68. *Studies in the Sterol Group. Part XLIV. The Oxidation of Phytosterols with the Oppenauer Reagent.*

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A comparative study of the behaviour of cholesterol and of the phytosterols, fucosterol, stigmasterol and β-sitosterol on oxidation by the Oppenauer method has been made. The αβ-unsaturated ketones, cholestenone, *fucostadienone* and stigmastadienone are all isolated in good yield, but β-sitosterol, with constants in agreement with those recorded in the literature, yields a mixture of ketones from which sitostenone, m. p. 83—84°, can be separated by chromatographic fractionation, in only 15% yield. The absorption spectra of the ketones and of their oximes, semicarbazones and 2 : 4-dinitrophenylhydrazones have been determined and the expected uniformity both in location and intensity of the maxima has been observed.

In a recent publication (Heilbron, Jones, Roberts, and Wilkinson, J., 1941, 344) the isolation of β-sitosterol from crepe rubber was described. On oxidation by the Oppenauer method (*Rec. Trav. chim.*, 1937, **56**, 137) this sterol gave in poor yield an αβ-unsaturated ketone, m. p. 92—93° (max. 2410 A., log ε = 4·23). This melting point is somewhat higher than that given in the literature for sitostenone from β-sitosterol and this fact, together with the knowledge that a similar oxidation of cholesterol yields Δ^4-cholestenone in excellent yield, made it desirable to study comparatively the oxidation of cholesterol and of the phytosterols, fucosterol, stigmasterol and β-sitosterol.

The αβ-unsaturated ketone, *fucostadienone*, m. p. 94°, was obtained in 50% yield from fucosterol by the Oppenauer method and similarly, stigmasterol yielded stigmastadienone, m. p. 125°, in 58% yield. The latter ketone (designated stigmastenone) was prepared previously by Marker and Wittle (*J. Amer. Chem. Soc.*, 1937, **59**, 2704) by dehydrogenation of stigmasterol with copper powder and by Marker and Rohrmann (*ibid.*, 1938, **60**, 1073) by dehydration of 4-hydroxystigmasterol, the melting point of the ketone being recorded as 94° in each case. The use of a modified Oppenauer method (acetone and aluminium *iso*propoxide; Schering-Kahlbaum A.-G., F.P. 822,551; *Chem. Zentr.*, 1938, II, 120) gave a stigmastadienone of m. p. 105°, but Fernholz and Stavely (*J. Amer. Chem. Soc.*, 1939, **61**, 2956), employing *cyclo*hexanone as the hydrogen acceptor, obtained a product (and semicarbazone) identical with that obtained by us. The same compound was also obtained by Fernholz and Stavely (*loc. cit.*) by Oppenauer oxidation and subsequent debromination of stigmasterol 22 : 23-dibromide (after purification through the semicarbazone).

The wheat-germ sitosterol employed in this investigation furnished a crude β-sitosteryl acetate which, after sixteen crystallisations from ethyl acetate, had m. p. 125°, $[\alpha]_D^{20°}$ − 35°. On hydrolysis this yielded β-sitosterol, m. p. 136—137°, $[\alpha]_D^{20°}$ − 32°, the constants of both sterol and acetate thus approximating closely to those usually quoted for pure β-sitosterol (for summary see Heilbron and Jones, *Ann. Rev. Biochem.*, 1940, **9**, 163). Oxidation of this β-sitosterol with the Oppenauer reagent gave a product, the heterogeneity of which was revealed by chromatographic analysis on alumina from light petroleum solution. By this method we have succeeded in isolating from the mixture, only however in 15% yield, sitostenone, m. p. 83—84°, comparing favourably in melting point and in melting point of the semicarbazone with specimens obtained by previous workers employing other methods (Marker and Wittle, *loc. cit.*; Marker, Kamm, and Wittle, *J. Amer. Chem. Soc.*, 1938, **60**, 1072; Heiduschka and Gloth, *Arch. Pharm.*, 1915, **253**, 415; Coffey, Heilbron, and Spring, J., 1936, 738). It should be noted that the intensity of light absorption at the maximum (2410 A.) is more than twice as high as that reported by the latter authors for a specimen of sitostenone melting only 3° lower. We have observed this phenomenon in other cases, particularly with materials purified by chromatographic fractionation.

