CCCLXXIX.—*Studies in the Sterol Group. Part VIII. The Reactions of* iso*Ergosterol.*

By Isidor Morris Heilbron and Frank Stuart Spring.

Reindel, Walter, and Rauch (*Annalen*, 1927, **452**, 34) have shown that the treatment of ergosterol β-acetate (see Heilbron and Sexton, this vol., p. 921) with dry hydrogen chloride results in the formation of the structurally isomeric *iso*ergosterol acetate, m. p. 129—131°. We have now found that this product is actually a mixture, separable by fractional crystallisation into (*a*) α-iso*ergosterol acetate*, m. p. 139°, and (*b*) β-iso*ergosterol acetate*, m. p. 111—112°. Both isomerides show the absorption band at 247 μμ hitherto regarded as characteristic of *iso*ergosterol * (van Wijk and Reerink, *Nature*, 1928, **122**, 648), but a slight difference exists in their molecular extinction coefficients, a fact compatible with the view that they are isomerides of the *cis-trans* type (Henri and Errera, *Compt. rend.*, 1925, **180**, 2049; **181**, 548).

On hydrolysis each acetate gives its respective sterol, α-*iso*ergosterol melting at 143—144° and the β-isomeride at 135°. Ergosterol can also be isomerised by means of alcoholic sulphuric acid, the product in this case consisting almost entirely of α-*iso*ergosterol.

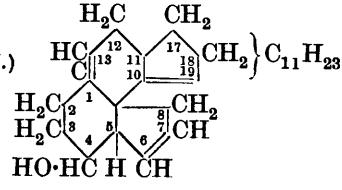
Hydrogenation of both isomerides in ethereal solution at room temperature leads to the formation of α-ergostenol. The hydrogenation is, however, selective, for if the absorption of hydrogen is interrupted when the volume required for the saturation of one ethenoid linkage has been reached, dihydro*iso*ergosterol, m. p. 181—182°, can be isolated. This substance can also be obtained by the reduction of β-*iso*ergosterol with sodium and ethyl alcohol; in the case of the α-isomeride, dihydro*iso*ergosterol is produced only when the reduction is carried out in boiling amyl-alcoholic solution.

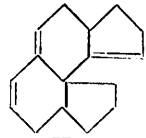
Mechanism of the Change Ergosterol ⟶ iso*Ergosterol.*—From the fact that hydrogenation of both α- and β-*iso*ergosterols gives rise to α-ergostenol, it may be inferred that the isomerisation of ergosterol has not involved the shift of its inert double bond, which according to Heilbron and Sexton (this vol., p. 921) is probably situated as shown in (I) at $\Delta^{10:19}$ (the alternative $\Delta^{10:11}$ is implied throughout). It is further evident that the ethenoid linkage $\Delta^{1:13}$ (or $\Delta^{1:2}$) is not involved in the change, since both α- and β-*iso*ergosterols give an immediate pink coloration with the Rosenheim reagent (*Biochem.*

* According to Windaus and Auhagen's suggested nomenclature (*Annalen*, 1929, **472**, 184) *iso*ergosterol would be termed ergosterol B; in view of the fact that this compound is a mixture we are retaining the name *iso*ergosterol as likely to cause less confusion than the use of terms α-ergosterol B and β-ergosterol B.

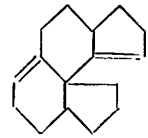
J., 1929, **23**, 47). We are thus forced to the conclusion that the isomerisation is due to a shift of the 6 : 7 double bond. The sugges-

(I.)





(II.)



(III.)

tion has been made by Reindel, Walter, and Rauch (*loc. cit.*), without any definite evidence being adduced, that " bei dieser Isomerisierung, zwei im Molekül des Ergosterins entfernter Doppelbindungen, sich in die energetisch begünstigte, konjugierte Lage begeben." To test this hypothesis, we have applied the elegant reaction of Diels and Alder (*Annalen*, 1928, **460**, 98), whereby a conjugated system combines with maleic anhydride, 1 : 4 addition taking place. Although α-*iso*ergosterol acetate fails to combine with this reagent, β-*iso*ergosterol acetate readily yields an addition *product*, m. p. 159°, and hence definite evidence is afforded that $\Delta^{6:7}$ must move to a position conjugated with either $\Delta^{1:13}$ or $\Delta^{10:19}$. As, however, reduction of either *iso*ergosterol results in the formation of dihydro*iso*ergosterol, which, in its turn, on further hydrogenation yields α-ergostenol, conjugation with $\Delta^{10:19}$ is excluded. We are consequently left with two possibilities : (*a*) if one of the double bonds in ergosterol occupies the $\Delta^{1:2}$ position, the shift would take place to position $\Delta^{12:13}$; (*b*) if it is in the $\Delta^{1:13}$ position, the shift would take place to $\Delta^{2:3}$. As has been explained in the case of the isomerisation of α- to β-dihydroergosterol (this vol., p. 2248), the latter seems undoubtedly correct, for space models show that the change from $\Delta^{6:7}$ to $\Delta^{12:13}$ is improbable. The *iso*ergosterols can thus be formulated as in (II); dihydro*iso*-ergosterol will consequently be (III) and in accordance with this structure shows the Rosenheim reaction.

