329. *Studies in the Sterol Group. Part XVIII. An Attempt to Define the Position of the Hydroxyl Group in Ergosterol.*

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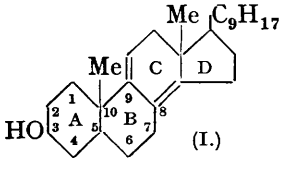
Chuang (*Annalen*, 1933, **500**, 270) has demonstrated that ergostane can be oxidised to give *allo*norcholanic acid, $C_{23}H_{38}O_2$, thus providing formal proof that the nuclear skeleton of ergosterol is identical with that of cholesterol and the bile acids. The above investigation fails to furnish, however, evidence as to the position of the secondary alcohol group in ergosterol, although from the fact that ergostanol yields on oxidation a dicarboxylic acid, $C_{28}H_{48}O_4$, from which a pyro-ketone, $C_{27}H_{46}O$, can be obtained (Reindel, *Annalen*, 1928, **466**, 131), it has been generally assumed that the hydroxyl group in ergosterol is situated in Ring A and attached to C_3 (I) as in cholesterol (compare Wieland and Dane, *Z. physiol. Chem.*, 1932, **212**, 41).



(I.)

At the same time many of the reactions of ergosterol are at variance with the assumption that its hydroxyl group is on C_3, and indicate rather that it is in close proximity to an activating system of double bonds. Examples of such reactions are afforded by (1) the mobility of the hydroxyl group in neoergosterol (Bonstedt, *Z. physiol. Chem.*, 1929,

185, 165), (2) the specific production from ergosterol of ergopinacol (Windaus and Borgeaud, *Annalen*, 1928, **460**, 235), and (3) the formation of ketones by the vacuum-distillation of ergosterol, α- and β-dihydroergosterols, and α- and β-ergostenols with copper–bronze, a reaction which does not occur with the saturated ergostanol (unpublished observation).

Prior to the appearance of Chuang's paper we had ourselves been engaged in an investigation dealing with the questions of the nuclear identity of ergosterol with the bile acids and also of the precise position of its hydroxyl group. With this end in view attempts were made to degrade the above-mentioned dibasic acid according to the method of Windaus and Riemann (*Z. physiol. Chem.*, 1923, **126**, 277) to either a tribasic acid, $C_{24}H_{38}O_6$, or its nor-derivative, but this line of attack failed, as it proved impossible to isolate a pure compound from the oxidation mixture. We therefore turned our attention to chloroergostane, $C_{28}H_{49}Cl$, from which we obtained on oxidation with chromic anhydride a *chloronorcholanic acid*, $C_{23}H_{37}O_2Cl$, m. p. 213° (Heilbron and Simpson, *Nature*, 1933, **131**, 438), which forms a *methyl* ester, m. p. 158—159°. Since the work of Chuang (*loc. cit.*) shows that ergostane belongs to the *allo*cholanic acid series, it follows that the acid, m. p. 213°, must be 3-chloro-*allo*norcholanic acid (or its epi-derivative) if the hydroxyl group in ergosterol be actually attached to C_3. In order to establish this point, an authentic specimen of 3-*chloro*allo*norcholanic acid* was prepared from 3-chloro*allo*cholanic acid (Windaus and Hossfeld, *Z. physiol. Chem.*, 1925, **145**, 177) according to the method of Wieland, Schlicting, and Jacobi (*Z. physiol. Chem.*, 1926, **161**, 80). This *acid*, m. p. 238° (regenerated from the ester), and its *methyl* ester, m. p. 176°, show strong depressions in m. p. on admixture with the corresponding compounds derived from chloroergostane. That, however, both acids possess the formula $C_{23}H_{37}O_2Cl$ is shown by the fact that reduction of their esters with sodium and propyl alcohol gives one and the same *allo*norcholanic acid identical in physical properties with that described by Chuang (*loc. cit.*).

These results, which definitely prove that the acid of m. p. 213° from chloroergostane is not 3-chloro*allo*norcholanic acid, admit of only two possible interpretations. Either a *cis-trans* isomeric change has occurred during the chlorination of one or other of the sterols, or alternatively the hydroxyl group in ergosterol is attached to a different carbon atom from that in cholesterol. Of these two possibilities we regard the former as the less probable, for it is difficult to believe that phosphoryl chloride should exhibit selective action with two such closely related secondary alcohols.

With the object of eliminating the possibility of stereoisomeric change it is our intention to degrade both methoxyergostane and methoxycholestane to their corresponding *allo*norcholanic acids. Further, we are also engaged in the attempted preparation of other chloro*allo*norcholanic acids in order to compare these compounds with the acid derived from chloroergostane.

