263. *Studies in the Sterol Group. Part XLI. A New Epimerisation Process.*

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A novel process for the conversion of sterols into their epimeric forms, in which the hydroxyl group assumes the alternative steric position to the ring plane, has been discovered, involving heating under reflux with aluminium *iso*propoxide in xylene solution. By this means *epi*cholesterol and *epi*lumisterol have now been obtained directly from the normal sterols, but attempts similarly to epimerise ergosterol, or to prepare *epi*ergosterol by other methods, have not been successful.

Dorée and Gardner (J., 1908, **93**, 1630) studied the action of sodium and boiling amyl alcohol on coprostanol and observed the production of an isomeric compound (*epi*coprostanol) and, since both sterols gave coprostanone on oxidation, concluded that the difference between these isomers lay solely in the position of the hydroxyl group relative to the ring plane. This phenomenon was examined further by Windaus and Uibrig (*Ber.*, 1914, **47**, 2384; 1915, **48**, 857) and Windaus (*Ber.*, 1916, **49**, 1724), who found that cholestanol under similar conditions (or with sodium in boiling xylene or sodium ethoxide at 180°) was partially converted (10%) into *epi*cholestanol (the mixture being resolved with digitonin), and that with coprostanol the equilibrium mixture contained some 90% of the epimeric form. Such methods of epimerisation, which have been extensively employed by numerous workers in the steroid field, suffer from the disadvantage that with unsaturated sterols simultaneous reduction or dehydration may occur. For example, saturated sterols are obtained by treating cholesterol with sodium ethoxide at 180° and ergosterol is converted under the same conditions into a mixture of the dihydrosterol and its epimer (Windaus, Anhagen, Bergmann, and Butte, *Annalen*, 1930, **477**, 268; Heilbron, Johnstone, and Spring, J., 1929, 2248), and fucosterol with sodium and amyl alcohol gives *epi*-β-dihydrofucosterol (Coffey, Heilbron, Spring, and Wright, J., 1935, 1205). Both dihydrolumisterol and *iso*lumisterol epimerise readily with sodium ethoxide at 200°, but lumisterol is dehydrated to a hydrocarbon by this method (Windaus, Dithmar, and Fernholz, *Annalen*, 1932, **493**, 259).

An alternative route to epimeric steroid alcohols involves the reduction, either catalytically or with aluminium *iso*propoxide, of the corresponding ketones; a mixture of both normal and *epi*-forms is usually produced, separation being effected either by fractional crystallisation or by resolution with digitonin. It is significant that in the catalytic process, when an *acid* medium is employed, cholestanone and coprostanone give mainly *epi*cholestanol and coprostanol respectively. On the other hand the latter two saturated compounds are the minor constituents (10%) of the equilibrium mixture obtained by epimerisation in an *alkaline* medium, *e.g.*, sodium and amyl alcohol.

*epi*Cholesterol has not been prepared previously by direct isomerisation of cholesterol, but has been obtained, together with cholesterol as a partial racemate, by oxidation of cholesterylmagnesium chloride (Marker, Kamm, Oakwood, and Laucius, *J. Amer. Chem. Soc.*, 1936, **58**, 1948), by partial hydrogenation of 3-keto-Δ^5-cholestene (Δ^5-cholestenone) with a Raney nickel catalyst (Ruzicka and Goldberg, *Helv. Chim. Acta*, 1936, **19**, 1407), and by Wolff–Kishner reduction of the product obtained by the action of potassium acetate in acetic acid on 7-ketocholesteryl chloride (Marker, Kamm, Fleming, Popkin, and

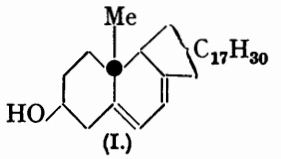
Wittle, *J. Amer. Chem. Soc.*, 1937, **59**, 619; U.S.P. 2,177,355, *Chem. Abstr.*, 1940, **34**, 1038). Resolution with digitonin is usually necessary to ensure the quantitative separation of pure *epi*cholesterol, but an alternative procedure involving fractional crystallisation of the mixed acetates and finally the benzoates has been devised (Marker, Kamm, Oakwood, and Laucius, *loc. cit.*).

