CXXII.—*Studies in the Sterol Group. Part IV. The Existence of Isomeric Ergosterols.*

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It was shown by Heilbron and Sexton (preceding paper) that the hydrogenation of yeast ergosterol in ethereal solution in the presence of palladium leads to the formation of the tetrahydro-derivative, α-ergostenol. The ergosterol employed in those experiments was prepared by Böhringer und Söhne of Hamburg. In a continuation of that work, a yeast ergosterol supplied by Messrs. Boot (Boot's A) was used, but to our surprise, working under identical conditions, we failed to obtain α-ergostenol, the reaction ceasing after the addition of approximately two atoms of hydrogen (compare Curves I and II).

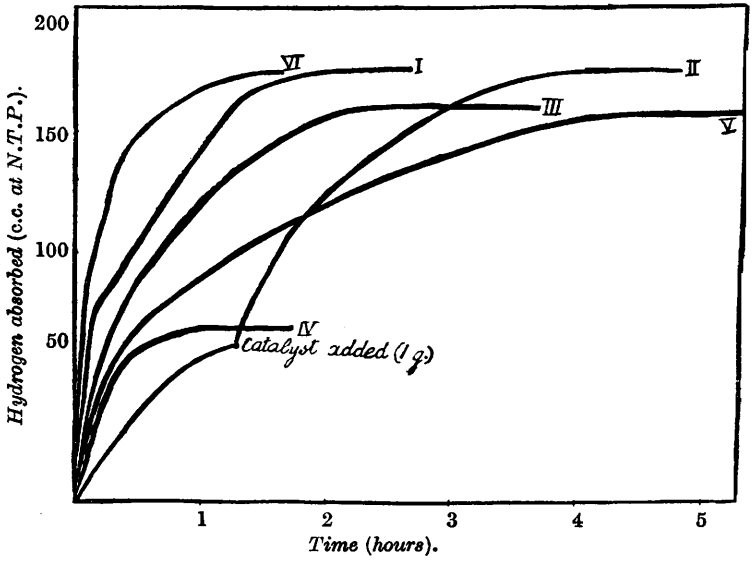
The first explanation which suggested itself to us was the presence of a catalyst poison in Boot's ergosterol. In order to test this, Boot's ergosterol was hydrogenated in ethereal solution until addition of hydrogen had ceased. The same catalyst was then employed with success in the hydrogenation of fresh Böhringer ergosterol, α-ergostenol being readily obtained with no diminution of the rate of absorption of hydrogen.

The homogeneity of the two sterols was demonstrated by fractionally crystallising each of them three times under identical conditions from the alcohol–benzene solvent recommended by Bills and Honeywell (*J. Biol. Chem.*, 1928, **80**, 15). The last two crystallisations effected no alteration of specific rotation, the melting points of the two sterols were identical at each stage, and no depression was noted in mixtures of them. The pure Boot's ergosterol had $[\alpha]^{22°}_{5461}$ −171°, and the Böhringer product $[\alpha]^{22°}_{5461}$ −159·3°. The absorption spectra of the two sterols showed the same characteristic absorption bands in the ultra-violet region. The extinction coefficients were, however, slightly different, the ratio being of the order 1·2 (Böhringer product) : 1·0 (Boot's sterol). As the ergosterol showing the higher extinction coefficient has the

lower specific rotation, it follows that, if Boot's ergosterol contains a non-absorbing impurity, this must have an exceptionally high lævorotation: the sterol commonly associated with ergosterol (zymosterol), however, is actually dextrorotatory (Smedley-Maclean, *Biochem. J.*, 1928, **22**, 22).

Hydrogenation of the purified Böhringer sterol gave α-ergostenol in the normal manner (Curve I), whereas the hydrogenation of the purified Boot's sterol proceeded in the same manner as that of the

FIG. 1.



I. *Böhringer's ergosterol* (1·5 *g.*) *with* 1 *g. of catalyst.*
II. *Boot's A ergosterol* (1·5 *g.*) *with* 1 *g. of catalyst and subsequent addition of* 1 *g. of catalyst.*
III. *Böhringer's ergosterol acetate with* 1 *g. of catalyst.*
IV. *Boot's A ergosterol acetate with* 1 *g. of catalyst.*
V. *Boot's A ergosterol acetate with* 2 *g. of catalyst.*
VI. *Boot's B ergosterol with* 2 *g. of catalyst.*

crude material. The more soluble portion of each preparation, obtained by evaporation of the filtrate from the first crystallisation, behaved in the same way on hydrogenation as the less soluble fraction. The quantity of hydrogen absorbed by Boot's ergosterol was slightly less than the volume required for complete saturation of one ethenoid linking. Attempts were made, however, to ascertain whether, as found in the case of Böhringer's sterol (Heilbron and Sexton, *loc. cit.*), pure dihydroergosterol could be isolated from the product; but these were unsuccessful, as ergosterol was still present.