The combination of β-*iso*ergosterol acetate with maleic anhydride is also in accordance with these views, the reaction being comparable with the combination of maleic anhydride with *cyclo*hexadiene (Diels and Alder, *loc. cit.*). The fact that α-*iso*ergosterol acetate fails to form a condensation product is ascribable to a difference in the spatial disposition of the respective ring systems, capable of demonstration only with the aid of solid models.

Experimental.

Preparation of α- *and* β-iso*Ergosterol Acetates.*—When ergosterol acetate was treated in chloroform solution with dry hydrogen

chloride as detailed by Reindel, Walter, and Rauch (*loc. cit.*), a product was obtained, m. p. 129—131°, as given by these authors. This (10 g.) was dissolved in 200 c.c. of benzene–alcohol (1 : 2) and left at 0°; α-iso*ergosterol acetate* (7 g.), m. p. 137—139°, then separated. After a further crystallisation from the same solvents the pure compound was obtained in colourless laminæ, m. p. 139°, $[\alpha]_{5461}^{21°}$ — 90·3° ($c = 1·6$ in chloroform) (Found: C, 82·1; H, 10·7. $C_{29}H_{44}O_2$ requires C, 82·1; H, 10·4%). The filtrate from which the α-compound had been removed was again left over-night at 0°; a second crop (2·8 g.), m. p. 108°, was then removed. On recrystallisation, a small amount (0·2 g.) of α-*iso*ergosterol acetate separated first; the filtrate later deposited pure β-iso*ergosterol acetate* as small plates, m. p. 111—112°, $[\alpha]_{5461}^{24°}$ — 58·9° ($c = 1·6$ in chloroform) (Found: C, 81·8; H, 10·7%).

α-iso*Ergosterol.*—α-*iso*Ergosterol acetate was hydrolysed by refluxing it with 5% alcoholic potash, and the product recrystallised from absolute alcohol. α-*iso*Ergosterol forms colourless plates, m. p. 143—144°, $[\alpha]_{5461}^{21°}$ — 134·2° ($c = 0·8$ in chloroform); it gives a deep yellow coloration with antimony trichloride and a pink colour with the Rosenheim reagent.

α-*iso*Ergosterol can be prepared directly by refluxing ergosterol with alcoholic sulphuric acid (10% H_2SO_4) for 1 hour. The solid obtained by precipitation of the green solution with water was recrystallised first from acetone (m. p. 139°) and finally from benzene–alcohol; pure α-*iso*ergosterol was then obtained in almost quantitative yield.

β-iso*Ergosterol.*—Hydrolysis of β-*iso*ergosterol acetate gives the free sterol, m. p. 135° (from alcohol), $[\alpha]_{5461}^{21°}$ — 95° ($c = 1·2$ in chloroform). A mixture of the α- and the β-sterol in the ratio of 4 : 1 melts at 135—136°, the melting point previously recorded for *iso*ergosterol.

Hydrogenation of α- *and* β-iso*Ergosterols.*—Each sterol (1 g.) was hydrogenated in ethereal solution (200 c.c.) in presence of a palladium catalyst. Four atoms of hydrogen were absorbed in each case, the products of both isomerides being α-ergostenol, m. p. 130—131°. Reindel, Walter, and Rauch (*loc. cit.*) state that hydrogenation with platinum effects saturation of the three ethenoid linkages, but this statement is erroneous (see Reindel and Walter, *Annalen*, 1928, **460**, 212).

*Dihydro*iso*ergosterol.*—A solution of α-*iso*ergosterol (5 g.) in amyl alcohol (500 c.c.) was treated with sodium (40 g.), added in small quantities during 2 hours; the whole was then gently refluxed for 3 hours. The solution was diluted with water, and the amyl alcohol removed in steam. The residual solid was repeatedly crystallised

from absolute alcohol, from which dihydro*iso*ergosterol separated in plates, m. p. 181—182°, $[\alpha]_{5461}^{17°} - 77{\cdot}7°$ ($c = 0{\cdot}26$ in chloroform). The *acetate* melts at 183° [Found (micro.): C, 82·0; H, 10·7. $C_{29}H_{46}O_2$ requires C, 81·7; H, 10·8%].

Condensation of β-iso*Ergosterol Acetate with Maleic Anhydride.*—β-*iso*Ergosterol acetate (1·8 g.) was dissolved in the minimum quantity of benzene and treated with a suspension of maleic anhydride (0·5 g.) in benzene. The solution was kept for 6 hours at room temperature, a yellow colour developing indicative of reaction (compare Farmer and Warren, this vol., p. 897). The reaction was completed by heating in a sealed tube for 6 hours at 100°. The pure *product* separated on cooling in plates, m. p. 159°; it gave a bright yellow coloration with antimony trichloride [Found (micro.): C, 75·9; H, 8·6. $C_{33}H_{46}O_5$ requires C, 75·9; H, 8·8%].

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