Experimental.

Chloroergostane.—Dried ergostanol was triturated with phosphoryl chloride according to the method of Reindel and Walter (*Annalen*, 1928, **460**, 212). After the reaction had ceased, the mass was thoroughly digested with hot water for 1½ hours, taken up in ether, washed with dilute sodium carbonate solution, dried, and evaporated. Crystallisation of the slightly coloured residue first from ether–alcohol and then from benzene–alcohol yielded chloroergostane in flattened needles having a constant m. p. 117—119° (Reindel gives m. p. 119—121°). Yield 50%.

?-*Chloro*allo*norcholanic Acid.*—A solution of the above chloride (6 g.) in glacial acetic acid (240 c.c.) was treated at 95° during 3½ hours with a solution of chromic anhydride (18 g.) in water (9 c.c.) and glacial acetic acid (36 c.c.). The whole was then maintained at the same temperature with mechanical stirring for a further 5½ hours. The cooled solution was diluted slightly with water, reduced with sulphur dioxide, concentrated under reduced pressure to a thick syrup to remove acetic acid, taken up in water, and thoroughly extracted with ether. The extract was first washed with water and then with 10% potassium hydroxide solution, when an infilterable precipitate of the potassium salt separated at the interface. This was drawn off with the alkaline layer and removed by centrifuging. The crude salt was purified by repeated centrifuging with water and finally by digestion with ether. The free *acid* was obtained by shaking a suspension of the salt in ether with dilute hydrochloric acid; the

ethereal solution was washed, dried, and evaporated, yielding an oil which separated in solid form from acetic acid or aqueous acetone. As it was found impossible to purify further the acid at this stage by direct crystallisation, it was reconverted into its potassium salt, which was further purified by centrifuging, etc. The acid regenerated as before was twice crystallised from ether–light petroleum (m. p. 205—208°) and then twice from glacial acetic acid, from which it separated in long glistening needles, m. p. 213—214°. The acid is easily soluble in ether, acetone, and hot acetic acid and very sparingly soluble in light petroleum; it forms an unstable ammonium salt. The yield of pure acid was extremely small (0·1 g. from 18 g. of chloroergostane) (Found: C, 72·7; H, 10·0; Cl, 9·7. $C_{23}H_{37}O_2Cl$ requires C, 72·5; H, 9·7; Cl, 9·3%). From the neutral portion of the oxidation product, 3—4 g. of unchanged chloroergostane were recovered. Acidification of the centrifuged alkaline liquors yielded an oily acid which has not yet been investigated.

The above procedure must be strictly adhered to in order to obtain reproducible results. In one experiment, in which the conditions of purification were slightly altered, the only product obtained was a minute quantity of a chlorine-free acid, m. p. 219—221° (methyl ester, m. p. 81°). Analysis of this showed it to be a dicarboxylic acid, probably identical with the acid (m. p. 217—219°; methyl ester, m. p. 81—83°) obtained by Reindel by oxidation of ergostanol (Found: acid, C, 75·3; H, 10·4; ester, C, 76·0; H, 10·8. $C_{28}H_{48}O_4$ requires C, 75·0; H, 10·7%. $C_{30}H_{52}O_4$ requires C, 75·7; H, 10·9%). It would thus appear that ring scission between the >CHCl–CH_2–group occurs to some extent during the oxidation.

Methyl ?-chloroallonorcholanate, obtained by treatment of the acid with ethereal diazomethane, crystallised from either chloroform–methyl alcohol or aqueous ethyl alcohol in long needles, m. p. 158—159° (Found: C, 73·3, 73·3; H, 9·5, 10·0; Cl, 9·1; OMe, 7·7. $C_{24}H_{39}O_2Cl$ requires C, 73·0; H, 9·9; Cl, 9·0; OMe, 7·9%).

Cholesteryl Chloride.—The following procedure was found to be satisfactory for the preparation of this compound in bulk. Recrystallised cholesterol (320 g. of m. p. 147°) was treated in 20 g. portions with thionyl chloride (13—14 c.c.) and left for 11 hours. The reaction product was taken up in methyl alcohol (50 c.c. per portion), and the cold solid filtered off, washed once with methyl alcohol, and well pressed. After two recrystallisations of the whole from acetone (2000 c.c.) the pure chloride was obtained in stout prisms, m. p. 95° (yield, 80%).