We have now found that a number of sterols are epimerised to varying extents by heating under reflux with aluminium *iso*propoxide in a neutral solvent. Thus a treatment of cholesterol in xylene for 48 hours gave, on resolution with digitonin, a 10% yield of *epi*cholesterol (*benzoate*, m. p. 99·5°). A similar treatment of cholestanol yielded some 4% of epimeric form, and *neo*ergosterol has been converted into *epineo*ergosterol in 15% yield, a process previously effected by the employment of sodium amyloxide (Windaus and Deppe, *Ber.*, 1937, **70**, 76).

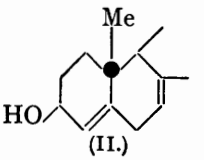
Lumisterol under these conditions is readily converted into *epi*lumisterol in yields as high as 40%, the crystalline racemate, m. p. 156—158° (Heilbron, Kennedy, Spring, and Swain, J., 1938, 869), of lumisterol and its epimer being resolved with digitonin. The high yields of epimer obtained in this case and the ease with which the racemate can be crystallised and identified, enabled us conveniently to discover the optimum conditions for this epimerisation process. Substitution of aluminium *tert.*-butoxide for the *iso*propoxide results in considerably diminished yields of *epi*lumisterol. The optimum temperature appears to be that of boiling xylene, since the use of benzene or toluene gives rather poorer yields and at higher temperatures (decalin) a marked tendency towards the formation of gums is observed, probably due to dehydration. That under given conditions an equilibrium is established has been demonstrated by the production of lumisterol from *epi*lumisterol by heating with aluminium *iso*propoxide in xylene.

Numerous attempts to epimerise ergosterol by this method have been unsuccessful. Treatment in xylene gave, after digitonin precipitation, only an impure ergostatetraene formed by dehydration of the sterol or its epimer; at lower temperatures (benzene) minute yields of material, m. p. about 180°, not precipitable with digitonin and exhibiting absorption at 2880 A., which may be impure *epi*ergosterol (I), have been obtained. The preparation of *epi*ergosterol claimed by Marker, Kamm, Laucius, and Oakwood (*J. Amer. Chem. Soc.*, 1937, **59**, 1840) has been refuted (Windaus and Buchholz, *Ber.*, 1938, **71**, 576), but recently the latter authors (*Ber.*, 1939, **72**, 1597) have described the isolation of traces of impure *epi*ergosterol from the reduction product of ergostatrienone. No details of the properties of this substance were given and it is therefore impossible for a comparison to be made.

An alternative approach to the preparation of *epi*ergosterol (I) appeared to be possible by the isomerisation of ergostatrienol (*epiallo*ergosterol) (II), a product of the reduction of ergostatrienone with aluminium *iso*propoxide.



(I.)



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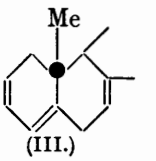

(III.)

Windaus and Buchholz (*Ber.*, 1939, **72**, 597) observed that this compound is dehydrated, either by treatment with boiling methyl alcohol containing a little hydrochloric acid or on attempted epimerisation with sodium ethoxide at 180°, to an ergostatetraene, m. p. 86·5°. We find that sublimation in a high vacuum (10^{-3} mm.) both of (II) and of its acetyl derivative in the presence of ferric chloride leads to the formation of the same hydrocarbon, m. p. 86—87°, as observed by the German authors (max. 2820 A., $\varepsilon = 32{,}000$). The strong dextrorotatory power and location of the absorption maximum suggest the constitution (III) for this hydrocarbon (cf. Bergmann and Hirschmann, *J. Org. Chem.*, 1939, **4**, 29). High-vacuum sublimation with traces of iodine, mercuric chloride, etc., irradiation in acetone solution, and shaking with platinum oxide in methyl alcohol were without effect on the ergostatrienol and adsorption of the acetate on alumina yielded a

small quantity of a substance, m. p. 131—132°, which may be *epi-iso*ergosteryl acetate (max. 2370 A., $E^{1\%}_{1\,cm.} = 330$).

