159. *Studies in the Sterol Group. Part XLV. Investigation of the Homogeneity of Sitosterol by Oxidation with the Oppenauer Reagent.*

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Oxidation of Tall-öl sitosterol by Oppenauer's method, followed by careful chromatographic purification, gives pure Δ^4-β-sitostenone, m. p. 88°. Similar treatment of sitosterol from wheat-germ oil indicates that this material is far from homogeneous, although the main product is the same $\alpha\beta$-unsaturated ketone. Wheat-germ sitosterol contains triacontane. Rather surprisingly, the small amount of saturated sterol present in both samples of sitosterol is oxidised to sitostanone.

Oppenauer oxidation, followed by chromatographic analysis of the ketones, has been shown to be of considerable value for examining the homogeneity of sitosterols, and in particular, for determining approximately the proportion of sitostanol present. It provides a far more delicate and convenient criterion of purity than the fractional crystallisation method employed hitherto and would appear to be generally applicable in the steroid field.

It was recently observed (Jones, Wilkinson, and Kerlogue, J., 1942, 391) that a specimen of β-sitosterol, with constants in good agreement with those reported in the literature, was actually far from homogeneous. The method described has now been examined in more detail with Tall-öl sitosterol (for the gift of a sample of which we are deeply indebted to Professor R. E. Marker of the Pennsylvania State College), which is reputed to contain a high percentage of the β-isomer (Sandqvist and Bengtsson, *Ber.*, 1931, **64**, 2167; see also Windaus, Werder, and Gschaider, *Ber.*, 1932, **65**, 1006).

Tall-öl sitosterol (m. p. 137—138°, $[\alpha]_D^{20°}$ −28·7°) was oxidised by Oppenauer's method (*Rec. Trav. chim.*, 1937, **56**, 137), and the crude ketone carefully chromatographed ("flowing chromatogram") on a column of alumina. It should be noted that much larger columns than usual were used in the experiments described in this paper, since reasonably good separations could only be achieved by using 100 times as much adsorbent as adsorbate. The results illustrated in Fig. 1 indicate that the oxidation product consists of four main portions, which for convenience are designated A, B, C, and D.

A, which accounted for 3% of the theoretical yield of ketonic material, was collected in four fractions with m. p.'s 113—100°, and will be discussed later. A second chromatographic analysis of B (three fractions of m. p.'s 155—152°), isolated in 2·5% yield, gave sitostanone, m. p. 157°.

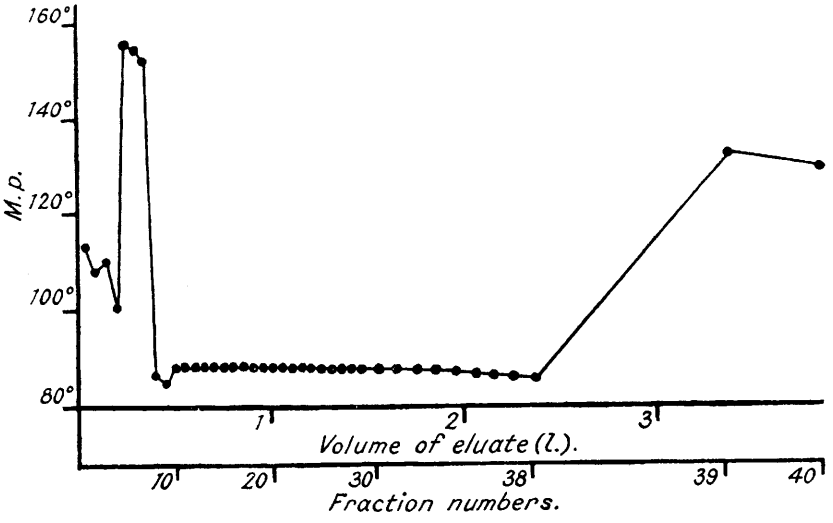
The main product of the oxidation, portion C, was represented by 32 fractions, m. p.'s 88—85°, which amounted to 66% of the theoretical yield, and consisted mainly of Δ^4-β-sitostenone. Fractions 10—13, which had m. p. 88° and $[\alpha]_D^{20°}$ +85·8°, were repeatedly recrystallised and rechromatographed without undergoing the slightest change in m. p. or rotation and must be pure Δ^4-β-sitostenone (cf. Marker and Wittle, *J. Amer. Chem. Soc.*, 1937, **59**, 2704; Marker, Kamm, and Wittle, *ibid.*, 1938, **60**, 1072; Heiduschka and Gloth, *Arch. Pharm.*, 1915, **253**, 415; Coffey, Heilbron, and Spring, J., 1936, 738). The highest m. p. previously recorded for the ketone is 83—84° (Jones, Wilkinson, and Kerlogue, *loc. cit.*). The m. p.'s of the oxime, semicarbazone, and 2 : 4-dinitrophenylhydrazone did not differ appreciably from those already recorded, but the intensities of the light absorption of both the ketone and its derivatives were somewhat higher than those obtained previously. Although the major portion (C) of the oxidation product of Tall-öl sitosterol undoubtedly consists of Δ^4-β-sitostenone, there was a slight but definite indication that the later fractions were contaminated with small amounts of a closely related sitostenone, since rechromatography yielded a sitostenone with m. p. 86° and $[\alpha]_D^{20°}$ +89·0°.