3-*Chloro*allo*cholanic Acid.*—For the oxidation of chlorocholestane it was found convenient to work with the quantities given below (compare Windaus and Hossfeld, *loc. cit.*). A solution of chlorocholestane (m. p. 112—113°) in quantities of 10 g. in glacial acetic acid (250 c.c.) was oxidised with chromic anhydride (30 g. in 40 c.c. of water) added during 3 hours, heating being then continued for a further 4 hours (stirring and temperature of 95° throughout). The solution was worked up as described in the oxidation of chloroergostane, and the pure acid obtained in needles, m. p. 175—176° after two or three crystallisations from acetic acid (95%). The yield of pure acid was only about 2% of the weight of chlorocholestane taken, but about 25% of the latter was always recovered unchanged.

Methyl 3-*Chloro*allo*cholanate.*—Windaus and Hossfeld record m. p. 128° for this compound. We now find that esterification (diazomethane) of the pure acid yields an ester having m. p. 133—134°. If, however, the reaction were carried out with a rather impure specimen of the acid, the m. p. of the ester could not be raised above 128—130°. Hydrolysis of this material, however, with alcoholic potassium hydroxide gave an acid which could now be obtained in pure form, and on re-esterification gave the pure ester, m. p. 133—134° (Found for ester, m. p. 133—134°: C, 73·6; H, 10·1; Cl, 8·8. Calc. for $C_{25}H_{41}O_2Cl$: C, 73·4; H, 10·0; Cl, 8·7%).

3-*Chloro*allo*norcholanic Acid.*—Methyl 3-chloro*allo*cholanate (0·5 g.) in ether (16 c.c.) was added to a Grignard solution prepared from magnesium turnings (0·1 g.) and bromobenzene (0·7 g.) in ether (4 c.c.), and the mixture refluxed for 3 hours; the ether was then driven off, and the product heated at 100° for 3 hours, decomposed under ether with dilute sulphuric acid, washed, evaporated, and steam-distilled. The non-volatile diphenylcarbinol was dissolved in ether, dried, and after removal of solvent obtained as a yellow gum which could not be crystallised; it was insoluble in methyl alcohol, moderately soluble in acetic acid, and dissolved readily in ether, ethyl acetate and light petroleum.

The diphenylcarbinol was therefore oxidised directly in glacial acetic acid solution (8 c.c.) at 87° with chromic anhydride (1 g.) in water (1 c.c.) and glacial acetic acid (4 c.c.) added during 1½ hours with frequent shaking. After a further 1½ hours at the same temperature the solution was cooled and worked up in the manner already described. The Grignard reaction and oxidation were repeated several times with different quantities of ester (reagents in proportion) and gave the same results. 3-*Chloro*allo*norcholanic acid* was obtained after two

crystallisations from acetic acid (in which it was less soluble than the acid, m. p. 213—214°) in glistening prismatic needles, m. p. 232—234°. A satisfactory analysis could not be directly obtained, since the acid held firmly some acetic acid of crystallisation, but after regeneration from its methyl ester and crystallisation from ethyl acetate it had m. p. 238° and was solvent-free (Found: C, 72·4; H, 10·0; Cl, 9·3. $C_{23}H_{37}O_2Cl$ requires C, 72·5; H, 9·7; Cl, 9·3%).

Methyl 3-*chloro*allo*norcholanate*, obtained from the acid by means of ethereal diazomethane, crystallised from chloroform–methyl alcohol in long needles, m. p. 176° (Found: C, 72·8; H, 9·8; Cl, 9·2. $C_{21}H_{39}O_2Cl$ requires C, 73·0; H, 9·9; Cl, 9·0%). A mixture of this ester with the isomeride from chloroergostane had m. p. 135—140°.

allo*Norcholanic Acid*.—(1) *From* 3-*chloro*allo*norcholanic acid*. The methyl ester of this acid (45 mg., m. p. 176°) was refluxed in *n*-propyl alcohol (50 c.c.) at 105—115°. Sodium (5 g.) was added in small pieces during 1½ hours, and heating was continued for a further 1½ hours. The propyl alcohol was removed in steam after dilution, and the solid residue was filtered off, acidified with dilute sulphuric acid, extracted with ether, and washed with water. The ethereal solution was then shaken with 10% potassium hydroxide solution, whereby a precipitate of potassium *allo*norcholanate was produced. This was removed, acidified with dilute sulphuric acid, and the acid taken up in ether. Evaporation of the washed and dried extract yielded a crystalline residue which after two crystallisations from acetone had a constant m. p. 170—170·5° and formed glistening plates. Chuang (*loc. cit.*) records m. p. 170° for *allo*norcholanic acid.

(2) *From* ?-*chloro*allo*norcholanic acid* (from chloroergostane). The ester, m. p. 158—159° (13 mg.), was reduced in a similar manner. The product after one crystallisation from acetone formed glistening plates, m. p. 168—169°, and a mixture of it with the product from (1) melted at 169—170°.

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