CCXC.—*Studies in the Sterol Group. Part VI. Dihydroergosterol and the Formation of Isomerides.*

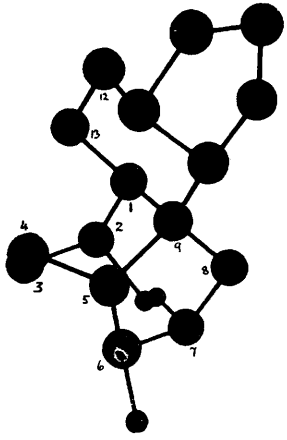
By Isidor Morris Heilbron, Frank Johnstone, and Frank Stuart Spring.

It has been shown by Windaus and Brünken (*Annalen*, 1928, **460**, 232) that the reduction of ergosterol by means of sodium and ethyl alcohol gives rise to a dihydroergosterol, m. p. 173—174° (regarding the acetate, see experimental portion); the same compound has also been obtained by the partial hydrogenation of certain specimens of ergosterol itself (Heilbron, Sexton, and Spring, this vol., p. 926).

Windaus and Brünken noted that, unlike the parent sterol, dihydroergosterol gives no positive colour reaction with antimony trichloride. We have confirmed this statement, and find further that the compound fails to give a coloration with Rosenheim's trichloroacetic acid reagent (*Biochem. J.*, 1929, **23**, 47), which fact leads us to the conclusion that in the conversion of ergosterol into the dihydro-derivative the ethenoid linkage between carbon atoms 1 and 13 (or 1 and 2) (formula I) must become saturated. If this be the case, two stereoisomeric dihydroergosterols should exist, corresponding to the formation of coprostane and cholestane from ψ-cholestene (Windaus, *Nachr. Ges. Wiss. Göttingen*, Jan., 1926). Although, despite repeated attempts, we have failed to obtain dihydroergosterol by Windaus and Brünken's method (*loc. cit.*), we have found that the reduction of ergosterol by means of sodium in boiling amyl alcohol gives rise both to this compound and to a

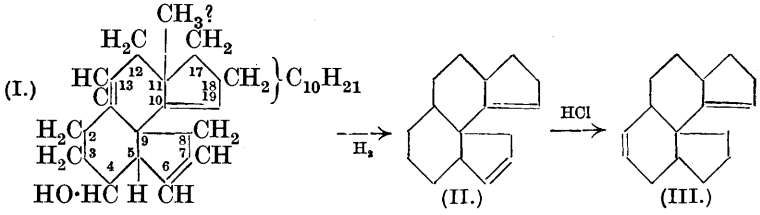
small quantity of an isomeride, *γ-dihydroergosterol*, m. p. 205—206°. The latter compound is also produced in small quantity by the action of sodium amyloxide on ordinary dihydroergosterol (α-dihydroergosterol). The hydrogenation of α-dihydroergosterol acetate in glacial acetic acid at 70° in presence of a platinum catalyst yields, according to Windaus and Brünken (*Annalen*, 1928, **460**, 235), *allo*-α-ergostanol acetate. If, however, the hydrogenation is carried out in ethereal solution, a palladium catalyst being used, the reaction ceases when two atoms of hydrogen are absorbed, α-ergostenol acetate being produced. As this compound cannot be further directly hydrogenated (Reindel and Walter, *Annalen*, 1928, **460**, 212), it follows that α-ergostenol cannot be a direct intermediate in the reduction of ergosterol or α-dihydroergosterol to *allo*-α-ergostanol.

FIG. 1.



