208. *Studies in the Sterol Group. Part XXV. An Investigation of Some Reactions of the Isomeric Ethers of Cholesterol.*

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The facile interaction of cholesteryl *p*-toluenesulphonate with alcohols, whereby isomeric cholesteryl ethers are formed (Stoll, *Z. physiol. Chem.*, 1932, **207**, 147), suggested this ester as a suitable subject for investigation in the search for a method for the preparation of the epimeride of cholesterol.

Stoll showed that when cholesteryl *p*-toluenesulphonate was refluxed with methyl alcohol a cholesteryl methyl ether, m. p. 84°, was obtained identical with that previously prepared by heating cholesteryl chloride, magnesium, and methyl alcohol in a sealed tube (Diels and Blumberg, *Ber.*, 1911, **44**, 2847). On the other hand, when the reaction was

carried out in the presence of potassium acetate, an isomeric methyl ether, m. p. 79°, was produced.

Although the relationship of these two ethers has not been defined, it appeared probable to us when this investigation was initiated that the lævorotatory isomer, m. p. 84°, was the methyl ether of "*trans*"-cholesterol, and that the dextrorotatory isomer, m. p. 79°, was that of "*cis*"-cholesterol (*epi*cholesterol).*

A study was therefore made of the hydrolysis of cholesteryl *p*-toluenesulphonate in the hope that under controlled conditions "*cis*"-cholesterol (cf. Marker, Oakwood, and Crooks, *J. Amer. Chem. Soc.*, 1936, **58**, 481) would be made available, but unfortunately this has not proved to be the case. Whereas treatment of the ester with either aqueous potassium hydroxide or aqueous potassium acetate produced a mixture of dicholesteryl ether and cholesterol, potassium acetate in acetic acid solution gave "*trans*"-cholesteryl acetate. Moist silver oxide suspended in water, or dilute sulphuric acid gave dicholesteryl ether, and alcoholic sulphuric acid yielded "*trans*"-cholesteryl ethyl ether.

Attention was next directed to the possibility of demethylating "*cis*"-cholesteryl methyl ether. To our great surprise, we observed that treatment of this ether with halogen acids in acetic acid at room temperature effects the replacement of the methoxyl group by the corresponding halogen atom. In the same way it was observed that "cis"-*cholesteryl ethyl ether*, m. p. 47°, and "cis"-*cholesteryl benzyl ether*, b. p. 170°/0·001 mm., prepared by us using the method of Stoll (*loc. cit.*), both react similarly with hydrogen halides. In this way, by employing hydrogen iodide, the hitherto unknown *cholesteryl iodide*, m. p. 107°, has now been prepared (cf. Kolm, *Monatsh.*, 1912, **33**, 447). No reaction of the "*trans*"-cholesteryl methyl, ethyl, and benzyl ethers with halogen acids is observed under these conditions.

The same facile replacement of the alkoxy-groups in these "*cis*"-cholesteryl ethers was observed on treatment with bromine at room temperature. In each case 3 : 5 : 6-tribromocholestane (Kolm, *loc. cit.*) was produced, a behaviour contrasting sharply with that of the "*trans*"-ethers, which reacted normally with bromine to give the corresponding 5 : 6-*dibromides*, from which on debromination with sodium iodide according to the method of Schönheimer (*J. Biol. Chem.*, 1935, **110**, 461) the original unsaturated ethers were regenerated.

The ease of replacement of the alkoxy-groups in the "*cis*"-ethers necessitates consideration of the possibility that during their formation the Δ^5-ethylenic linkage of cholesterol has migrated to the Δ^4-position (cf. Stoll, *loc. cit.*). We consider this unlikely, however, since the "*cis*"-ethers fail to give a positive colour reaction with the antimony trichloride reagent in contrast to the authentic Δ^4-compounds, *allo*cholesterol, *allo*cholesteryl methyl ether (unpublished work), and ψ-cholestene, each of which gives an immediate deep pink coloration. The investigation is proceeding.

Experimental.

