CCCLIII.—*Studies in the Sterol Group. Part X. The Relationship of the Fully Saturated Derivatives of Ergosterol and Sitosterol.*

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THE similarity in physical constants of corresponding derivatives of *allo*-α-ergostanol and sitostanol was first pointed out by Reindel and Walter (*Annalen*, 1928, **460**, 212) and more recently shown to be specially marked in the case of γ-sitostanol and its derivatives (Heilbron and Sexton, *Nature*, 1929, **123**, 567). A new ergostanol is now described which, as will be shown, strengthens the case regarding the identity of the fully saturated derivatives of the ergosterol and sitosterol series. The possibility of complete demonstration of identity of corresponding members of these two series is remote owing to the capacity of the sitosterol group to retain isomeric impurities in admixture (compare Rhyg, *Z. physiol. Chem.*, 1929, **185**, 99). The work of Anderson and his collaborators (*J. Amer. Chem. Soc.*, 1926, **48**, 2972, 2976, 2987) has shown that sitosterol as usually isolated is a mixture of isomerides, the only component of constant physical properties being γ-sitosterol, although in all probability the β-sitosterol described is a nearly pure substance.

A very intensive fractionation of the sitosterol from cotton-seed oil carried out in this laboratory by Dr. A. Thompson (unpublished work) showed that this was free from γ-sitosterol. After bromination of the acetate of the sterol, debromination, and subsequent hydrolysis by the method of Windaus and Hauth (*Ber.*, 1907, **40**, 3682), a sitosterol, m. p. 137—137·5° and $[\alpha]_D^{20°}$ — 38°, was isolated.

Hydrogenation of ergosterol in ethereal solution gives rise to α-ergostenol, which is isomeric with the sitosterols but differs from them in having a positive rotation. Further, it gives a positive reaction with the Tortelli–Jaffé reagent, to which no sitosterol responds (Heilbron and Spring, *Biochem. J.*, 1930, **24**, 133). Similarly the isomeric β-ergostenol, although failing to give a Tortelli–Jaffé reaction, also differs from the sitosterols both in melting point and in optical rotation.

In connexion with work being carried out in these laboratories on the oxidation of the ergostenes (unpublished work) it became necessary to prepare a large quantity of α-ergostenol. The crude product prepared by hydrogenation of ergosterol in the usual manner was fractionally crystallised, the first three crops proving to be nearly pure α-ergostenol. From the fourth crop, however, a new substance, m. p. 129—130°, $[\alpha]_D^{22°}$ + 5·1°, was isolated which gave a green colour with the Tortelli–Jaffé reagent; on admixture

with α-ergostenol a depression of over 10° in melting point was observed. Analysis of the acetate of the new compound showed that the latter was isomeric with α-ergostenol and for it the name *γ-ergostenol* is proposed.

Treatment of *γ-ergostenyl acetate* (m. p. 140°) with hydrogen chloride resulted in the formation of iso-*γ-ergostenyl acetate* (m. p. 103°). Whereas γ-ergostenol, like α-ergostenol, resists further direct hydrogenation, iso-*γ-ergostenol* readily absorbs two atoms of hydrogen in ethereal solution at room temperature, giving rise to a substance, isomeric with *allo*-α-ergostanol, for which the name *γ-ergostanol* is suggested. The purified product, which gave no Liebermann–Burchard reaction, melted at 137°, had $[\alpha]_D^{22°}$ + 29°, and showed no depression on admixture with the sitostanol, m. p. 136°, $[\alpha]_D^{23°}$ + 26°, prepared from cotton-seed oil sitosterol. It would thus appear reasonable to infer that the two substances are identical. These results point to the conclusion that the ergosterol employed was a mixture of isomerides and that the new γ-ergostenol has not arisen during the hydrogenation process (compare Heilbron, Johnstone, and Spring, J., 1929, 2248). In support of this view fractionation of the ergosterol and hydrogenation of the less and the more soluble fractions showed that a greater yield of γ-ergostenol was obtained from the latter source, *i.e.*, the isomeride responsible for the appearance of γ-ergostenol is concentrated in the more soluble fractions of yeast ergosterols.

The following table shows the physical constants of the sitosterols, the isomeric ergostenols, and the saturated alcohols reviewed above.

TABLE I.

	M. p.	$[\alpha]_D$.	
Sitosterol	136—137°	−33·9°	Ritter, *Z. physiol. Chem.*, 1902, **34**, 461.
γ-Sitosterol	145—146	−46	Anderson and Shriner, *J. Amer. Chem. Soc.*, 1926, **48**, 2976.
β-Sitosterol	139—140	−36	,, ,, ,,
Sitosterol from cotton-seed oil	137—137·5	−38	Liverpool (unpublished work).
α-Ergostenol	130—131	+17·8	Reindel, Walter, and Rauch, *Annalen*, 1927, **452**, 34.
β-Ergostenol	114—118	+15·89	,, ,, ,,
γ-Ergostenol	129—130	+ 5·1	This paper.
γ-Sitostanol	144—145	+17·82	Anderson and Shriner, *loc. cit.*
Sitostanol (cotton-seed sitosterol)	136	+26	This paper.
γ-Ergostanol	137	+29	,, ,,
allo-α-Ergostanol	145	+15·9	Reindel and Walter, *Annalen*, 1928, **460**, 212.