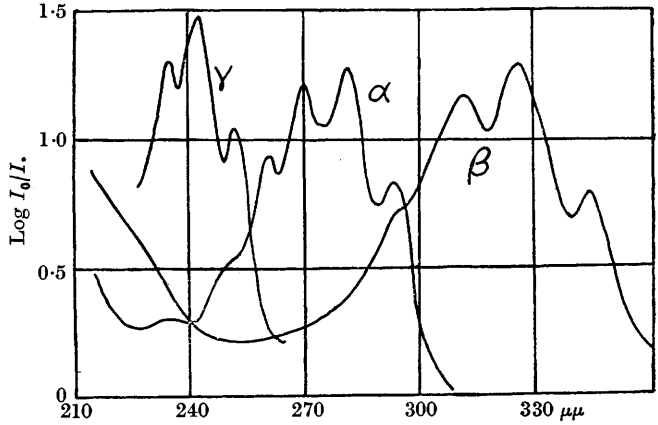
β-*Dihydroergosterol*.—Reindel, Walter, and Rauch (*Annalen*, 1927, **452**, 34) found that α-ergostenol acetate could be isomerised by means of dry hydrogen chloride to the structurally isomeric β-ergostenol acetate. α-Dihydroergosterol acetate behaves similarly, yielding an *isomeride*, m. p. 108°, which on hydrolysis gives β-dihydroergosterol, m. p. 124°. Hydrogenation of this isomeride in ether also leads to the production of α-ergostenol, and consequently its formation could not have involved the inert ethenoid linkage present in ergosterol (10—19 in formula I), but must have been occasioned by a shift of the 6 : 7 double bond. In order to account for this change, we assume in the first place that the isomerisation is preceded by the addition of hydrogen chloride at positions 6 : 7, followed by its elimination from a different part of the molecule. As shown in Fig. 1, the addition of hydrogen chloride (chlorine on C_7) and its elimination from positions 2 : 7 would give rise to a new ethenoid linkage, either between carbon atoms 1 and 2 (production of *ψ*-cholestene from cholestene and of *allo*-cholesterol from cholesterol; see also Windaus, *loc. cit.*) or between carbons 2 and 3. We suggest it is the latter change which is involved in the conversion of α- into β-dihydroergosterol, since the β-compound fails to give any coloration either with antimony trichloride or with the Rosenheim reagent, and consequently cannot have a $\Delta^{1:2}$-linkage. Assuming that the structure of ergosterol is represented as in (I) (Heilbron and

Sexton, this vol., p. 921), the nuclear changes on hydrogenation and isomerisation may be represented as follows :



Dehydrogenation of α-Dihydroergosterol.—Windaus has shown (*Annalen*, 1928, **465**, 148) that treatment of ergosterol with mercuric

FIG. 2.



α . . . . . *Ergosterol* (0·02%; 2 *mm.*).
β . . . . . *Dehydroergosterol* (0·02%; 2 *mm.*).
γ . . . . . *Ergosterol D* (0·21%; 2 *mm.*).

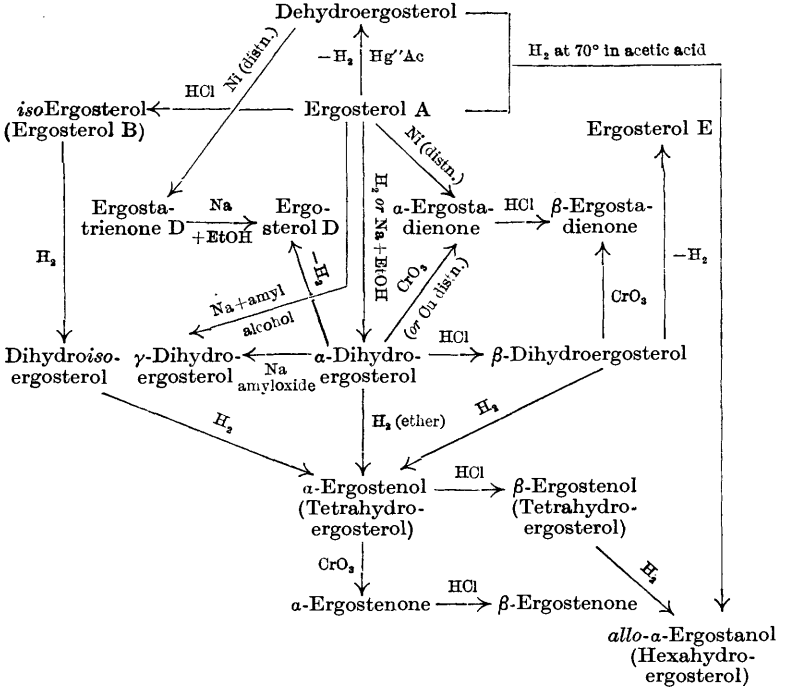
acetate yields dehydroergosterol. We have applied this reaction to α-dihydroergosterol, and find that here also two hydrogen atoms are eliminated with formation of a new isomeride * of ergosterol. The compound melts at 167°, but whereas ergosterol is strongly lævorotatory, the new sterol has $[\alpha]^{18°}_{5461}$ + 17·8°. We have also determined its absorption spectrum, and find that it is characterised by three bands at 235 μμ, 243 μμ, and 252 μμ (γ-curve, Fig. 2),

