92. *The Sterols of* Calycanthus floridus.

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A. The phytosterol fraction of the oil extracted from the seeds of *Calycanthus floridus* has been found to consist mainly of β-sitosterol, together with a small amount of α-sitosterol, and possibly sitostanol.
B. The hydrogenation of β-cholestenol to cholestanol has been confirmed.

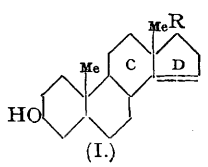
A supply of *Calycanthus floridus* seeds purchased by the late Professor Barger for isolation of their alkaloidal constituents (compare Barger, Jacob, and Madinaveitia, *Rec. Trav. chim.*, 1938, **57**, 548) became available to us, and as these seeds, like those of *Calycanthus glaucus*, contain a large proportion of fixed oil (Eccles, *Proc. Amer. Pharm. Assoc.*, 1888, **84**, 382; Wiley, *Amer. Chem. J.*, 1889, **11**, 557), it seemed of interest to investigate the sterols of the unsaponifiable matter from the oil. Extraction of the seeds with cold benzene gave about 33% of a pale yellow oil of low alkaloid content. This was hydrolysed with methyl-alcoholic potash, the unsaponifiable components being obtained as a semicrystalline mass representing some 1·6% of the original oil. Treatment with light petroleum yielded colourless crystals of the phytosterol mixture.

Fractional crystallisation of this mixture from alcohol afforded a number of fractions very similar in melting point, although subsequent examination of the fractions showed that some separation had occurred. Each fraction was submitted to the action of the Tortelli–Jaffé reagent according to the procedure of Westphal (*Ber.*, 1939, **72**, 1243); a green colour developed only in the most soluble fraction, suggesting the presence, in low concentration, of one or more of the α-sitosterols. Examination of the least soluble fraction, in which stigmasterol and sitostanol should be concentrated, showed the absence of stigmasterol but yielded a very small quantity of material which, from its m. p., appeared to be sitostanol.

As phytosterol mixtures are more efficiently separated by crystallisation of their benzoates and 3 : 5-dinitrobenzoates than of their acetates (Wallis *et al.*, *J. Amer. Chem. Soc.*, 1936, **58**, 2446; 1939, **61**, 1903; Gloyer and Schuette, *ibid.*, 1939, **61**, 1901; Anderson *et al.*, *ibid.*, 1924, **46**, 1450; 1926, **48**, 2972, 2976, 2987), the least soluble fraction was benzoylated. The m. p. of the product corresponded closely with that reported for β-sitosteryl benzoate, and there was no indication of the presence of γ-sitosterol. Hydrolysis of this benzoate gave the free sterol, which was converted into its acetate and 3 : 5-dinitrobenzoate. The specific rotation of the free sterol, and the m. p.'s of its three esters confirmed the view that this was β-sitosterol, and the m. p. of the sterol was not depressed by admixture with an authentic specimen of β-sitosterol prepared from tall oil; for this specimen we are indebted to Dr. E. R. H. Jones. Circumstances did not permit of the application of the elegant test of homogeneity of sitosterols devised by Barton and Jones (J., 1943, 599). Each of the other sterol fractions was likewise benzoylated and yielded β-sitosteryl benzoate.

The results indicated that at least 70% of the phytosterol mixture consisted of β-sitosterol. Calycanthus oil thus resembles cottonseed oil (Wallis and Chakravorty, *J. Org. Chem.*, 1937, **2**, 335) rather than soya bean oil (Rosenheim and Webster, *Biochem. J.*, 1941, **35**, 929) and wheat germ oil (Anderson *et al.*, *loc. cit.*), both of which contain γ-sitosterol as their principal steroid component.