Experimental.

The aluminium *iso*propoxide was freshly distilled before use.

epi*Cholesterol.*—(a) A solution of cholesterol (20 g.) and aluminium *iso*propoxide (53 g.) in dry xylene (500 c.c.) was refluxed for 48 hours. The cooled mixture was poured into dilute hydrochloric acid and extracted with ether, the ethereal solution being washed with dilute acid and water and dried. The solvents were removed under diminished pressure and the crude solid, m. p. 122—127°, obtained was twice crystallised from alcohol, yielding unchanged cholesterol (9·5 g.), m. p. 145—146°. Concentration of the combined mother-liquors gave a second fraction (4·5 g.), m. p. 127—129°, which was acetylated, and, on crystallisation from acetone, cholesteryl acetate (2·5 g.) was isolated. The acetate mother-liquors were then hydrolysed and combined with the cholesterol residue and the total material was resolved with digitonin (7 g.) in the usual manner. The solid fraction (4 g.), not precipitable with digitonin, was adsorbed on alumina (100 g.) from light petroleum (b. p. 40—60°), the chromatogram being developed with this solvent. After separation of a portion containing a small quantity of brown oil, further elution and crystallisation of the residue from methyl alcohol gave pure *epi*cholesterol (2 g.), m. p. 140·5°, $[\alpha]_D^{20°}$ — 34° ($l = 1$, $c = 2·7$ in chloroform) (lit., m. p. 141°, $[\alpha]_D^{20°}$ — 35°). (The remainder of the non-precipitable portion could not be crystallised and, since it gave a red coloration with antimony trichloride in chloroform, it probably contained some cholestadiene, formed by dehydration of the sterol.) Treatment with acetic anhydride for 2 hours on the steam-bath gave *epi*cholesteryl acetate, which separated from alcohol in flat needles, m. p. 84—85° (lit., m. p. 85°). epi*Cholesteryl benzoate*, prepared in the usual manner with benzoyl chloride and pyridine in the cold and purified by percolation through alumina from benzene solution, separated from methyl alcohol in plates, m. p. 99·5°, $[\alpha]_D^{20°}$ — 29° ($l = 1$, $c = 1·8$ in chloroform) (Found: C, 83·45; H, 10·55. $C_{34}H_{50}O_2$ requires C, 83·2; H, 10·3%).

(b) A mixture of cholesterol (2 g.), aluminium *iso*propoxide (5 g.), and dry benzene (70 c.c.) was refluxed for 24 hours. The crude product, isolated as described above, was immediately resolved with digitonin, yielding, as non-precipitable portion after crystallisation from methyl alcohol, *epi*cholesterol (350 mg.), m. p. 138—139°, undepressed on admixture with a specimen prepared by method (a).

3-*Keto*-Δ^4-*cholestene from* epi*Cholesterol.*—*epi*Cholesterol (200 mg.) in dry acetone (6 c.c.) was refluxed for 24 hours with freshly distilled (at 10^{-3} mm.) aluminium *tert.*-butoxide (800 mg.) and dry benzene (18 c.c.). The solvents were removed in a vacuum, and the residue repeatedly extracted with ether. The oil obtained from the washed and dried extracts crystallised in contact with ether–methyl alcohol at 0° and after one recrystallisation from methyl alcohol gave 3-keto-Δ^4-cholestene, m. p. 80°, not depressed on admixture with authentic material.