The unoxidised sterol, which comprised portion D, had m. p. 135—136°, $[\alpha]_D^{20°}$ −19·0°, indicating that no marked concentration of the strongly dextrorotatory sitostanol had occurred. This unexpected finding is further discussed below.

* Throughout the work on diarylpyrroles a tendency has been found for carbon analyses to be low. Compare analyses for diphenylpyrrole itself (this vol., p. 594) and Gabriel's analytical results (p. 598).

FIG. 1.

Chromatogram from oxidation of Tall-öl sitosterol.

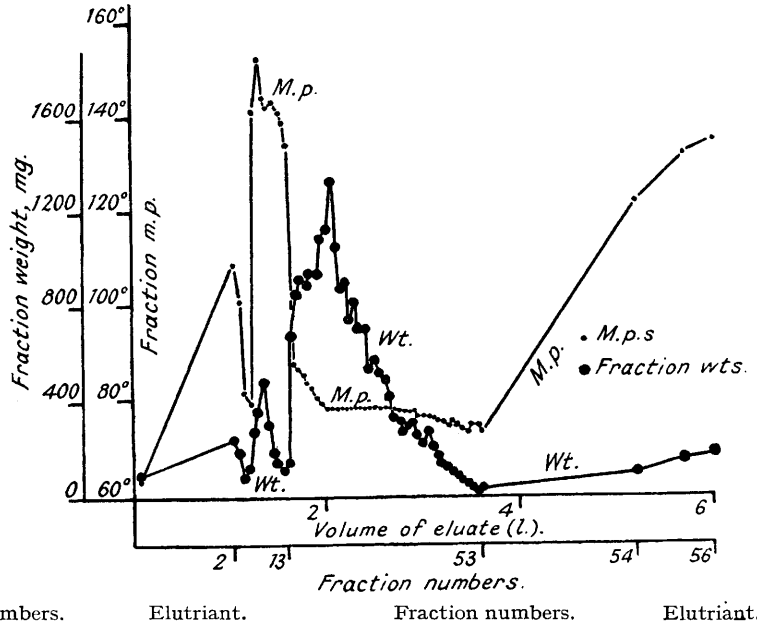


Fraction numbers.	Elutriant.		Fraction numbers.	Elutriant.	
1—4	Benzene . . .	A	31—38	95 Benzene : 5 Ether .	C
5—7	Benzene . . .	B	38—39	Ether	—
8—30	97½ Benzene : 2½ Ether .	C	39—40	Methyl alcohol . .	D

The chromatogram obtained when the above procedure was repeated with wheat-germ oil sitosterol is illustrated in Fig. 2, and is seen to be conveniently divisible into five sections, designated E, F, G, H, and I. E was easily eluted from the column with light petroleum, and crystallisation gave triacontane in 0·3% yield.

FIG. 2.

Chromatogram from oxidation of wheat-germ oil sitosterol.



Fraction numbers.	Elutriant.		Fraction numbers.	Elutriant.	
1	Light petroleum .	E	14—53	90 Benzene : 10 Ether .	H
2—5	Benzene . . .	F	53—54	Ether	—
6—13	95 Benzene : 5 Ether .	G	54—56	Methyl alcohol . .	I

It was also obtained by chromatography of the original sterol. This hydrocarbon has frequently been isolated from plant sources, and Ichiba (*Sci. Papers Inst. Phys. Chem. Res. Tokyo*, **1935**, **28**, 112) obtained an impure hydrocarbon from wheat-germ sitosterol.

Further elution gave a 1·8% yield of fractions (F), with m. p.'s varying from 110° to 80°. Rechromatography and crystallisation of this material, essentially similar to the corresponding fractions (A) isolated from Tall-öl sitosterol, failed to give any pure substance, and the poor yields precluded a more detailed examination. It was ascertained, however, that the main constituent is probably an αβ-unsaturated ketone, and one fraction exhibited intense absorption in the ultra-violet ($E^{1\%}_{1\,cm.}$ at 2400 A. = 365). Chromatographic examination failed to reveal the presence of any similar material in the original sterol.