There is evidence that rings C and D of the natural sterols are locked by *trans* valency bonds (Wieland and Schlichting, *Z. physiol. Chem.*, 1924, **134**, 276; Wieland and Dane, *ibid.*, 1933, **216**, 91; Dimroth and Jonsson, *Ber.*, 1941, **74**, 520). With the simple dicyclic hydrindanes the *cis*-configuration is the more stable, and in cases where interconversion is possible *trans*-compounds tend to pass into their *cis*-isomerides (compare *Annual Reports*, 1935, **32**, 310). In view of these relationships it is interesting that hydrogenation of β-ergostenol (I, R = C_9H_{19}) should give ergostanol (Heilbron and Wilkinson, J., 1932, 1708) and that β-cholestenol (I, R = C_8H_{17}) should give cholestanol (Schenck, Buchholz, and Wiese, *Ber.*, 1936, **69**, 2703) rather than the stereoisomerides in which the fusion of rings C and D is *cis* (compare Peak, *Nature*, 1937, **140**, 280). The product obtained by Schenck by hydrogenation of β-cholestenyl acetate was identified as cholestanyl acetate merely by m. p. and mixed m. p. determinations, and in view of the theoretical importance of this experiment it seemed worth while to seek confirmation and to complete the identification of the saturated sterols by specific rotation measurements. This we have done. Hydrogenation of β-cholestenyl benzoate over Adams's catalyst afforded *cholestanyl hexahydrobenzoate*, which was hydrolysed to cholestanol, identical with a specimen prepared by direct hydrogenation of cholesterol. It must therefore be concluded that attachment of the additional rings to the hydrindane system to give the tetracyclic sterol molecule modifies the relative stabilities of the *cis*- and *trans*-forms. The *trans*-configuration assigned to the C–D ring fusion of the sterols is probably correct, but it would be desirable to have a simpler and more direct proof of this than has hitherto been furnished.



(I.)

Further confirmation of the identity of hydrogenated β-cholestenol with cholestanol was provided by the work of Heath-Brown, Heilbron, and Jones, who, in a paper published at the time when our experiments were almost complete (J., 1940, 1482), showed that zymosterol was readily convertible into β-cholestenol, which these authors likewise hydrogenated to cholestanol.

Experimental.

Extraction of Calycanthus floridus *Seeds.*—(*a*) The roughly ground seeds (kernels and hulls, 250 g.) were covered with benzene (800 c.c.) and kept at room temperature for a week with occasional agitation. The filtered solution was

concentrated under reduced pressure. The residual pale yellow oil (83 g.) contained only traces of alkaloid. (*b*) A similar yield of oil was obtained when a large batch of the seeds (44 kg.) was extracted with benzene (90 kg.). We are greatly indebted to Messrs. Roche Products, Ltd., for carrying out this extraction. Through a misunderstanding the extraction was done with hot benzene and the resulting oil (32·5% of the weight of seeds) contained about 3·8% of alkaloid, which was removed by dilute hydrochloric acid, after addition of light petroleum (2 vols.). Most of the following experiments were carried out with the brown oil obtained in this way. The products obtained seemed identical with those from the pale yellow oil resulting from cold extraction of the seeds.

Saponification of the oil (580 g.) was effected by 8 hours' boiling with methyl-alcoholic potash (190 g. in 2·5 l.). The solution was concentrated four-fold under reduced pressure; the whole was then diluted with water (2 l.), and the unsaponifiable matter extracted with ether. The ethereal extract was washed with dilute hydrochloric acid and with water, dried, and the ether removed. The semi-solid residue (1·15—1·6% from various batches) was dissolved in hot light petroleum, the filtered solution kept at 0° for 24 hours, and the crystals collected. They had m. p. 135—137°. (These amounted to some 60% of the crude unsaponifiable matter.)

Examination of phytosterols. The crystalline mixture (5·8 g.) was submitted to a triangle scheme of fractional crystallisation from absolute alcohol, involving 36 crystallisations. The m. p.'s and yields of the final series of fractions are shown in the table:

		more soluble fractions →				
Fraction.	A.	B.	C.	D.	E.	F and G.
M. p.	134—135·5°	134·5—135·5°	133—135°	135—136·5°	132·5—135°	132—134·5°
Yield, g.	1·33	0·66	0·39	0·09	0·32	0·13

Total yield, 2·92 g.

