66. *Studies in the Sterol Group. Part XLIII. The Unsaponifiable Portion of the Acetone Extract of Plantation Rubber.*

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In previous studies of this material the isolation and identification of a sterol, $C_{27}H_{46}O$ (Whitby, Dolid, and Yorston, J., 1926, 1448), and octadecyl alcohol (Bruson, Sebrell, and Vogt, *Ind. Eng. Chem.*, 1927, **19**, 1187) have been described. It is now shown that the former is a mixture of isomeric sterols which includes β-sitosterol and probably a 24 : 28-dehydrostigmastanol, and that the aliphatic alcohol is eicosyl alcohol. No other pure constituent has as yet been isolated in sufficient quantity for investigation, since the remainder of the neutral fraction undergoes extensive pyrolysis on distillation even in a high vacuum. Evidence has, however, been obtained that a portion of the unsaponifiable matter becomes increasingly intractable as rubber ages, and it is possible that the present failure to obtain the ketone $C_{15}H_{24}O$ and the so-called "liquid sterols," $C_{27}H_{42}O_3$ and $C_{20}H_{30}O$, reported by Bruson, Sebrell, and Vogt (*loc. cit.*) may be due to this cause.

The raw material of the present investigation was the acetone extract of 100 lb. of plantation crepe rubber. On concentration, this deposited a few grams of sparingly soluble material consisting of quebrachitol, sterol glucoside, and nitrogenous and phosphatic matter. The unsaponifiable portion, isolated by the usual methods, yielded on treatment with alcohol a crystalline solid and a mother-liquor from which a reddish gum was obtained.

The solid fraction, after repeated crystallisation, had m. p. 135·5° (Whitby, Dolid, and Yorston, *loc. cit.*, give m. p.'s 133—134°, 134—135°, and 125° for various preparations), and was characterised readily by the preparation of acetyl, benzoyl, and *p*-nitrobenzoyl derivatives. Analytical evidence suggested the composition $C_{29}H_{50}O$; this was confirmed by quantitative hydrolysis of the acetate and by a molecular-weight determination based on *X*-ray data, for which we are indebted to Miss D. Crowfoot. Titration with perbenzoic acid indicated the presence in the molecule of one double bond, and the isolation of a substance indistinguishable from stigmastanyl acetate on catalytic hydrogenation of the acetate revealed that the material belonged to the phytosterol group. Further evidence of this association was forthcoming from a selenium dehydrogenation carried out at a high temperature, hydrocarbons similar to those obtained from sterols under like conditions (Ruzicka, Thomann, Brandenburger, and Goldberg, *Helv. Chim. Acta*, 1934, **17**, 200; Ruzicka, Goldberg, and Thomann, *ibid.*, 1933, **16**, 812) being obtained. These included a *chrysene*, m. p. 172—173°, and a *picene*, m. p. 274—276°, their respective struc-

tures being deduced from their absorption spectra, which are closely analogous to those described by Mayneord and Roe (*Proc. Roy. Soc.*, 1935, *A*, **152**, 319) for chrysene and picene. In addition there was isolated a *hydrocarbon*, m. p. 227° (2 : 7-dinitroanthraquinone derivative, m. p. 242—243°), which, although quite similar to, is not identical with either of the hydrocarbons obtained by Ruzicka *et al.* (*loc. cit.*) from phytosterols.

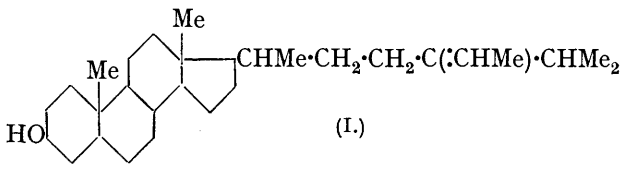
Despite the apparent homogeneity of the sterol as outlined above, chromatographic analysis demonstrated that it was actually a mixture, although a pure component could not be obtained by this means. This was also suggested by *X*-ray diffraction photographs, which showed a very close relationship with certain phytosterol mixtures (private communication from Miss D. Crowfoot). A more effective resolution was obtained by repeated fractional crystallisation of the acetate, which resulted in the separation of two distinct components. For convenience the corresponding sterols will be referred to as sterols A and B, and their physical constants, together with those of the corresponding acetyl and benzoyl derivatives, are summarised in the accompanying table with similar data for β-sitosterol (Wallis and Chakravorty, *J. Org. Chem.*, 1937, **2**, 335; see also Heilbron and Jones, *Ann. Rev. Biochem.*, 1940, **9**, 163) :

	Sterol.		Acetyl derivative.		Benzoyl derivative.	
	M. p.	$[\alpha]_D^{20°}$	M. p.	$[\alpha]_D^{20°}$	M. p.	$[\alpha]_D^{20°}$.
β-Sitosterol	136—137°	−37°	125—126°	−41°	146—147°	−13·8°
Sterol A	135·5	−37	123·5	−41	147	−15·2
Sterol B	134	−26	114—116	−28	145	−14·9

The results recorded above indicate that sterol A is identical with β-sitosterol and no depression in melting point was observed on admixture with authentic material from wheat germ oil. Further, catalytic hydrogenation of sterol A gave stigmastanol indistinguishable from specimens obtained by the authors by the complete hydrogenation of fucosterol (cf. Coffey, Heilbron, and Spring, J., 1936, 738).