Accompanying this sitostenone was some unoxidised sterol (10%), together with a similar quantity of a second ketonic substance, m. p. 143—145°, $[\alpha]_D^{20°}$ + 52°. Its absorption spectrum (see Experimental) indicates that this material is probably a mixture in which a saturated ketone predominates along with

some 10% of an αβ-unsaturated ketone. The fact that this material is dextrorotatory, together with its facile elution from alumina and the light absorption of its 2 : 4-dinitrophenylhydrazone, supports the above contention, although not wholly eliminating the possibility of the presence of an ethenoid linkage in the ketone in a non-conjugated position. It is possible that this material is impure sitostanone either present as an impurity in the β-sitosterol, or having been produced from sitostanol present as a contaminant. Such oxidations of saturated sterols under these conditions have not been reported previously, but we have observed (with B. Heath-Brown) that under more vigorous conditions (aluminium *iso*propoxide and *cyclo*hexanone) the Oppenauer method can effect partial conversion of cholestanol into cholestanone. In a recent publication Reich and Reichstein (*Arch. Intern. Pharmacodynamie Thérapie*, 1941, **65**, 415) report the oxidation of the 3-hydroxyl group of saturated steroids by a modification of the Oppenauer method employing aluminium phenoxide. The chromatographic analysis of the crude oxidation product has revealed yet another αβ-unsaturated ketone (max. at 2410 A., $E^{1\%}_{1cm.}$ = 380), the further investigation of which will be reported in a subsequent communication.

The results described above raise the question as to whether β-sitosterol as described in the literature is indeed a homogeneous substance and the wider issue as to whether crystallisation alone can be relied upon for the resolution of phytosterol mixtures. We believe that oxidation by the Oppenauer method, followed by chromatographic resolution of the product, provides a reliable method of determining the homogeneity or otherwise of a phytosterol and this is being tested by further experimental work.

The four main unsaturated ketones described above have been characterised by the preparation of oximes, semicarbazones and 2 : 4-dinitrophenylhydrazones, the light absorption data for these substances being indicated in the accompanying table.

			Oxime.		Semicarbazone.		2 : 4-Dinitrophenylhydrazone.	
	A.	ε.	A.	ε.	A.	ε.	A.	ε.
Cholestenone	2405	18,000						
	3120	100	2400	23,000	2705	26,000	3910	32,000
Fucostadienone	2400	17,000						
	3100	70	2405	23,000	2715	27,000	3920	32,000
Stigmastadienone	2410	17,000						
	3075	75	2400	22,000	2710	29,000	3920	30,000
Sitostenone	2410	17,000						
	3070	75	2400	20,000	2720	25,000	3920	32,000

(The absorption spectra of the ketones and oximes were determined in alcoholic solution, those of the semicarbazones and dinitrophenylhydrazones in chloroform.)

The absorption spectra of cholestenone, its oxime and semicarbazone have been recorded by other workers (Heilbron and Morton, J., 1928, 48; Menschick, Page, and Bossert, *Annalen*, 1932, **495**, 225; Mohler, *Helv. Chim. Acta*, 1937, **20**, 289) and the significance of the location of the main band in the spectrum of cholestenone and other αβ-unsaturated ketones has been discussed by Woodward (*J. Amer. Chem. Soc.*, 1941, **63**, 1123) and by Evans and Gillam (J., 1941, 815). The position of the principal band in the absorption spectra of 2 : 4-dinitrophenylhydrazones of saturated aldehydes and ketones lies between 3500 and 3750 A., and for αβ-unsaturated aldehydes and ketones, between 3800 and 3950 A.