Only in the case of the combined fraction F and G was a faint green colour developed with the Tortelli-Jaffé reagent. Fraction A was acetylated by boiling for an hour with acetic anhydride (10 c.c.). A solution of the resulting acetate (1·45 g.), m. p. 118·5—123·5°, in ether (15 c.c.) was mixed with a solution of bromine (0·3 c.c.) in acetic acid (20 c.c.) and kept at room temperature for a few hours. The amount of bromide which separated was insufficient for a m. p. determination, indicating the almost complete absence of stigmasterol (Windaus and Hauth, *Ber.*, 1906, **39**, 4378). The material from the filtered solution was debrominated by boiling with zinc dust and acetic acid, and the regenerated phytosteryl acetate was hydrolysed with 10% methyl-alcoholic potash, giving the phytosterol as elongated leaflets (0·85 g.), m. p. 133·5—135°. Some of this was treated according to the procedure of Anderson and Nabenhauer (*J. Amer. Chem. Soc.*, 1924, **46**, 1957) for the removal of unsaturated sterols. The residual saturated sterol, after two crystallisations from alcohol, had m. p. 137—138° and probably consisted of sitostanol. The remainder of the phytosterol regenerated from the acetate-bromide was treated with benzoyl chloride in pyridine at 100° (1 hour) and the resulting benzoate was recrystallised three times from alcohol–benzene, giving pure β-sitosteryl benzoate. This was hydrolysed to the sterol, from which was prepared the pure acetate. Fractions B to E of the phytosterol mixture were then purified through the benzoate, which was hydrolysed and the pure sterol then converted into its 3 : 5-dinitrobenzoate. The following table gives the constants of the pure sterol and its derivatives, together with those recorded for β-sitosterol:

Sterol.		Acetate,	Benzoate,	3 : 5-Dinitrobenzoate,
M. p.	$[\alpha]_D$.	m. p.	m. p.	m. p.
137·5—138·5°	−34·0° [1]	126·5—127·5°	145·5—146·5°	208—209° [2]
136—137	−36·6	125—126	146—147	202—203 [3]
135—135·5	−34·2	126—127	145—146	207—209 [4]
136—137	−31·5	122—123	146—147	— [5]

[1] At 18°; *c*, in chloroform, 2·267. [2] Present authors' values. [3] Wallis and Chakravorty, *loc. cit.* [4] Simpson and Williams, J., 1937, 733. [5] Ichiba, *Sci. Papers Inst. Phys. Chem. Res., Tokyo*, 1935, **28**, 112.

Benzoylation of fraction F–G gave β-sitosteryl benzoate (0·14 g.), m. p. 144·5—145·5°, and a fraction, m. p. 101—123°, was recovered from the mother-liquors of the crystallisation. Hydrolysis of this gave crystals, m. p. 130—132·5°, which gave an intense green colour with the Tortelli–Jaffé reagent, indicating the presence of the α-sitosterol mixture.

Hydrogenation of β-Cholestenyl Benzoate.—7-Hydroxycholesterol was prepared as described by Windaus *et al.* (*Annalen*, 1935, **520**, 98) Its dibenzoate was converted into 7-dehydrocholesteryl benzoate in 70% yield by boiling dimethylaniline (8 hours) (compare Haslewood, J., 1938, 224). In the preparation of the dibenzoate of 7-hydroxycholesterol some *7-ketocholesteryl benzoate* was isolated owing to incomplete Meerwein–Ponndorff reduction of 7-ketocholesteryl acetate. This was identified by direct comparison with a specimen prepared from 7-ketocholesterol (1 g.) and benzoyl chloride (0·5 g.) in dry pyridine (15 c.c.) at room temperature (18 hours). It formed long colourless needles (from alcohol) which melted at 159·5—161° to an opaque liquid. This became pea-green at 182·5° and formed a clear colourless liquid at 183·5° (Found: C, 81·0; H, 9·7. $C_{34}H_{48}O_3$ requires C, 80·95; H, 9·5%).

7-Dehydrocholesteryl benzoate was converted into α-cholestenyl benzoate and thence into β-cholestenyl benzoate by a procedure which was essentially that of Schenck, Buchholz, and Wiese (*loc. cit.*). A solution of β-cholestenyl benzoate (3 g.) in ether was added to a platinum catalyst prepared by reducing a suspension of platinic oxide (0·1 g.) in acetic acid (50 c.c.), and the whole was shaken with hydrogen until absorption ceased (2½ hours) The resulting *cholestanyl hexahydrobenzoate* (2·9 g.) formed colourless needles (from alcohol), m. p. 158·5—159° (Found: C, 82·2: H, 11·7. $C_{34}H_{58}O_2$ requires C, 81·9; H, 11·65%). Hydrolysis gave hexahydrobenzoic acid, m. p. 29—30°, and cholestanol, m. p. 141·5—142·5°, alone or mixed with a specimen prepared by hydrogenation of cholesterol. Both specimens of cholestanol were purified by the method of Anderson and Nabenhauer (*loc. cit.*). The dihydro-β-cholestenol had $[\alpha]_{5500}^{19°} = +26·7°$ (*c*, in chloroform, 0·6) and the authentic cholestanol had $[\alpha]_{5500}^{19°} = +25·0°$ (*c*, in chloroform, 0·6).

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