	Alcohol.		Acetyl derivative.		Phenylurethane.	
	M. p.	$[\alpha]_D^{20°}$.	M. p.	$[\alpha]_D^{20°}$.	M. p.	$[\alpha]_D^{20°}$.
Stigmastanol	133°	+23°	128·5—129·5°	+13·0°	169—170°	+13·8°
Dihydro-sterol A	133—133·5	+23	128—128·5	+11·5	169—170	+13·9
Dihydro-sterol B	126·5	+ 5·8	122—123	− 8·2		

Sterol B, composing rather less than 10% of the original sterol mixture, on titration with perbenzoic acid gave a value corresponding to one ethenoid linkage, and on ozonolysis of the acetate, acetaldehyde was obtained in 22% yield (estimated as the 2 : 4-dinitrophenylhydrazone), indicating the presence of an ethylidene group in the side chain and suggesting the constitution (I) for this sterol. Only a trace of formaldehyde and no acetaldehyde could be detected on ozonolysis of the hydrogenation product of the acetyl derivative of sterol B. The latter is not, however, identical with stigmastanyl acetate (see table), a fact which is not entirely unexpected, since reduction of the ethenoid linkage involves the introduction of an asymmetric centre at C_{24}. In this connection it might be noted that campesterol (Fernholz and Ruigh, *J. Amer. Chem. Soc.*, 1941, **63**, 1157) differs from 22 : 23-dihydrobrassicasterol only in the optical configuration about C_{24}. We would emphasise that sterol B has not yet been obtained completely free from β-sitosterol, since a small quantity of an αβ-unsaturated ketone was isolated from the product of oxidation by the Oppenauer method. Limited by the availability of material, attempts to isolate in a pure condition the 24 : 28-dehydrostigmastanol (I), which is almost certainly the major constituent of the sterol B complex, have been unsuccessful and, as it is impossible meantime to continue this work, it has been decided to place the results on record. It has recently been demonstrated that the unsaturated side chains of brassicasterol (Fernholz and Stavely, *J. Amer. Chem. Soc.*, 1940, **62**, 1875) and α-spinasterol (Fernholz and Ruigh, *ibid.*, p. 2341) are identical with those of ergosterol and stigmasterol respectively and that the side chain of zymosterol



(I.)

contains an *iso*propylidene group (Heath-Brown, Heilbron, and Jones, J., 1940, 1482). The suggestion that the non-saponifiable portion of the acetone extract of rubber contains a sterol in which an ethylidene group is located in the side chain is of interest in that it points to the existence of yet another type of unsaturated side chain in naturally occurring sterols.

When the reddish gum from the original mother-liquor was distilled in a high vacuum (10^{-3} mm.), a colourless waxy substance passed over at 80—90°. This was readily purified and its constants together with those of the acetyl derivative and *phenylurethane* prove it to be eicosyl alcohol and not octadecyl alcohol, as suggested by Bruson, Sebrell, and Vogt (*loc. cit.*), who actually call attention to the fact that their "octadecyl alcohol" depressed slightly the melting point of an authentic specimen. The crystalline distillate obtained at 120—130° is probably steroid in nature. Above this temperature extensive decomposition occurred, and the small quantity of viscous oil which distilled contained ketonic material, part of which was identified by the preparation of a 2 : 4-dinitrophenylhydrazone giving the analysis required for the derivative of a steroid ketone. The remainder of the ketonic fraction yielded a complex dinitrophenylhydrazone which could not be resolved; that this is a product of pyrolytic decomposition was revealed by the fact that treatment of the original gum with the Girard reagent P failed to reveal the presence of any ketonic matter. Since high-vacuum distillation yielded no satisfactory separation, attention was directed to the removal of hydroxylic compounds from the original gum by means of the acid succinates, but this only resulted in the isolation of a further quantity of the sterol mixture and eicosyl alcohol. Careful fractionation of the non-hydroxylic residue both by distillation in a high vacuum and by solvent treatment failed to yield any tractable material. Examination of the acetone extract of crepe rubber twelve months older than that employed in the above investigation revealed an even lower proportion of volatile non-hydroxylic matter.