Experimental.

Melting points are uncorrected. Rotations were carried out in a 1 dcm. tube in chloroform solutions. Specimens for analysis were dried at a suitable temperature in a high vacuum for some hours.

Cholestenone.—A solution of cholesterol (10 g.) in dry acetone (120 c.c.) was mixed with aluminium *tert.*-butoxide (12 g.) in dry benzene (300 c.c.) and refluxed for 18 hours. The reaction mixture was washed with 2N-sulphuric acid, water, and sodium bicarbonate solution and the gum obtained after drying and evaporation was heated in a vacuum for an hour at 100°. The residue solidified on cooling and on crystallisation from methyl alcohol (60 c.c.) and ether (30 c.c.) yielded cholestenone (8·6 g.), m. p. 77—81°. This m. p. was raised to 81—82° after adsorption on and fractional elution from alumina. The semicarbazone, prepared by refluxing an alcoholic solution of the ketone for 15 minutes with aqueous alcoholic semicarbazide acetate (10%), was twice crystallised from alcohol–ethyl acetate (1 : 1) and had m. p. 234—235°. Cholestenoneoxime, prepared in a similar manner from hydroxylamine acetate, after crystallisation from ethyl acetate, had m. p. 152—153°. 2 : 4-Dinitrophenylhydrazone : A hot solution of the ketone in alcohol was added to a slight excess of the hydrazine hydrochloride in alcohol. The flocculent red precipitate was collected after 3 hours, washed, dried, and adsorbed on a column of activated alumina from benzene solution. The main red band obtained on developing the chromatogram was washed through with benzene and the solid remaining after evaporation was crystallised from benzene–alcohol (4 : 1), giving the cholestenone-2 : 4-dinitrophenylhydrazone in fine red needles (plates from benzene alone), m. p. 233° (Found : N, 10·4. Calc. for $C_{33}H_{48}O_4N_4$: N, 9·9%).

Fucostadienone.—Fucosterol (10 g.), oxidised as above, gave a ketone which was purified by adsorption on alumina from solution in light petroleum (b. p. 40—60°). Elution with benzene yielded a residue, which was crystallised from methyl alcohol and finally from acetone to give *fucostadienone* (5 g.) in elongated plates, m. p.

94—94·5°, $[\alpha]_D^{20°}$ + 76° (*c* = 1·5) (Found: C, 84·9; H, 11·3. $C_{29}H_{46}O$ requires C, 84·8; H, 11·2%). The *semicarbazone*, crystallised from alcohol–chloroform, had m. p. 238° (decomp.) (Found: N, 9·25. $C_{30}H_{49}ON_3$ requires N, 9·0%). The *oxime* was purified by crystallisation from alcohol and sublimation in a high vacuum, forming needles, m. p. 166—167° (Found: N, 3·3. $C_{29}H_{47}ON$ requires N, 3·3%). The 2 : 4-*dinitrophenylhydrazone* after purification by percolation of a benzene solution through activated alumina separated from benzene in flat needles, m. p. 237° (Found: N, 9·9. $C_{35}H_{50}O_4N_4$ requires N, 9·5%).

