CXXI.—*Studies in the Sterol Group. Part III. The Acetylation and Catalytic Hydrogenation of Ergosterol.*

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We suggested in a previous paper, on the evidence adduced from spectrographic results (J., 1928, 47), that two of the ethenoid linkings in ergosterol occupy the same position in the molecule as those in α-cholesterilene, and in a second contribution (*ibid.*, p. 347) it was pointed out that one of the double bonds in the latter compound must be attached to carbon atom 1. In an attempt definitely to establish the existence in ergosterol of a double bond in this position, a study has now been made of its catalytic hydrogenation.

Previous investigators on the hydrogenation of ergosterol have always employed the well-known acetyl derivative in their experiments. This choice does not seem to us altogether happy, because the acetate is abnormal in that its melting point, values of which have been recorded between 172° and 180°, is higher than that of the free sterol, whereas in the case of cholesterol, sitosterol, and stigmasterol the melting points of the acetates are all lower.

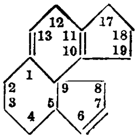
Hydrogenation of the free sterol both in ether at room temperature and in alcohol at 70° in the presence of palladium-black gives rise, when carried to completion, to the α-ergostenol of Reindel and Walter (*Annalen*, 1928, **460**, 212). A study of the rate of addition of hydrogen in ethereal solution revealed a sharp break after the addition of two atoms, and by arresting the process at this stage it has been found possible to isolate in good yield the intermediate dihydroergosterol identical with that obtained by Windaus and Brünken (*Annalen*, 1928, **460**, 225) by the reduction of ergosterol with sodium and alcohol.

The employment of glacial acetic acid as solvent at a temperature of 70° resulted in the formation of the saturated *allo*-α-ergostanol acetate, identical with that obtained by Reindel and Walter (*loc. cit.*) by hydrogenation of β-ergostenol acetate. Windaus and Linsert (*Annalen*, 1928, **465**, 148) have also brought about the addition of six atoms of hydrogen to ergosterol in hot glacial acetic acid containing a little hydrochloric acid in the presence of platinum-black; these authors do not, however, state the nature of the product.

That the complete saturation of ergosterol under our conditions is not prefaced by any shift of an ethenoid linking prior to hydrogenation has been demonstrated by experiments on the action of acetic acid upon the sterol in an inert atmosphere. These show that acetylation takes place, giving rise to a new acetate (m. p. 132—133°) the absorption spectrum of which is identical with that of ergosterol and of the already well-known acetyl derivative. We consider that this product is the normal acetate of the sterol and consequently propose to designate it as *ergosterol α-acetate*; the acetate prepared by the action of acetic anhydride will be referred to as ergosterol β-acetate. Ergosterol α-acetate yields ordinary ergosterol on hydrolysis with alcoholic potash, and hydrogenation in ethereal solution in the presence of palladium-black converts it into α-ergostenol acetate. The above evidence points to the isomerism between the two being stereochemical: this view is supported by the fact that the α-isomeride changes into the β-form either when heated above its melting point or when boiled with acetic anhydride.

Of the three double bonds in ergosterol, the one which resists hydrogenation in ether or alcohol must be the one which is not present in cholesterilene, since no difficulty is experienced in fully hydrogenating the latter substance in ether at room temperature. It follows, therefore, from the work of Windaus (*Nach. Ges. Wiss. Göttingen*, Jan., 1926) that in the hydrogenation of ergosterol it should be possible to obtain stereoisomeric forms of both ergostenol and ergostanol. Although only one ergostenol has so far been observed as a direct product of hydrogenation, two ergostanols have been prepared, for hydrogenation of ergosterol in acetic acid gives rise, as pointed out above, to *allo*-α-ergostanol acetate and hydrogenation of ergosterol β-acetate in ethereal solution in the presence of Adams's platinum oxide results in the formation of the isomeric α-ergostanol acetate (Reindel and Walter, *loc. cit.*), which these authors consider differs from its isomeride by asymmetry in the nucleus.