Reactions of Cholesteryl p-*Toluenesulphonate.*—(a) *Aqueous potassium acetate.* The ester (2 g.) was refluxed for 4 hours with aqueous potassium acetate (5%; 100 c.c.), and the solid product filtered off and triturated with hot acetone. The residue was crystallised from ether, dicholesteryl ether (1 g.) separating in long felted needles, m. p. 197—198°, $[\alpha]_D^{19°}$ − 38·4° † (l = 1; c = 3·28). Mauthner and Suida (*Monatsh.*, 1896, **17**, 38) record m. p. 194—195° for this compound (Found: C, 86·1; H, 12·0. Calc. for $C_{54}H_{90}O$: C, 85·9; H, 12·0%). Concentration of the acetone extract yielded a solid, repeated crystallisation of which from methyl alcohol

* The nomenclature developed by Ruzicka and Wettstein (*Helv. Chim. Acta*, 1935, **18**, 986) being adopted, "*trans*"-cholesterol is normal Δ^5-cholesterol, the hydroxyl group of which has the same configuration relative to ring A as that in cholestanol, which is *trans*-oriented relative to the C_5 hydrogen atom. The terms "*cis*" and "*trans*" will be used throughout this communication in order to differentiate the isomeric pairs of cholesteryl ethers with the reservation that the assumed configurations still require confirmation.

† All rotations recorded were measured in chloroform solution; the analyses were carried out by Mr. W. F. Boston.

gave cholesterol in plates, $[\alpha]_D^{20°}$ − 39·0° (l = 1; c = 3·8), m. p. 148°, showing no depression on admixture with an authentic specimen.

(b) *Potassium acetate in acetic acid.* A solution of cholesteryl *p*-toluenesulphonate (2 g.) in glacial acetic acid (50 c.c.) was refluxed for 3 hours with potassium acetate (2 g.). After dilution with water, the separated solid was collected and crystallised from ethyl acetate, giving cholesteryl acetate (1·1 g.) in needles, $[\alpha]_D^{20°}$ − 29·6° (l = 1; c = 3·76), m. p. 115°, unchanged by admixture with an authentic specimen.

(c) *Aqueous potassium hydroxide.* Cholesteryl *p*-toluenesulphonate (2 g.) was refluxed for 4 hours with potassium hydroxide solution (10%; 100 c.c.). Subsequent procedure as described under (a) gave dicholesteryl ether (1·1 g.), m. p. 197—198° (Found: C, 85·9; H, 11·8%), and cholesterol, m. p. 148°.

(d) *Silver oxide.* A suspension of cholesteryl *p*-toluenesulphonate (2 g.) and silver oxide (4 g.) in water (10 c.c.) was heated for 3 hours at 150° in a sealed tube. Extraction with ether, followed by removal of the solvent from the dried extract, gave dicholesteryl ether, m. p. 197—198°.

(e) *Aqueous sulphuric acid.* Cholesteryl *p*-toluenesulphonate (1 g.) in suspension in 4*N*-sulphuric acid (20 c.c.) was heated for 3 hours at 130° in a sealed tube. Crystallisation of the product from ether gave dicholesteryl ether (0·6 g.) in needles, m. p. 197—198°.

(f) *Alcoholic sulphuric acid.* A solution of the ester (1 g.) in alcohol (15 c.c.) and 4*N*-sulphuric acid (15 c.c.) was refluxed for 6 hours and then diluted with water. The precipitated solid was collected and crystallised from acetone, giving "*trans*"-cholesteryl ethyl ether (0·6 g.) in needles, m. p. 88·5°, not depressed on admixture with the ether described below.

(g) *Sulphuric acid in acetic acid.* The ester (0·5 g.) was refluxed for 5 hours with glacial acetic acid (15 c.c.) and 5% sulphuric acid (5 c.c.) and then diluted with water. The solid was collected and crystallised from acetone, giving dicholesteryl ether (0·1 g.), m. p. 197—198°.

