275. *Studies in the Sterol Group. Part XLII. The Constitution of Zymosterol.*

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The constitution of the diethenoid secondary yeast sterol, zymosterol, has been conclusively proved. The identity of the sterols obtained by the various processes employed by previous workers has been confirmed (see Table I), and the fully saturated zymostanol has been shown to be identical with cholestanol by a careful comparison of the melting points and optical rotations of a large number of derivatives (Table II).

That the readily reducible double bond of zymosterol is located in the side chain in an *iso*propylidene group (not previously observed in the sterol series) is proved by the isolation of acetone in 50% yield on ozonolysis, none being obtained in a control experiment on the dihydro-sterol, α-zymostenol. The isomerisation of the inert ethenoid linkage of the latter with hydrogen chloride to yield β-*zymostenol* (reducible to zymostanol) and the facile oxidation of α-zymostenol with selenium dioxide to *dehydro-α-zymostenol* are analogous to the behaviour of α-ergostenol with these reagents. It is thus evident that in zymosterol the second double bond is in the 8 : 14-position and that we have the first authenticated example of a natural sterol without the familiar 5 : 6-ethenoid linkage.

The close resemblance in properties between the α- and β-cholestenols and α- and β-zymostenol and their derivatives (Table III) indicates that these compounds are respectively identical and provides verification of the suggested locations of the ethenoid linkage in the zymostenols.

The first authenticated discovery of a sterol other than ergosterol in yeast is due to Smedley-MacLean (*Biochem. J.*, 1920, **14**, 484; 1928, **22**, 22, 980; *Chem. and Ind.*, 1929, **48**, 295), who isolated a dextrorotatory sterol of m. p. 108—109°, to which the name zymosterol and the formula $C_{27}H_{42}O$ were assigned. Subsequent workers (Wieland and Asano, *Annalen*, 1929, **473**, 300; Wieland and Gough, *ibid.*, 1930, **482**, 36; Wieland and Kanaoka, *ibid.*, 1937, **530**, 146; Häussler and Brauchli, *Helv. Chim. Acta*, 1929, **12**, 187; Heilbron and Sexton, J., 1929, 2255; Gesellschaft für Chemische Industrie in Basel, D.R.P. 549,110; Reindel and Weickmann, *Annalen*, 1929, **475**, 86; 1930, **482**, 120), employing a variety of methods of isolation, have confirmed its homogeneity, but hitherto no conclusive information as to its constitution has been obtained. The analytical data of Reindel and Weickmann (*loc. cit.*, 1930) indicated a formula $C_{30}H_{50}O$ for zymosterol, but Wieland and Kanaoka (*loc. cit.*), by quantitative hydrolysis of the acetate and benzoate, eventually established the formula $C_{27}H_{44}O$, suggesting that it is a diethenoid C_{27}-sterol, although Reindel and Weickmann (*loc. cit.*, 1930) were unable to identify zymostanol with any known saturated sterol.

We have isolated zymosterol in reasonable yield from ergosterol residues by the convenient method evolved by Heilbron and Sexton (*loc. cit.*), involving the separation of the insoluble dibromide from ethereal solution; the debromination, either by the original method of treatment with zinc and hot alcohol, or by the rather slower process with zinc and acetic acid in the cold (Reindel and Weickmann, *loc. cit.*, 1930), leads to identical products. The properties of the zymosterol so obtained are in close agreement with those described by other workers and as additional proof we have confirmed the identity of our product, prepared from the dibromide, with zymosterol obtained by fractional crystallisation of the steryl benzoates according to the method of Wieland and Asano (*loc. cit.*).

That zymosterol contains two ethenoid linkages was revealed by titration with perbenzoic acid (Reindel and Weickmann, *loc. cit.*), but on catalytic hydrogenation only one of the ethenoid linkages is reduced, yielding α-zymostenol. Isomerisation of the latter compound with hydrogen chloride in dry chloroform gives β-*zymostenol*, which can be hydrogenated further to zymostanol, previously prepared by Reindel and Weickmann (*loc. cit.*, 1930). We have been able to prove conclusively that pure zymostanol is identical with cholestanol by a comparison of the properties of a number of derivatives prepared

TABLE I.