epi*Cholestanol.*—A solution of cholestanol (7 g.; m. p. 140—141°; $[\alpha]_D^{20°}$ + 23° in chloroform) and aluminium *iso*propoxide (25 g.) in dry xylene (350 c.c.) was refluxed for 48 hours. The crude product, m. p. 127—130°, isolated in the usual manner, after two crystallisations from alcohol gave cholestanol (4·5 g.), m. p. 139—140°. Attempts fractionally to crystallise the remainder of the product being unsuccessful, the mixture of epimers was resolved with digitonin, and from the non-precipitable portion, after two crystallisations from alcohol, *epi*-cholestanol (300 mg.) was isolated, m. p. 182—184°, undepressed on admixture with an authentic specimen; $[\alpha]_D^{20°}$ + 32·2° ($l = 1$, $c = 2·0$ in chloroform) (lit., m. p. 184°, $[\alpha]_D^{20°}$ + 33·9°). The acetate had m. p. 92—94°, both alone and mixed with *epi*cholestanyl acetate.

epineo*Ergosterol.*—A mixture of *neo*ergosterol (300 mg., m. p. 152°), aluminium *iso*propoxide (1 g.), and dry xylene (20 c.c.) was refluxed for 24 hours. Recrystallisation of the crude product from ether–alcohol gave *epineo*ergosterol (50 mg.), m. p. 170° (lit., m. p. 176°).

epi*Lumisterol.*—A mixture of lumisterol (10 g., m. p. 116°), aluminium *iso*propoxide (50 g.), and dry xylene (350 c.c.) was refluxed for 48 hours. The crude product was isolated as previously described and the material from four such experiments (40 g. of lumisterol) was crystallised from methyl alcohol, from which solvent the lumisterol–*epi*lumisterol complex (31 g.) separated in clusters of fine needles, m. p. 156—158°, $[\alpha]_D^{20°}$ + 199° ($l = 1$, $c = 0·6$ in chloroform). On resolution of the complex with digitonin in the usual way, recovery from the precipitated digitonide yielded *epi*lumisterol (15 g.) in needles from methyl alcohol, m. p. 113°, undepressed on admixture with a specimen prepared by the reduction of lumistatrienone (Heilbron, Kennedy, Spring, and Swain, *loc. cit.*). *Light absorption in alcohol:* Maximum, 2780 A., $\log \varepsilon = 4·08$.

Epimerisation of epi*Lumisterol.*—A mixture of *epi*lumisterol (1 g.), aluminium *iso*propoxide (5 g.), and dry benzene (40 c.c.) was refluxed for 24 hours. The crude product, m. p. 108—130°, on recrystallisation from methyl alcohol yielded the lumisterol–*epi*lumisterol complex (130 mg.), m. p. 149—155°.

Attempted Epimerisation of Ergosterol.—The two following experiments are representative of the numerous attempts which have been made to prepare *epi*ergosterol. (*a*) A mixture of ergosterol (10 g.), aluminium *iso*propoxide (50 g.), and dry xylene (300 c.c.) was refluxed for 48 hours, and the crude product isolated in the usual manner; resolution with digitonin yielded unchanged ergosterol (8·4 g.). The non-precipitable fraction crystallised slowly from alcohol to yield an impure ergostatetraene (1·3 g.), m. p. 83—93°, which was recovered unchanged on attempted benzoylation. *Light absorption in alcohol:* Maxima, 2800, 3170, 3310 A., $E^{1\%}_{1cm.}$ 150.

(*b*) A solution of ergosterol (20 g.) and aluminium *iso*propoxide (50 g.) in dry benzene (700 c.c.) was refluxed in the dark in an atmosphere of nitrogen for 160 hours. The crude product was crystallised thrice from alcohol and the ergosterol obtained (14·5 g.), m. p. 155—156°, was neglected. From the residual mother-liquors a solid fraction (5 g.), m. p. 153—156°, was isolated and this was resolved with digitonin (5 g.). The non-precipitable portion (100 mg.) had m. p. 175—182° and after adsorption on alumina yielded two fractions, m. p. 185—190° and 173—176°, exhibiting similar light absorption in alcohol: Maximum, 2880 A., $E^{1\%}_{1cm.}$ 100.

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