The behaviour of ergosterol on hydrogenation is thus reminiscent of that of abietic acid, where the use of hot glacial acetic acid as solvent is necessary for complete saturation (compare Ruzicka and Meyer, *Helv. Chim. Acta*, 1922, **5**, 315; Ruzicka and Pfeiffer, *ibid.*, 1925, **8**, 632; Madinaveita, *Chem. Abstr.*, 1923, **17**, 288). The resistant double bond in this case joins two quaternary carbon atoms, and we venture tentatively to suggest by analogy that in ergosterol the third double bond may be similarly disposed. Such a position is to be found between carbon atoms 10 and 11 (see formula, where a suggested nuclear structure is shown). With regard to this possibility, attention must be directed to the recent work of Wieland and Vocke on the bile acids (*Z. physiol. Chem.*, 1928, **177**, 68). These authors consider that their results indicate the presence of a methyl group attached to carbon 11 (see also *Ann. Reports*, 1928, **25**, 162). In such a case the resistant double bond may be between carbon atom 10 and the side chain bearing carbon atom 19.



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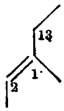

EXPERIMENTAL.

Hydrogenation of Ergosterol in Ether and in Alcohol.—Ergosterol (1 g.) was dissolved in dry ether (150 c.c.) and hydrogenated at a pressure slightly greater than atmospheric in the presence of palladium-black (1 g.). The solution was filtered from the catalyst and, after addition of about one-third of its volume of methyl alcohol, left in a covered beaker to crystallise. The first crop (0·5 g.) consisted of flat needles, m. p. 128—130°. Recrystallisation from ether–methyl alcohol gave a product, m. p. 130—131°, the value recorded in the literature for α-ergostenol. The second crop (0·2 g.), third crop (0·1 g.), and fourth crop (0·1 g.) all consisted of less pure forms of the same substance.

Ergosterol (2 g.) was dissolved in absolute alcohol (150 c.c.) at 70° and hydrogenated for 5 hours in the presence of palladium-black. The filtered solution was concentrated and allowed to crystallise. The first crop (0·5 g.) melted at 122—126°, the second (0·5 g.) at 125—127°, and the third (0·2 g.) at 116—124°. (The loss of material is attributed to its absorption by the palladium.) The three crops were combined and recrystallised from ether–methyl alcohol, giving flat needles, m. p. 130—131°, showing no depression in admixture with the α-ergostenol prepared by hydrogenation in ethereal solution.

Dihydroergosterol.—Ergosterol (5 g.) was dissolved in ether (400 c.c.) and hydrogenated in the presence of palladium-black (2 g.) until 350 c.c. of hydrogen were absorbed (calc. for one double bond, 295 c.c.). Excess of hydrogen was purposely used, as the separation of traces of ergosterol from dihydroergosterol is a tedious process, whereas α-ergostenol may be removed from dihydroergosterol by crystallisation with comparative ease. The product was fractionally crystallised from ether–methyl alcohol and the first two crops were combined (3·2 g.) and repeatedly crystallised from alcohol. Dihydroergosterol was thus obtained in plates, m. p. 173°, $[\alpha]_{5461}^{20^\circ}$ −21·7° (c = 1·248 in chloroform). The acetate, prepared by the action of acetic anhydride, melted at 180° [Windaus and Brünken (*loc. cit.*) give for dihydroergosterol m. p. 173—174°, $[\alpha]_D$ −19°, and for the acetate m. p. 180—181°]. The third and fourth crops (1·5 g.) consisted of α-ergostenol.