EXPERIMENTAL.

Hydrogenation of Ergosterol.—Hydrogenation of ergosterol (m. p. 160°; $[\alpha]_D^{22°}$ − 131°, $c = 3$ in chloroform) was effected in a narrow

closed cylindrical steel vessel of about 400 c.c. capacity, provided with an efficient high-speed rotary stirrer, and gas inlet and outlet tubes, both fitted nearly flush with the lid. Ergosterol (25 g.) was suspended in ethyl acetate (250 c.c.) and introduced together with palladium-black (4 g.) into the pot. The gas flow was measured by means of a water-meter; after displacement of the air with hydrogen the outlet cock was screwed down, the pot being surrounded by a water jacket maintained at 40—50°. Absorption of hydrogen ceased in about 6 hours (2800 c.c.).

Fractionation of Tetrahydroergosterol.—The product (100 g.) after removal of the catalyst was dissolved in industrial alcohol (1000 c.c.) and allowed to crystallise. The crystals (65 g.) proved to be α-ergostenol, m. p. 131°, $[\alpha]_D^{20°}$ + 15° (c = 2·5 in chloroform). The filtrate was concentrated to 500 c.c. and again allowed to cool; needles then separated (4·5 g.), m. p. 115°, $[\alpha]_D^{22°}$ + 12° (c = 2 in chloroform). This fraction was recrystallised from industrial alcohol, giving α-ergostenol, $[\alpha]_D^{22°}$ + 15·2° (c = 1·8 in chloroform), showing no depression on melting in admixture with the first crop. The filtrate was again concentrated to half bulk, and allowed to crystallise; the crop obtained (3·5 g.), m. p. 128°, $[\alpha]_D^{22°}$ + 8° (c = 2 in chloroform), was repeatedly crystallised from ethyl alcohol, giving a homogeneous *substance*, m. p. 129—130°, $[\alpha]_D^{22°}$ + 5·1° (c = 3·2 in chloroform) (on admixture with an authentic sample of α-ergostenol, m. p. 120—122°) [Found: (micro) C, 80·0; H, 12·1. $C_{27}H_{46}O,H_2O$ requires C, 80·2; H, 11·9%].

Fractionation of Ergosterol.—The sterol (100 g.) was dissolved in boiling benzene–alcohol (1 : 2; 2000 c.c.) and after 6 hours the separated solid (60 g.) was removed (Fraction A). The filtrate was concentrated to half its bulk; a second crop then separated (15 g.). The procedure was repeated, and a third crop (10 g.), m. p. 156°, $[\alpha]_D^{22°}$ — 125°, obtained (Fraction B). Fractions A and B were hydrogenated separately in ethyl acetate solution at 40° in presence of palladium-black and gave α-ergostenol and the γ-isomeride (yield, 1 and 5% respectively).

γ-Ergostenyl Acetate.—γ-Ergostenol (0·5 g.) was refluxed for 30 minutes with acetic anhydride (2·5 c.c.), and the product recrystallised from ethyl alcohol. The *acetate* crystallised in plates, m. p. 140° [Found: (micro) C, 81·4; H, 11·2. $C_{29}H_{48}O_2$ requires C, 81·3; H, 11·2%].

iso-*γ-Ergostenyl Acetate.*—γ-Ergostenyl acetate (0·5 g.) in dry chloroform (10 c.c.) was treated with a rapid stream of hydrogen chloride for 2 hours. The chloroform was evaporated, and the residual oil dissolved in absolute alcohol; large plates slowly separated, m. p. 103—104°, $[\alpha]_D^{22°}$ + 4·05° (c = 3·1 in chloroform)

[Found: (micro) C, 81·3; H, 10·8. $C_{29}H_{48}O_2$ requires C, 81·3; H, 11·2%].

iso-*γ*-*Ergostenol.*—*iso*-*γ*-Ergostenyl acetate was refluxed for 2 hours with 5% alcoholic potash. The product crystallised from ethyl alcohol in plates, m. p. 129°, $[\alpha]_D^{22°}$ + 3·7° (*c* = 1·75 in chloroform).

Hydrogenation of iso-*γ*-*Ergostenol.*—iso-*γ*-Ergostenol (1 g.) was dissolved in dry ether (150 c.c.) and shaken with hydrogen in presence of palladium-black. Absorption proceeded slowly and regularly, and ceased after 6 hours (absorption 54 c.c.; calc. for the presence of one double bond, 58 c.c.). The product still showed a faint blue colour with the Liebermann–Burchard reagent; it was dissolved in 5 c.c. of carbon tetrachloride and acetic anhydride (2·5 c.c.) was added, followed by concentrated sulphuric acid (2·5 c.c.). The solution was shaken and kept for 5 minutes, water added, and the carbon tetrachloride layer run off. The product on crystallisation from alcohol now showed a negative Liebermann–Burchard reaction and had m. p. 137° and $[\alpha]_D^{23°}$ + 29·0°.

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