Experimental.

All rotations were carried out in chloroform solution in a 1 dcm. tube. Melting points are uncorrected.

The crude acetone extract from crepe rubber (100 lb.), concentrated to 2·5 l., was filtered and evaporated to dryness under reduced pressure. The residue (1100 g.) was shaken for 72 hours with methyl-alcoholic potassium hydroxide (5 l.; 5%), the alcohol removed under reduced pressure, and the residue extracted with ether. Washing the extract with water yielded a deep red, voluminous aqueous phase and a yellow ethereal phase which contained the bulk of the unsaponifiable matter. A considerable further quantity of the latter, however, was obtained by re-extracting the aqueous phase with much ether. The total unsaponifiable fraction (210 g.) in hot alcohol (4 l.) was decanted from a small amount of tarry matter and cooled; the crude sterol (85 g.) then separated. Removal of solvent from the mother-liquor under diminished pressure yielded an oily red gum.

The Sterol Mixture.—The crude sterol, purified by several recrystallisations from alcohol, formed colourless plates, m. p. 133·5°. $\equiv$ (perbenzoic acid), 0·97 (Found : C, 82·7; H, 12·0. Calc. for $C_{29}H_{50}O,\frac{1}{2}C_2H_5{\cdot}OH$: C, 82·3; H, 12·2%). (The presence of alcohol of crystallisation is confirmed by the *X*-ray investigations made by Miss D. Crowfoot.) Acetate : needles from alcohol, m. p. 123·5° [Found : C, 81·7; H, 11·5; *M* (by hydrolysis of acetate), 456, 457, 455. Calc. for $C_{31}H_{52}O_2$: C, 81·5; H, 11·6%; *M*, 456]. Benzoate : plates from alcohol, m. p. 147·5° (Found : C, 83·4; H, 10·6. Calc. for $C_{36}H_{54}O_2$: C, 83·3; H, 10·5%). *p*-Nitrobenzoate : pale yellow micro-needles from alcohol, m. p. 183—184° (Found : C, 76·8; H, 9·6. Calc. for $C_{36}H_{53}O_4N$: C, 76·7; H, 9·5%). Dihydro-derivative of acetate (by reduction of acetate in the presence of Adams's catalyst at room temperature and pressure) : plates from acetone, m. p. 130·5°, $[\alpha]_D^{20°}$ + 14·3° (*c*, 2·0) (Found : C, 81·4; H, 11·7. Calc. for $C_{31}H_{54}O_2$: C, 81·1; H, 11·9%). The dihydro-sterol obtained by alkaline hydrolysis of the dihydro-acetate formed plates from acetone, m. p. 134·5—135·5°, $[\alpha]_D^{20°}$ + 24° (*c*, 1·8), and gave a slight Liebermann–Burchard test (Found : C, 83·7; H, 12·7. Calc. for $C_{29}H_{52}O$: C, 83·6; H, 12·6%).

Selenium Dehydrogenation of the Sterol Mixture.—The dry mixed sterol (35 g.) was heated with dry selenium (60 g.) in a nitrite bath, the temperature of which varied between 320° and 400° during 72 hours. The pulverised product was extracted (Soxhlet) first with ether and then with benzene. Traces of phenolic substances were removed on washing the diluted ethereal extract with alkali; it was subsequently washed with water, dried, and evaporated, and the residue adsorbed from benzene solution on alumina. Elution of the chromatogram with

benzene gave a pale yellow, green-fluorescent solution and the subsequent ethereal washings were added to the original benzene extract. The solid product obtained on evaporation of the benzene eluate on trituration with cold ether gave a pale yellow substance, which after an elaborate purification yielded an almost colourless *picene* derivative, m. p. 274—276° (Found: C, 93·8; H, 6·15. $C_{25}H_{20}$ requires C, 93·7: H, 6·3%).

The original benzene extract, together with the ethereal eluate from the chromatogram, was evaporated and a benzene solution of the residue was adsorbed on alumina. The solid (3·1 g.) from the first 2500 c.c. of eluate was taken up in light petroleum (b. p. 40—60°)–benzene (1 : 1) and refractionated through alumina. The initial and the final runnings, which contained waxy and gummy matter respectively, were neglected; two of the remaining fractions, after elaborate processes involving washing, crystallisation, sublimation, and readsorption on alumina, ultimately yielded two substances. (a) A *chrysene* derivative, m. p. 172—173° [Found: C, 93·6; H, 6·5; *M* (Rast), 279. $C_{21}H_{18}$ requires C, 93·3; H, 6·7%; *M*, 270]. (b) A *hydrocarbon*, m. p. 227° (Found: C, 92·6; H, 7·4. $C_{25}H_{24}$ requires C, 92·5; H, 7·4%), giving a 2 : 7-dinitroanthraquinone derivative, m. p. 242—243°.