Sitostanone (6% yield) was isolated without difficulty from the eight fractions (m. p.'s 152—136°) comprising portion G of the chromatogram. It was ascertained by chromatographic analysis that neither of the original sterols contained any sitostanone. Since it is well recognised that saturated sterols are not readily oxidised under the standard Oppenauer conditions (Jones, Wilkinson, and Kerlogue, *loc. cit.*; Reich and Reichstein, *Arch. Intern. Pharm. Ther.*, 1941, **65**, 415), it can only be concluded that the oxidation of the sitostanol present in the starting materials is brought about by the intervention of the αβ-unsaturated ketone, which must act as a powerful hydrogen acceptor.

The main ketonic portion (H), consisting of 40 fractions with m. p.'s varying from 88° to 75°, representing a 69·5% yield, was by no means as homogeneous as that obtained from Tall-öl sitosterol. Repeated rechromatography resulted in the isolation in a pure state of only Δ⁴-β-sitostenone, m. p. 88°, $[\alpha]_D^{20°}$ +85·9°, identical in all respects with that obtained from the alternative source. The accompanying ketones appeared to be αβ-unsaturated and, as was observed with the ketones from Tall-öl sitosterol, had somewhat higher rotations than the pure Δ⁴-β-sitostenone. After recrystallisation, the unchanged sterol (I) (1·5% yield) had m. p. 134·5—135·5°, $[\alpha]_D^{20°}$ −24·1°, and, like the material recovered in the previous experiment, showed little evidence of increased content of saturated sterol (sitostanol).

Although the method now described has led to the isolation of only one pure sitostenone, it furnishes, nevertheless, an excellent means of ascertaining the degree of homogeneity of a sitosterol, a process far more convenient and reliable than the tedious fractional crystallisation methods hitherto employed. The value of the results obtained is clearly illustrated in the accompanying table. The total yields based on the original

	Wheat-germ oil sitosterol.		Tall-öl sitosterol.	
	Yield, %, based on original sterol.	Proportion of ketonic product, %.	Yield, %, based on original sterol.	Proportion of ketonic product, %.
Triacontane	0·3	0·4	—	—
Unidentified ketone, m. p. *ca.* 115°	1·8	2·3	3·2	4·5
Sitostanone	5·9	7·6	2·5	3·2
Sitostenones	69·5	89·7	66·2	92·3
Recovered sterol	1·5	—	3·8	—

sterol do not total 100%, a discrepancy occasioned by the inevitable losses incurred in the crystallisation of each fraction from the chromatogram.

Experimental.

M. p.'s are uncorrected. Rotations were measured in a 1-dcm. tube in chloroform solutions, made up in carefully calibrated flasks. Specimens for analysis were dried at a suitable temperature in a high vacuum for some hours. Chromatogram fractions were all crystallised from either methyl or ethyl alcohol before determination of the m. p. Except where stated otherwise, light petroleum refers to that fraction of b. p. 40—60°.

Oxidation of Tall-öl Sitosterol.—To a solution of the commercial sterol [10 g.; m. p. 137—138°, $[\alpha]_D^{20°}$ −28·7° (*c* = 5·030)] in dry benzene (300 c.c.) and dry acetone (120 c.c.), freshly sublimed aluminium *tert.*-butoxide (12 g.) was added, and the mixture refluxed for 18 hours. The resulting solution was washed repeatedly with dilute sulphuric acid and water, and the benzene distilled off; the residue, after being heated for 2 hours at 100° in a vacuum to remove mesityl oxide, readily solidified on cooling. This residue was dissolved in light petroleum and chromatographed on a 3·6 × 150 cm. column of " Birlec " alumina (see Fig. 1).

Portion A (323 mg.) was not investigated further. Portion B (249 mg.) was rechromatographed on a 3·0 × 40 cm. column of the same adsorbent and readily yielded sitostanone, m. p. 157°, unchanged on repeated recrystallisation from alcohol (Found: C, 83·6; H, 12·1. Calc. for $C_{29}H_{50}O$: C, 84·0; H, 12·1%). This exhibited no high-intensity absorption in the ultra-violet, and gave a yellow 2 : 4-*dinitrophenylhydrazone*, m. p. 223° (decomp.) (Found: N, 9·1. $C_{35}H_{54}O_4N_4$ requires N, 9·4%).

From C (6·62 g.), fractions 10—13 were combined and repeatedly crystallised, without any change in m. p., giving pure Δ⁴-β-sitostenone, m. p. 88°, $[\alpha]_D^{20°}$ +85·8° (*c* = 4·475). The analytical data for the ketone and its derivatives, prepared in the usual manner, are given in the following table.