Stigmastadienone.—The crude solid from the oxidation of stigmasterol (700 mg., m. p. 166—168°) was dissolved in benzene–light petroleum (b. p. 40—60°) (1 : 1) and fractionated on activated alumina. Ten fractions were collected and from seven of these crystalline material, m. p. 123—125°, was obtained, which after two crystallisations from acetone–methyl alcohol yielded stigmastadienone (400 mg.), m. p. 124·5—125° (Fernholz and Stavely, *loc. cit.*, give m. p. 125°), $[\alpha]_D^{20°}$ + 56° (*c* = 1·59) (lit., $[\alpha]_D^{20°}$ + 63°). The *oxime* was crystallised twice from light petroleum (b. p. 40—60°), separating in needle clusters, m. p. 187—188° (Found: C, 82·0; H, 11·1. $C_{29}H_{47}ON$ requires C, 81·8; H, 11·1%). The semicarbazone was crystallised from alcohol–chloroform (1 : 1) and had m. p. 238—239° (Fernholz and Stavely, *loc. cit.*, give m. p. 235—237°). The 2 : 4-*dinitrophenylhydrazone* after purification by percolation of a benzene solution through activated alumina, crystallised from alcohol–benzene (1 : 1) in fine red needles, m. p. 244—245° (decomp.) (Found: N, 9·3. $C_{35}H_{50}O_4N_4$ requires N, 9·5%).

Sitostenone.—Crude sitosteryl acetate (50 g.) (*ex* wheat germ oil) was crystallised from ethyl acetate in such a manner that the less soluble and the more soluble fractions were eliminated. After 16 crystallisations a β-sitosteryl acetate (10 g.) was obtained, m. p. 125°, $[\alpha]_D^{20°}$ − 35° (*c* = 2·10). On hydrolysis with alcoholic potassium hydroxide, this yielded a β-sitosterol in long flat needles from acetone–alcohol (1 : 1), m. p. 136—137°, $[\alpha]_D^{20°}$ − 32° (*c* = 1·99). Oxidation of this sterol (5 g.) as previously described yielded a pale yellow gum which solidified on cooling. A solution of this in light petroleum (b. p. 40—60°) was run through a column of activated alumina (18 × 2 cm.); by variation of the proportion of benzene in the mixture used to develop the chromatogram, 27 fractions (each of 50 c.c.) were obtained. The product from each of these was crystallised from methyl alcohol and adjacent fractions of similar m. p. were combined and recrystallised from the same solvent. The five main fractions resulting from this procedure, in the order in which they were eluted from the adsorbent, were: (A) m. p. 115—122° (650 mg.); (B) m. p. 95—97° (150 mg.); (C) m. p. 86—88° (450 mg.); (D) m. p. 79—81° (800 mg.); (E) m. p. 131—133° (550 mg.).

Further crystallisation of (A) gave a crystalline solid, m. p. 143—145°, $[\alpha]_D^{20°}$ + 52° (*c* = 1·30). *Light absorption in alcohol:* Maxima, 2405 and 2900 A., $E_{1\,cm.}^{1\%}$ 70 and 2·5 respectively. The 2 : 4-*dinitrophenylhydrazone*, purified by adsorption on and fractional elution from alumina, was obtained as a red amorphous powder from ethyl acetate, m. p. 208—209° (Found: N, 9·0. $C_{35}H_{51}O_4N_4$ requires N, 9·4%). *Light absorption in chloroform:* Maxima, 3730 and 2540 A., $E_{1\,cm.}^{1\%}$ 430 and 265 respectively.

Since fractions (B) and (C) exhibited similar light absorption in alcohol and gave the same 2 : 4-dinitrophenylhydrazone, m. p. 245°, they were combined and twice crystallised from methyl alcohol, yielding a small quantity of a product, m. p. 112—115°, which was not examined further.

Fraction (D) on crystallisation from ethyl alcohol yielded sitostenone, m. p. 83—84° (Found: C, 84·6; H, 11·6. Calc. for $C_{29}H_{48}O$: C, 84·4; H, 11·7%). Prepared in the usual manner and separating from benzene–alcohol (1 : 1) as a dark red powder, the 2 : 4-*dinitrophenylhydrazone* had m. p. 247—248° (Found: N, 9·4. $C_{35}H_{52}O_4N_4$ requires N, 9·45%).

Fraction (E), which required benzene–methyl alcohol (20 : 1) for elution from the alumina, after crystallisation had m. p. 133—134·5°, $[\alpha]_D^{20°}$ − 20·2° (*c* = 1·53).

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