Resolution of the Sterol Mixture.—The sterol mixture (950 mg.) was refluxed for 1 hour with acetic anhydride (10 c.c.) and pyridine (10 c.c.). The product, isolated by pouring into water, yielded two acetates on fractional crystallisation: A (600 mg.), m. p. 123·5°, $[\alpha]_D^{20°}$ − 41° (*c*, 2·3), and B (50 mg.), m. p. 114—116°, $[\alpha]_D^{20°}$ − 28° (*c*, 1·5). A mixture of these two acetates in approximately equal amounts had m. p. 116—119°.

Sterol A (β-*Sitosterol*).—Hydrolysis of acetate A with methyl-alcoholic potassium hydroxide (5%) yielded sterol A, which formed plates from methyl alcohol–acetone, m. p. 135—135·5°, $[\alpha]_D^{20°}$ − 37° (*c*, 1·6). $\mid^{=}$ (perbenzoic acid), 1·09. Benzoate: m. p. 147°, $[\alpha]_D^{20°}$ − 15·2° (*c*, 2·6). Reduction of acetate A in acetic acid with hydrogen and Adams's catalyst at room temperature and pressure yielded the corresponding dihydro-acetate, which formed plates from methyl alcohol–acetone, m. p. 128—128·5°, $[\alpha]_D^{20°}$ + 11·5° (*c*, 1·8). This on alkaline hydrolysis was converted into the dihydro-sterol, m. p. 133—133·5°, $[\alpha]_D^{20°}$ + 23° (*c*, 2·5). Phenylurethane: m. p. 169—170°, $[\alpha]_D^{20°}$ + 13·9° (*c*, 1·6).

Sterol B.—The acetate B similarly gave sterol B in plates, m. p. 134°, $[\alpha]_D^{20°}$ − 26° (*c*, 2·1). $\mid^{=}$ (perbenzoic acid), 1·02. Benzoate: m. p. 145°, $[\alpha]_D^{20°}$ − 14·9° (*c*, 2·0). Reduction of acetate B under the above conditions gave a crystalline product, m. p. 122—123°, $[\alpha]_D^{20°}$ − 8·2° (*c*, 2·0), which, after alkaline hydrolysis, had m. p. 126·5—127°, $[\alpha]_D^{20°}$ + 5·8° (*c*, 1·2).

Ozonolysis of acetate B gave a volatile aldehyde, which was converted into the 2 : 4-dinitrophenylhydrazone. This, after purification through alumina and crystallisation from alcohol, had m. p. 158—159°, undepressed by authentic acetaldehyde-2 : 4-dinitrophenylhydrazone (yield, 22%) (Found: N, 25·1. Calc. for $C_8H_8O_4N_4$: N, 25·0%). Ozonolysis of the dihydro-acetate described above gave no acetaldehyde.

Sterol B on oxidation with aluminium *tert.*-butoxide and acetone in the usual manner produced in small yield a ketone, m. p. 92—95°. Light absorption in alcohol: Max. 2410 A.; log ε = 3·9.

The Red Gum.—This material, when heated in a retort at 80—90/10^{-3} mm., gave a waxy distillate of eicosyl alcohol, which crystallised from methyl alcohol in leaflets, m. p. 62° (Adam and Dyer, J., 1925, **127**, 70, give m. p. 65·0—65·5°). Acetate: needles, m. p. 41° (lit., 39·5—40·5°). *Phenylurethane:* plates from methyl alcohol, m. p. 75—76° (Found: C, 77·8; H, 11·3. $C_{27}H_{47}O_2N$ requires C, 77·6; H, 11·3%). At 110—130° some crude sterol sublimed from the gum and at 140—150° a pale yellow, viscous oil with ketonic properties distilled slowly. Volatile products of decomposition were also evident, and the viscid residue in the retort had darkened in colour and become insoluble in acetone. The distillate obtained at 140—150° was treated with 2 : 4-dinitrophenylhydrazine hydrochloride in alcohol and the resulting red solid, dissolved in benzene, was towered on alumina. The benzene washings were evaporated, and the residue crystallised fron benzene–methyl alcohol, giving scarlet prisms, m. p. 239—240° (Found: C, 70·9; H, 8·8. $C_{35}H_{52}O_4N_4$ requires C, 70·9; H, 8·8%). The mother-liquor from the crystallisation of the above substance contained a second 2 : 4-dinitrophenylhydrazone, which crystallised from light petroleum (b. p. 60—80°) in golden leaflets, m. p. 121—123°, but the analytical data for this material could not be interpreted.

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