Concentration of the mother-liquors and crystallisation of the solid separating on cooling gave cholesterol in plates (from methyl alcohol), m. p. 147—148°.

"*trans*"-Cholesteryl ethyl ether prepared by the method of Stoll (*loc. cit.*) separated from alcohol in leaflets, m. p. 88·5°, $[\alpha]_D^{21°}$ − 39·0° (l = 1; c = 4·2). Müller and Page (*J. Biol. Chem.*, **1933**, **101**, 127) give m. p. 88·5° and $[\alpha]_D^{22°}$ − 39·4° for this ether (Found: C, 83·95; H, 12·1. Calc. for $C_{29}H_{50}O$: C, 84·0; H, 12·2%).

"cis"-*Cholesteryl Ethyl Ether.*—Cholesteryl *p*-toluenesulphonate (11 g.) was added to a solution of potassium acetate (12 g.) in absolute alcohol (500 c.c.) and refluxed for 6 hours. After concentration and cooling to − 10° a crystalline mass separated, which on repeated crystallisation from acetone yielded "cis"-*cholesteryl ethyl ether* (5 g.) in laminæ, m. p. 47°, $[\alpha]_D^{20°}$ + 49·78° (l = 1; c = 1·607) (Found: C, 84·2; H, 12·2. $C_{29}H_{50}O$ requires C, 84·0; H, 12·2%).

"*trans*"-Cholesteryl benzyl ether prepared by the method of Stoll (*loc. cit.*) separated from acetone in needles, m. p. 118·5°, $[\alpha]_D^{20°}$ − 25·8° (l = 1; c = 3·76). Steinkopf and Blümner (*J. pr. Chem.*, 1911, **84**, 460) give m. p. 118·5°, $[\alpha]_D$ − 26·0° (Found: C, 85·3; H, 10·6. Calc. for $C_{34}H_{52}O$: C, 85·6; H, 11·0%).

"cis"-*Cholesteryl Benzyl Ether.*—A solution of cholesteryl *p*-toluenesulphonate (15 g.) and potassium acetate (15 g.) in benzyl alcohol (500 c.c.) was heated for 6 hours on the steam-bath. The solid which separated on cooling was crystallised from acetone, giving "*trans*"-cholesteryl benzyl ether (2 g.), m. p. 118°.

The benzyl alcohol mother-liquor was distilled in steam, and the residue extracted with ether. After removal of solvent from the dried extract the residue was distilled, giving "cis"-*cholesteryl benzyl ether* (5 g.) as an oil, b. p. 170°/0·001 mm., $[\alpha]_D^{20°}$ + 15·77° (l = 1; c = 17·94) (Found: C, 84·8; H, 11·0. $C_{34}H_{52}O$ requires C, 85·6; H, 11·0%).

The Action of Hydrogen Halides on "cis"-*Cholesteryl Methyl Ether.*—(a) *Hydrogen chloride.* A solution of "*cis*"-cholesteryl methyl ether (2 g.) in glacial acetic acid (400 c.c.) and concentrated hydrochloric acid (20 c.c.) was kept over-night at room temperature. The reaction mixture was diluted with water, and the precipitated solid crystallised from acetone, giving cholesteryl chloride (2 g.) in prisms, $[\alpha]_D^{20°}$ − 27·4° (l = 1; c = 4·41), m. p. 95°, unchanged by admixture with an authentic specimen prepared by the method of Mauthner and Suida (*Monatsh.*, **1894**, **15**, 85).

(b) *Hydrogen bromide.* "*cis*"-Cholesteryl methyl ether (2 g.), on treatment with hydrogen bromide (*d* 1·4; 20 c.c.) in glacial acetic acid under the conditions described under (a), gave cholesteryl bromide (2·2 g.) in laminæ (from acetone), $[\alpha]_D^{20°}$ − 20·8° (l = 1; c = 8·85), m. p. 98°, unchanged by admixture with cholesteryl bromide, m. p. 98°, prepared by the method of

Kolm (*loc. cit.*) (Found: C, 72·2; H, 10·0; Br, 17·8. Calc. for $C_{27}H_{45}Br$: C, 72·1; H, 10·1; Br, 17·8%).