* Since this work was completed, a description of this compound (ergosta-trienol D) has appeared in a paper published by Windaus and Auhagen (*Annalen*, 1929, **472**, 185). The properties given by them closely agree with our findings except as regards the absorption spectrum, in which they have failed to observe the band at 235 μμ. In order to avoid unnecessary confusion we meanwhile accept their arbitrary nomenclature for the various isomeric ergosterols. In strict accordance with this, ergosta-trienol D should be simply ergosterol D.

resembling in its banded structure ergosterol A (α-curve). We have also re-examined the absorption spectrum of dehydroergosterol (β-curve) and find, in contradistinction to Windaus and Linsert's observation (*Annalen*, 1928, **465**, 148), who only record a single broad band at 320 μμ, that this substance also possesses a fine structure with three definite bands. It will be noted that the extinction coefficients of all three substances are remarkably similar.

β-Dihydroergosterol also is converted by mercuric acetate into an *alcohol*, m. p. 124°, isomeric with ergosterol. For this we tentatively suggest the name *ergosterol E*, pending further work on its hydrogenation products and absorption spectrum.

As the various changes undergone by ergosterol are exceedingly complex, we append herewith a scheme summarising the more important of these :



Formation of α-Ergosta-dienone.—This *compound* (m. p. 182—183°) is readily obtained as main product on oxidation of α-dihydroergosterol with chromic anhydride. It can also be readily prepared in good yield by distilling α-dihydroergosterol with copper-bronze according to Sexton's method (J., 1928, 2825), and Windaus and Auhagen (*loc. cit.*) have recently described its formation by the

action of nickel on ergosterol. On hydrogenation the ketone yields α-ergostenol. When α-ergosta-dienone is treated with hydrogen chloride in chloroform solution it is converted into β-*ergosta-dienone*, m. p. 125°, which can also be prepared by direct oxidation of β-dihydroergosterol. Hydrogenation of the ketone also results in the formation of α-ergostenol, a reaction in complete agreement with our suggested formulation of the isomeric dihydroergosterols.

Experimental.

α-Dihydroergosterol was prepared by the partial hydrogenation of ergosterol according to Heilbron and Sexton's method (*loc. cit.*). It could readily be freed from traces of ergosterol by boiling the crude product (4 g.) in acetone solution with potassium permanganate (0·4 g.) for 1 hour (compare Bills, Honeywell, and McNair, *J. Biol. Chem.*, 1928, **76**, 251). After the removal of manganese dioxide the filtrate was concentrated; almost pure α-dihydroergosterol then crystallised in large plates from the cold solution. Traces of ergosterol still remaining, indicated by the coloration with antimony trichloride, were removed by a further crystallisation from acetone in presence of blood charcoal.