			Found, %.			Calc., %.		
Substance.	M. p.	Formula.	C.	H.	N.	C.	H.	N.
Δ⁴-β-Sitostenone	88°	$C_{29}H_{48}O$	84·4	11·7	—	84·4	11·7	—
Oxime	175·5	$C_{29}H_{49}ON$	81·45	11·7	—	81·45	11·55	—
			81·45	11·5	—	—	—	—
Semicarbazone	250 *	$C_{30}H_{51}ON_3$	76·7	10·8	—	76·7	10·9	—
2 : 4-Dinitrophenylhydrazone	253 *	$C_{35}H_{52}O_4N_4$	—	—	9·35	—	—	9·45

* With decomposition.

Light-absorption data for the ketone and its derivatives were as follows:

Substance.	$\lambda_{max.}$, A.	$\varepsilon_{max.}$	Solvent.	Substance.	$\lambda_{max.}$, A.	$\varepsilon_{max.}$	Solvent.
Δ⁴-β-Sitostenone	2405	20,000	EtOH	Semicarbazone	2730	30,000	$CHCl_3$
Oxime	2410	23,500	,,	2 : 4-Dinitrophenyl-hydrazone	3890	32,000	,,

The last 7 fractions of portion C were combined and rechromatographed on a 3·0 × 40 cm. column of alumina;

14 fractions were collected, the last 4 of these being recrystallised twice from aqueous methyl alcohol, giving a ketone with m. p. 86°, $[\alpha]_D^{20°}$ +89·0° (c = 3·950).

The two fractions of unchanged sterol comprising portion D (383 mg.) were combined, and on recrystallisation from alcohol had m. p. 135—136°, $[\alpha]_D^{20°}$ −19·0° (c = 1·870).

Chromatography of Tall-öl Sitosteryl Acetates.—The crude acetates [10 g.; m. p. 118·5—119·5°, $[\alpha]_D^{20°}$ −33·0° (c = 8·485)], prepared with acetic anhydride and pyridine, were adsorbed and fractionally eluted from a 3·1 × 105 cm. column of "Birlec" alumina, 14 fractions with m. p.'s ranging from 122° to 114° being obtained. None of these fractions was pure, for on repeated crystallisation the m. p. slowly rose and the rotations changed.

Oxidation of Wheat-germ Oil Sitosterol.—The crude sterol {30 g.; m. p. 137—138°, $[\alpha]_D^{20°}$ −26·6 (c = 14·11)} was oxidised as described above, and the product, which solidified on cooling, was dissolved in light petroleum and chromatographed, giving the results illustrated in Fig. 2. After several recrystallisations from alcohol–benzene, the triacontane (98 mg.) had m. p. 65° (lit., m. p. 65°) (Found: C, 85·2; H, 14·9. Calc. for $C_{30}H_{62}$: C, 85·3; H, 14·7%).

Portion F (554 mg.), corresponding to A from the Tall-öl chromatogram, was rechromatographed on a 3·0 × 40 cm. column, giving 7 fractions with m. p.'s ranging from 115—116° to 97—98°, all of which gave red precipitates with an acid solution of 2 : 4-dinitrophenylhydrazine. The second fraction, the only one so examined, exhibited high-intensity light absorption (see p. 601). Unfortunately, none of the fractions was homogeneous, the m. p.'s rising on further chromatography or recrystallisation.

Rechromatography of G (1·78 g.) on a 3·1 × 105 cm. column gave 11 fractions, and pure sitostanone, identical with the material described above, was readily obtained.

The 40 fractions (20·85 g.) comprising portion H were chromatographed twice more in three groups on 2·9 × 45 cm. columns, whereby a total of 78 fractions was obtained. Only one pure substance, however, *viz.*, Δ^4-β-sitostenone, was isolated by this procedure. It had m. p. 88°, $[\alpha]_D^{20°}$ +85·9° (c = 2·070), which constants remained unchanged after repeated crystallisation, and it gave the same derivatives as the ketone already described. Crystallisation of any of the remaining fractions from these chromatographic experiments invariably resulted in an increase in the m. p. Two typical fractions had the following constants: (*a*) m. p. 85°, $\varepsilon_{max.}$ at 2405 A. = 19,500; (*b*) m. p. 80°, $[\alpha]_D^{20°}$ +88·8° (c = 2·605), $\varepsilon_{max.}$ at 2405 A. = 19,500.

The last fraction of the unchanged sterol (I) (461 mg.) had m. p. 134·5—135·5°, $[\alpha]_D^{20°}$ −24·1°.

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