(c) *Hydrogen iodide.* Treatment of "*cis*"-cholesteryl methyl ether with hydrogen iodide (*d* 1·7; 20 c.c.) as described above gave *cholesteryl iodide*, which separated from ethyl acetate in needles, m. p. 106·5—107°, $[\alpha]_D^{20°}$ — 11·94° ($l = 1$; $c = 3·6$) (Found: C, 65·3; H, 9·1; I, 25·0. $C_{27}H_{45}I$ requires C, 65·3; H, 9·1; I, 25·6%).

The Action of Bromine on "cis"*-Cholesteryl Methyl Ether.*—A solution of dry bromine in glacial acetic acid (9·6%; 50 c.c., 1·2 mols.) was added to "*cis*"-cholesteryl methyl ether (10 g., 1 mol.) in ether (100 c.c.) and kept over-night. The solid (8 g.) precipitated with alcohol (100 c.c.) was crystallised twice from ether–alcohol, 3 : 5 : 6-tribromocholestane being obtained in colourless prisms, $[\alpha]_D^{19°}$ — 49·3° ($l = 1$; $c = 4·966$), m. p. 112—113°, unchanged by admixture with tribromocholestane, m. p. 112—113°, prepared by the method of Kolm (*loc. cit.*) (Found: C, 53·2; H, 7·5; Br, 39·5. Calc. for $C_{27}H_{45}Br_3$: C, 53·2; H, 7·4; Br, 39·4%). Tribromocholestane is obtained quantitatively when "*cis*"-cholesteryl methyl, ethyl, or benzyl ether is treated with excess of bromine.

"trans"*-Cholesteryl Methyl Ether Dibromide.*—A solution of bromine in glacial acetic acid (8%; 50 c.c., 2 mols.) was added to "*trans*"-cholesteryl methyl ether (5 g., 1 mol.) in ether (50 c.c.). The solid (6 g.) separating over-night was crystallised twice from ether–alcohol, yielding "trans"*-cholesteryl methyl ether dibromide* in plates, m. p. 107°, $[\alpha]_D^{20°}$ — 52·95° ($l = 1$; $c = 3·607$) (Found: C, 60·0; H, 8·7; Br, 29·0. $C_{28}H_{48}OBr_2$ requires C, 60·0; H, 8·6; Br, 28·6%).

"trans"*-Cholesteryl ethyl ether dibromide*, obtained from "*trans*"-cholesteryl ethyl ether by the same procedure, crystallised from ether–alcohol in hexagonal plates, m. p. 80°, $[\alpha]_D^{20°}$ — 50·75° ($l = 1$; $c = 4·9$) (Found: C, 60·8; H, 8·9; Br, 28·05. $C_{29}H_{50}OBr_2$ requires C, 60·6; H, 8·8; Br, 27·9%).

"trans"*-Cholesteryl benzyl ether dibromide*, similarly obtained from "*trans*"-cholesteryl benzyl ether, separated from ether–alcohol in silky needles, m. p. 107°, $[\alpha]_D^{20°}$ — 50·2° ($l = 1$; $c = 4·76$) (Found: C, 64·4; H, 8·0; Br, 25·15. $C_{34}H_{52}OBr_2$ requires C, 64·1; H, 8·2; Br, 25·1%).

The Action of Sodium Iodide on Tribromocholestane.—A solution of tribromocholestane (3 g.) in dry benzene (50 c.c.) was refluxed for 6 hours with sodium iodide (6 g.) in absolute alcohol (30 c.c.). After washing with aqueous sodium sulphite and water and drying, the solvent was removed, and the residue crystallised from acetone, giving cholesteryl bromide in laminæ, $[\alpha]_D^{19°}$ — 21·4° ($l = 1$; $c = 3·2$), m. p. 98°, unchanged by admixture with that obtained by the action of hydrogen bromide on the "*cis*"-cholesteryl ethers.

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