α-Dihydroergosterol Acetate.—Windaus and Brünken (*loc. cit.*) prepared this compound (m. p. 181—182°) by treating α-dihydroergosterol with acetic anhydride. We have now ascertained that, as in the case of ergosterol β-acetate (Heilbron, Sexton, and Spring, this vol., p. 926), the melting point and specific rotation of the acetate depend upon the duration of the acetylation process as shown below :

Period of treatment (mins.).	M. p.	$[\alpha]^{21°}_{5461}$.
10	179—180°	−25·3°
30	175—176	−27·3
55	173	−30·3

Neither pure α-dihydroergosterol nor its acetate shows selective absorption.

γ-Dihydroergosterol.—Ergosterol (10 g.) was dissolved in hot amyl alcohol (100 c.c.), and sodium (80 g.) added in small pieces during 1 hour. The solution was gently boiled under reflux for a further 4 hours and then poured into a large volume of hot water. The amyl alcohol layer was repeatedly washed with water, and the alcohol removed by steam-distillation. The residual solid was fractionally crystallised from boiling absolute alcohol. The first crop (0·8 g.; m. p. 201—203°) was repeatedly crystallised from alcohol, from which *γ-dihydroergosterol* separated in long needles, m. p. 205—206°, $[\alpha]^{20°}_{5461}$ −10° (*c* = 1 in chloroform) [Found (micro.) : C, 84·1; H, 11·5. $C_{27}H_{44}O$ requires C, 84·3; H, 11·6%]. γ-Di-

hydroergosterol gives no colour either with antimony trichloride or with the Rosenheim reagent; it forms an insoluble digitonide. The acetate melts at 179°. The subsequent crops from the original alcoholic solution consisted of α-dihydroergosterol.

γ-Dihydroergosterol was also prepared by refluxing its α-isomeride (1 g.) with a solution of sodium amyloxide (12 g. of sodium in 200 c.c. of amyl alcohol) for 4 hours. When the product was worked up as described above, γ-dihydroergosterol was isolated in small amount.

β-*Dihydroergosterol.*—A solution of α-dihydroergosterol acetate (1·5 g.) in chloroform (20 c.c.) was subjected to a rapid stream of dry hydrogen chloride for 1 hour; the solution then acquired a faint violet colour. The residue obtained after removal of the solvent was crystallised from alcohol and from benzene–alcohol, from which β-*dihydroergosterol acetate* separated in plates, m. p. 108—109°, $[\alpha]_{5461}^{20°}$ −25·2° (*c* = 1·07 in chloroform) (Found: C, 81·4; H, 10·8. $C_{29}H_{46}O_2$ requires C, 81·7; H, 10·8%).* β-Dihydroergosterol was obtained by hydrolysis of the acetate with alcoholic potash and precipitation with water; it crystallised from absolute alcohol in flat plates containing water of crystallisation, m. p. 124°, $[\alpha]_{5461}^{18°}$ −7·0° (*c* = 1 in chloroform). It gives no colour with antimony trichloride and shows only general absorption.

Ergosterol D.—α-Dihydroergosterol (5 g.), dissolved in boiling alcohol (250 c.c.), was refluxed for 40 minutes with a solution of mercuric acetate (11·5 g.) in a mixture of glacial acetic acid (20 c.c.) and alcohol (10 c.c.). After removal of mercurous acetate, the yellow solution was evaporated under reduced pressure. The product was separated from further traces of inorganic matter by extraction with boiling chloroform and crystallised from benzene–alcohol, flat plates of ergosterol D being obtained (yield, about 40%). The sterol gives a yellow coloration with antimony trichloride and a faint pink colour with the Rosenheim reagent. The *acetate* melts at 172° and has $[\alpha]_{5461}^{20°}$ +15·9° (*c* = 1·32 in chloroform) [Found (micro.): C, 81·7; H, 10·9. $C_{29}H_{44}O_2$ requires C, 82·1; H, 10·4%].