allo-α-*Ergostanol Acetate.*—Ergosterol (1 g.) was dissolved in glacial acetic acid (120 c.c.), and the solution hydrogenated at 70° in presence of palladium-black (1 g.) for 8 hours. The resulting colourless solution was decanted from the catalyst, diluted with water, and extracted with ether. The ethereal extract, after being washed with dilute alkali solution and with water, was mixed with methyl alcohol ($\frac{1}{3}$ vol.) and kept in a covered beaker, crops of crystals being removed from time to time. The first crop (0·4 g.) consisted of flat needles, m. p. 120—129°. Recrystallisation from ether–methyl alcohol gave a constant m. p. of 144—145° (Reindel and Walter, *loc. cit.*, give 145°) [Found : (micro) C, 81·0 ; H, 11·8. Calc. for $C_{29}H_{50}O_2$: C, 80·9 ; H, 11·6%].

allo-α-*Ergostanol.*—The acetate (1 g.) was refluxed for 1 hour with 10% alcoholic potash (100 c.c.) : crystalline plates separated on cooling. Repeated crystallisation from ether–methyl alcohol gave a m. p. of 144° (Reindel and Walter, *loc. cit.*, give 144—145°). The substance was dried at 100° [Found: (micro) C, 80·3; H, 12·5; H_2O, 3·8. Calc. for $C_{27}H_{48}O,H_2O$: C, 80·2; H, 12·4; H_2O, 4·4%. Reindel, Walter, and Rauch (*Annalen*, 1927, **452**, 34) found " about " 1 H_2O].

Ergosterol α-Acetate.—A solution of ergosterol (2 g.) and anhydrous potassium acetate (5 g.) in glacial acetic acid (200 c.c.) was maintained at 100° for 5—6 hours, a stream of carbon dioxide being passed through the flask to minimise any loss by atmospheric oxidation. The crystals which separated on cooling (1·0 g.) contained a considerable amount of unchanged sterol. The filtrate was poured into water, and the product extracted with ether. The washed and dried extract was evaporated, and the residue purified by repeated crystallisation from absolute alcohol. The *α-acetate* formed small leaves, m. p. 132—133° [Found: (micro) C, 81·8; H, 10·8. $C_{29}H_{44}O_2$ requires C, 82·1; H, 10·4%].

A further quantity of the acetate was recovered by fractional crystallisation of the first crop from alcohol–ether, the acetyl derivative being found in the more soluble portion. The acetylation can also be carried out in the absence of potassium acetate; nevertheless the presence of this substance is desirable. In some cases a product, m. p. 133°, was obtained which was shown by analysis to contain unchanged ergosterol. This is probably a eutectic mixture of the sterol and the acetyl derivative.

Conversion of Ergosterol α-Acetate into the β-Isomeride.—Ergosterol α-acetate (1 g.) was heated under reflux for 1 hour with acetic anhydride (4 c.c.), and the product treated with alcohol to destroy the excess of the anhydride. On recrystallisation of the resulting compound from absolute alcohol, the characteristic leaves of ergosterol β-acetate (m. p. 172—174°) separated. The conversion was also brought about by maintaining the molten compound for ½ hour at 170—180° and repeatedly recrystallising the product from absolute alcohol.

Hydrogenation of Ergosterol α-Acetate.—The acetate (1·2 g.) was dissolved in ether (100 c.c.) and hydrogenated in the presence of palladium-black until absorption of hydrogen ceased. The volume absorbed was 135 c.c. (N.T.P.) (Calc. for 2 mols. of hydrogen, 127 c.c.). The product after repeated crystallisation from ether–methyl alcohol had m. p. 109—110° and gave no depression in admixture with α-ergostenol acetate prepared by acetylation of the free alcohol.

Hydrolysis of Ergosterol α-Acetate.—The acetate (1·5 g.) was refluxed for an hour with 5% alcoholic potash (100 c.c.), and the solution allowed to cool. The crystals obtained melted after recrystallisation at 160° and gave no depression in admixture with ergosterol.

We desire to express our thanks to Mr. F. S. Spring, B.Sc., for his help in a portion of the experimental work.

THE UNIVERSITY, LIVERPOOL. [*Received, March* 9*th*, 1929.]