohol of the dextrorotatory cholesteryl methyl ether would be formed. We find that, using these conditions, *i*-cholesterol is obtained in high yield, and its relationship to the dextrorotatory methyl ether is established by its conversion into the latter by direct methylation. In its behaviour *i*-cholesterol closely resembles the dextrorotatory ethers in that, on treatment with the halogen acids or with bromine, it yields the corresponding cholesteryl halide and tribromocholestane respectively (cf. Beynon, Heilbron, and Spring, *loc. cit.*, 1936). The recognition of the pentacyclic nature of the isomeric cholesteryl ethers and of *i*-cholesterol leaves unsettled their molecular configuration. We cannot subscribe to the provisional structure proposed by Wallis, Fernholz, and Gephart (*loc. cit.*), which is based upon the observation that on oxidation of *i*-cholesterol with chromic acid a ketone was isolated (as oxime). In view of the susceptibility of *i*-cholesterol to rearrangement, this evidence cannot be taken to indicate the presence of a secondary hydroxyl group.



(I.)

A crystallographic examination of *i*-cholesterol and its derivatives, undertaken at our request by Miss F. Bell, B.A., in the Physics Department of this University, has unequivocally demonstrated that the hydroxyl group of the former is attached to C_3; the detailed results of this investigation will be communicated later. In view of the lability of the C_3-alkoxy-group in the *i*-cholesteryl ethers, and the ease with which *i*-cholesterol derivatives are isomerised to the corresponding derivatives of cholesterol (Δ^5), it is tempting to postulate that *i*-cholesterol contains a labile bridge joining C_3 and C_5 (I). Experiments designed to test the validity of this representation are in progress.

Experimental.

i-Cholesteryl Acetate.—A solution of cholesteryl *p*-toluenesulphonate (3 g.) and anhydrous potassium acetate (4 g.) in aqueous acetone (100 c.c.; 50%) was heated under reflux for 6 hours. The oil separating on dilution with water was isolated by means of ether and heated for 2 hours with alcoholic potassium hydroxide solution (60 c.c.; 5%), diluted with water, and extracted with ether. After removal of the solvent from the washed and dried extract, the residual oil was taken up in warm 90% alcohol (15 c.c.) and set aside overnight with a solution of digitonin in 90% alcohol (500 c.c.; 1%). The separated digitonide was collected, and the solvent removed from the filtrate under diminished pressure. The residual solid was triturated with cold ether, the ethereal extract washed with water, and dried (sodium sulphate). After removal of the solvent, the crude *i*-cholesterol was heated on the steam-bath for 2 hours with acetic anhydride (20 c.c.), and the solid separating on cooling was crystallised from alcohol, from which *i*-cholesteryl acetate (1·2 g.) separated in clusters of needles, $[\alpha]_D^{20°} + 48{\cdot}1°$ ($l = 1$, $c = 1{\cdot}6$ in chloroform), m. p. 73° showing no depression on admixture with that obtained by the method of Wallis, Fernholz, and Gephart (*loc. cit.*) (Found: C, 81·4; H, 11·1. Calc. for $C_{29}H_{48}O_2$:

C, 81·2; H, 11·3%). *i*-Cholesteryl acetate is more soluble than cholesteryl acetate in the usual organic solvents; it does not give a coloration with tetranitromethane or antimony trichloride in chloroform, or with the Rosenheim reagent.

i-*Cholesterol.*—*i*-Cholesteryl acetate (1 g.) was heated under reflux for 1 hour with alcoholic potassium hydroxide solution (40 c.c.; 4%). The oil separating on dilution with water was isolated by means of ether and crystallised from alcohol at −10°, *i*-cholesterol being obtained in needles, $[\alpha]_D^{20°}$ + 27° (l = 1, c = 1·5 in chloroform), m. p. 68—69°, not depressed on admixture with that obtained by the method of Wallis, Fernholz, and Gephart (*loc. cit.*) (Found: C, 84·0; H, 11·7. Calc. for $C_{27}H_{46}O$: C, 83·8; H, 12·0%).

Cholesteryl Chloride from i-*Cholesterol.*—A solution of *i*-cholesterol (0·1 g.) in glacial acetic acid (30 c.c.) was treated with concentrated hydrochloric acid (0·1 c.c.). After standing at room temperature overnight, the mixture was diluted with water, and the resulting solid was crystallised from acetone, from which cholesteryl chloride separated in laminæ, $[\alpha]_D^{20°}$ − 27·0° (l = 1, c = 1 in chloroform), m. p. 95°, unchanged by admixture with an authentic specimen.

Tribromocholestane.—A solution of bromine in ether (15 c.c.; 1%) was added to *i*-cholesterol (0·1 g.) in ether (40 c.c.), and the mixture set aside at room temperature for 12 hours. The solvent was removed from the colourless solution, and the residue crystallised from alcohol to give tribromocholestane in prisms, $[\alpha]_D^{20°}$ − 49·0° (l = 1, c = 1·08 in chloroform), m. p. 112—113°, not depressed by admixture with an authentic specimen.

Methylation of i-*Cholesterol.*—*i*-Cholesterol (0·5 g.) was added to a suspension of finely divided potassium (0·2 g.) in dry benzene (30 c.c.), and the mixture heated under reflux for 1 hour. After addition of methyl iodide (30 c.c.), the heating was continued for a further 4 hours. Treatment with alcohol, followed by removal of the solvent mixture under diminished pressure, gave a solid which was extracted with ether, the extract washed with water, and dried (sodium sulphate). The oil obtained after removal of the solvent was crystallised from acetone, giving *i*-cholesteryl methyl ether in needles, $[\alpha]_D^{19°}$ + 54° (l = 1, c = 1·3 in chloroform), m. p. 79°, not depressed in admixture with the dextrorotatory cholesteryl methyl ether prepared by the method of Stoll (*loc. cit.*, 1937) (Found: OMe, 7·8. Calc. for $C_{28}H_{48}O$: OMe, 7·75%).

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