Ergosterol E.—This was prepared from β-dihydroergosterol in a similar manner to ergosterol D. The product separated from methyl alcohol–ethyl acetate in colourless plates, containing water of crystallisation, m. p. 124—125°, $[\alpha]_{5461}^{18°}$ −22·9° (*c* = 1·09 in chloroform). It develops a yellow colour with antimony trichloride and a pale pink with Rosenheim's reagent. The *acetate* has m. p. 119—120°, and $[\alpha]_{5461}^{18°}$ −38·0° (*c* = 1·03 in chloroform) [Found (micro.): C, 82·1; H, 10·3. $C_{29}H_{44}O_2$ requires C, 82·1; H, 10·4%].

* As in nearly all cases the free sterols contain indefinite solvent addenda, analyses of the solvent-free acetates have been made.

α-*Ergosta-dienone.*—A solution of α-dihydroergosterol (5 g.) in glacial acetic acid was oxidised with chromic anhydride (5 g. in 1 c.c. of water and 14 c.c. of glacial acetic acid) at 70°. The reagent was added during 1 hour, the whole being mechanically stirred. After a further hour the product was precipitated with water, collected, and dissolved in ether, and the solution shaken with dilute alkali. The ethereal solution was dried, concentrated to small bulk, and mixed with an equal volume of absolute alcohol; crude α-ergosta-dienone then separated. The pure *ketone* was obtained after repeated crystallisation from alcohol in colourless plates, m. p. 182—183°. It gives no coloration with antimony trichloride and shows no selective absorption [Found (micro.): C, 85·0; H, 11·2. $C_{27}H_{42}O$ requires C, 84·8; H, 11·0%]. From the alkaline extract an oily acid was obtained, but the quantity was too small to allow of further work being carried out.

The *semicarbazone*, prepared in the usual manner, separated from methyl alcohol, in which it was very sparingly soluble, in colourless crystals, m. p. 254° (decomp.) (Found : N, 9·5. $C_{28}H_{45}ON_3$ requires N, 9·6%).

Catalytic Oxidation of α-Dihydroergosterol.—α-Dihydroergosterol was distilled at 2 mm. pressure in presence of an equal weight of copper-bronze. The main fraction was collected between 250° and 270° and consisted of a bright yellow oil which solidified on cooling. Crystallisation of this product from absolute alcohol yielded α-ergosta-dienone identical with the product obtained by oxidation of the alcohol with chromic anhydride.

Hydrogenation of α-Ergosta-dienone.—The ketone (1 g.) in ether (100 c.c.) was shaken with hydrogen in presence of palladium-black. The absorption of the hydrogen ceased after 80 minutes, four atoms having been absorbed. The product, after two recrystallisations from ether–methyl alcohol, had m. p. 130—131° and gave no depression in melting point in admixture with an authentic sample of α-ergostenol.

β-*Ergosta-dienone.*—This compound was obtained by the oxidation of β-dihydroergosterol with chromic anhydride in exactly the same manner as that of α-dihydroergosterol. The pure *ketone* separated from glacial acetic acid in long needles, m. p. 125°. β-Ergosta-dienone was also obtained by passing a rapid stream of dry hydrogen chloride for 1 hour into a chloroform solution of the α-ketone. The oil remaining after removal of the solvent was taken up in alcohol, and the separated solid recrystallised from glacial acetic acid (Found : C, 84·5; H, 11·2. $C_{27}H_{42}O$ requires C, 84·8; H, 11·0%).

The *semicarbazone* separated from methyl alcohol in needles, m. p. 266° (Found : N, 9·7. $C_{28}H_{45}ON_3$ requires N, 9·6%).

Hydrogenation of β-Ergosta-dienone.—The ketone (1 g.) was hydrogenated as described under the α-isomeride (volume of hydrogen absorbed = 108 c.c.; 2 mols. H_2 require 112 c.c.). The product melted at 131° and proved to be α-ergostenol.

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