On the other hand, we were invariably able to complete the hydrogenation to tetrahydroergosterol by the addition of more catalyst at this stage, but, as shown in Curve II, the reaction was very slow. An examination of the product obtained under these conditions revealed a marked difference, for whereas Böhringer's ergosterol gave rise solely to α-ergostenol, the tetrahydro-derivative from Boot's sterol was separable into α- and β-ergostenols. The isolation of the β-isomeride in this way is at first sight curious, as Reindel and Walter (*Annalen*, 1927, **460**, 212) have shown that β-ergostenol acetate is hydrogenated without difficulty in the presence of platinum to the saturated *allo*-α-ergostanol acetate. Experiments carried out by us, however, on free β-ergostenol with a palladium catalyst showed that the reaction was difficult to carry to completion.

A series of experiments was also carried out with twice the initial amount of catalyst, in order to see whether four atoms of hydrogen could be directly added to Boot's ergosterol. Unfortunately, a fresh supply of the sterol (Boot's B) was used which, after purification as already described, had a specific rotation $[\alpha]_{5461}^{22°}$ −161·2°. Four atoms of hydrogen were absorbed with no marked break at the dihydro-stage (Curve VI). It was sometimes difficult to free the product entirely from dihydroergosterol, and although α-ergostenol was readily isolated, we failed to obtain evidence of the formation of the β-isomeride.

The two sterols, Böhringer's and Boot's A, were each converted into the β-acetates (Heilbron and Sexton, *loc. cit.*) by means of acetic anhydride. Although the two acetates had identical melting points and specific rotations, Curve IV shows that the difficulty of hydrogenating Boot's sterol persists after acetylation. Both in this case and also when a large initial excess of catalyst was used (Curve V) we failed to detect β-ergostenol acetate, the α-isomeride alone being isolated. It may well be, however, that β-ergostenol acetate was formed in small quantity, but could not be separated from the α-compound. Hydrolysis of Boot's A acetate with alcoholic potash gave a sterol which after purification had a specific rotation $[\alpha]_{5461}^{22°}$ −160°, the value for the Böhringer product. On hydrogenation this hydrolysed material behaved precisely as the original Böhringer ergosterol, the velocity break appearing at the dihydro-stage and α-ergostenol being the sole product. It is thus possible by acetylation and hydrolysis to convert Boot's ergosterol into an ergosterol the behaviour of which on hydrogenation is in harmony with the recorded result of previous workers, and the failure of previous investigators to observe the results described above may be due to their having subjected the ergosterol to a

purification process involving acetylation and hydrolysis (compare Reindel and Walter, *loc. cit.*).

The melting point of ergosterol β-acetate recorded by various investigators shows considerable discrepancies, *e.g.*, 170—172° by Reindel and Walter (*loc. cit.*) and 180° by Bills and Honeywell (*loc. cit.*). We have found that the melting point is dependent on the duration of the treatment with acetic anhydride: the longer the treatment, the lower is the m. p. and the higher the specific rotation. Heilbron, Morton, and Sexton (J., 1928, 37) have directed attention to the susceptibility of the sterol nucleus to racemisation, and we regard it as probable that under the rather violent conditions of its preparation ergosterol β-acetate undergoes partial racemisation, giving rise to the observed difference in physical constants.

All the ergosterols so far examined are converted on irradiation into vitamin-*D*.

Experimental.

Fractionation of Böhringer's and of Boot's A Ergosterols.—Each sterol (25 g.) was dissolved in 500 c.c. of a boiling mixture of industrial alcohol (2 parts) and benzene (1 part), and the filtered solution kept over-night; the ergosterol had then crystallised in glistening flat needles. These were removed and the filtrate was concentrated to half its bulk to obtain a second crop. The first crop was recrystallised twice from the same proportion of solvent and the m. p. and the specific rotation of the products were determined at each stage. These are shown, together with the weights of the crops, in the table.

		Boot's A.		Böhringer.	
Fraction.	Wt. (g.).	M. p.	$[\alpha]_{5461}^{21\cdot5^\circ}$.	M. p.	$[\alpha]_{5461}^{21\cdot5^\circ}$.
Crude	25·0	158—159°	−155°	158—159°	−145·3°
1st Cryst.	18·5	160—161	−171·2	160—161	−159·2
2nd ,, ...	13·2	160·5—161·5	−171·0	160·5—161·5	−159·2
3rd ,, ...	9·5	161—162	−171·0	161—162	−159·3
2nd crop from filtrate of 1st crystallisation	5·0	157—159	−150·0	158—159	−134·5

Catalytic Hydrogenation.—The hydrogenations were all carried out in presence of palladium-black prepared by a modification of Seng's method (Diss., Göttingen, 1918), for which we are indebted to Mr. W. Doran, M.Sc.

Palladous chloride (1 g.) was digested with water (1000 c.c.) on a steam-bath for 1 hour with continual stirring. Formic acid (2 c.c. of 80%) was then added to the hot solution, followed after 5 minutes by a solution of potassium hydroxide (50 c.c. of 10%). The liquid was kept on the steam-bath with continual stirring for

2 hours and the precipitated palladium was then filtered off, thoroughly washed with hot water, and dried over calcium chloride in a desiccator.

Hydrogenation of Böhringer's Ergosterol.—The sterol (1·5 g.) was dissolved in ether (250 c.c.) and hydrogenated in the presence of the catalyst (1 g.), conversion into the tetrahydro-derivative being effected in 1½—2 hours. The product (m. p. 126°), after twice crystallising from ether–methyl alcohol, had m. p. 130—131° and $[\alpha]^{21°}_{5461}$ +18·0° (c = 1·104 in chloroform). The rate of addition of hydrogen is indicated in Curve I, where the velocity break at the dihydro-stage is evident.

Hydrogenation of Boot's A Ergosterol.—Under precisely the same conditions as those described above, the absorption of hydrogen had almost ceased after 80 minutes (Curve II) (Found : H_2 absorbed, 65 c.c. Calc. for one double bond, 88 c.c.). At this stage fresh catalyst was added (1 g.); the absorption of hydrogen was then resumed and continued slowly until the tetrahydro-stage was reached. The filtered solution was concentrated to half its bulk, treated with a little methyl alcohol, and kept in a covered beaker. The first crop (0·5 g.) melted at 114° and on further crystallisation the m. p. could not be raised above 114—116°. On treatment with acetic anhydride, it gave β-ergostenol acetate which, alone or mixed with β-ergostenol acetate prepared by Reindel, Walter, and Rauch's method (*Annalen*, 1927, **452**, 34), melted at 94—96°. The second crop (0·8 g.) melted at 126°, and at 130—131° after several recrystallisations from ether–methyl alcohol. It then had $[\alpha]^{21°}_{5461}$ +19° (c = 1·104 in chloroform) and showed no depression of m. p. when mixed with α-ergostenol prepared from Böhringer's ergosterol.

Acetylation of the Ergosterols with Acetic Anhydride.—Boot's A sterol (1 g.) was heated at 135—145° with acetic anhydride for various times, and the product recrystallised from ether–acetone (1 : 3). The physical constants were as follows :

Time of treatment (mins.)	10	30	90
$[\alpha]^{21°}_{5461}$	−109·0°	−115·6°	−119·3°
M. p.	176—177°	172—174°	171—173°

A purified sample of Böhringer's ergosterol, after being treated for 30 minutes, gave exactly the same m. p. and specific rotation as Boot's isomeride.

Hydrogenation of the β-Acetates.—The same quantities were employed as in the case of the free sterols. Curve III represents the addition of hydrogen to the Böhringer acetate, and Curves IV and V show the rate of hydrogenation of the Boot acetate. The product in each case consisted entirely of α-ergostenol acetate,

which after two crystallisations from ether–methyl alcohol had m. p. 109—110° and $[\alpha]^{21°}_{5461}$ +6·3° (c = 1·74 in chloroform).

Hydrolysis of Ergosterol β-Acetate.—Boot's ergosterol β-acetate (m. p. 172—174°) (5 g.) was refluxed with 5% alcoholic potash (100 c.c.) for 1 hour. The product which separated on cooling was recrystallised from alcohol–benzene; it then had m. p. 161—162° and $[\alpha]^{21°}_{5461}$ −160·0° (c = 1·194 in chloroform). Hydrogenation of this ergosterol (1·5 g.) in the presence of palladium-black (2 g.) was complete in about 1 hour. After two recrystallisations of the crude product from ether–methyl alcohol, pure α-ergostenol, m. p. 130—131°, $[\alpha]^{22°}_{5461}$ +19° (c = 1·74 in chloroform), was obtained.

Hydrogenation of β-Ergostenol.—β-Ergostenol (0·5 g.), prepared by Reindel, Walter, and Rauch's method (*loc. cit.*), was hydrogenated in the presence of palladium-black (2 g.) until absorption of hydrogen ceased (21 c.c.) (Calc. for one double bond : 29 c.c.). The product when tested by the Liebermann–Burchard reaction gave a strong colour indicating the presence of unsaturated material.

Hydrogenation of Boot's B Ergosterol.—The sterol (1·5 g.), which had $[\alpha]^{22°}_{5461}$ −161·2° (c = 2·1 in chloroform), was hydrogenated in the presence of palladium (2 g.), absorption of 4 atoms being complete in 1½ hours (Curve VI). The product was fractionally crystallised from ether–methyl alcohol in the usual manner. The first four crops (1·2 g.) consisted of impure α-ergostenol (m. p. 124—126°). One crystallisation of the combined crops from ether–methyl alcohol gave a substance having m. p. 129—131° and showing no depression in m. p. when mixed with an authentic specimen of α-ergostenol.

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