Compound.	M. p.	$[\alpha]_D^{20°}$.	Lit. m. p.	Lit. $[\alpha]_D^{20°}$.	Ref.
Zymosterol	107—109°	+50°	107—110°	+52·2°	(1)
			108—110	+47·3	(2)
			108—110	+38·6 (Hg)	(3)
			105—107	+44·0	(4)
			108—109	+34·1 (Hg)	(5)
Zymosteryl acetate	107—108	+35	102—104	+33·6	(1)
			104—106	+33·5	(2)
			106—107		(3)
			115		(5)
Zymosterol dibromide	157	+ 7·4	157—158	+ 7·1 (Hg)	(1)
			168		(3)

(1) Reindel and Weickmann, *loc. cit.* (2) Wieland and Asano, *loc. cit.* (3) Heilbron and Sexton, *loc. cit.* (4) Haüssler and Brauchli, *loc. cit.* (5) Smedley-MacLean, *loc. cit.*

TABLE II.

Compound.	M. p.	$[\alpha]_D^{20°}$.	Lit. m. p.	Lit. $[\alpha]_D^{20°}$.	Ref.
Zymostanol	140—141°	+24·8°	139—140°	+20·6° (Hg)	(1)
Cholestanol	140—141	+24·1	140—141	+23	(6)
Zymostanyl acetate	114—116 / 109—110	+10·9	130—131	—	(1)
Cholestanyl acetate	109—110	+11·5	110	—	(7)
Zymostanyl benzoate	131—133	+17·8	—	—	—
Cholestanyl benzoate	131—133	+18·5	135	+19·3	(6)
Zymostanyl phenylurethane	155—156	+11·3	—	—	—
Cholestanyl phenylurethane	151	+11·8	—	—	—
Zymostanone	125—126	+40	—	—	—
Cholestanone	127—128	+40	127—128	+43·7	(6)
2-Bromozymostanone	166—167	—	—	—	—
2-Bromocholestanone	167—168	—	169—170	—	(8)
Zymostanedicarboxylic acid	196—197	+33·4	—	—	—
Dimethyl ester	50	+23·7	—	—	—
Cholestanedicarboxylic acid	195—196	+35·7	195—196	—	(9) (10)
Dimethyl ester	58—60	+23·3	60	—	
Zymostane	74—76	+20·9	—	—	—
Cholestane	79—80	+22·1	80	+24·4	(11)

(6) Vavon and Jakubowicz, *Bull. Soc. chim.*, 1933, **53**, 581. (7) Schenck, Buchholz, and Wiese, *Ber.*, 1936, **69**, 2696. (8) Butenandt and Wolff, *Ber.*, 1935, **68**, 2091. (9) Ruzicka and Plattner, *Helv. Chim. Acta*, 1938, **21**, 1717. (10) Windaus and Kuhr, *Annalen*, 1937, **532**, 52. (11) Mauthner, *Monatsh.*, 1909, **30**, 635.

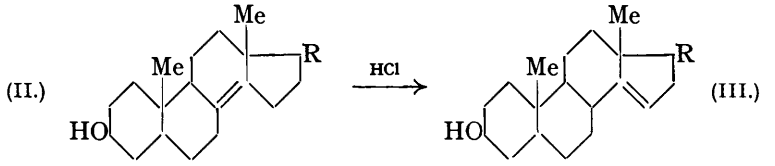
under identical conditions (Table II). It will be observed that there is some disagreement in the properties of the acetates of the saturated sterols. The previous workers observed a remarkably high melting point for zymostanyl acetate (130—131°) which we have been unable to confirm, the two samples obtained by the present authors being prepared, in the one case, directly by reduction of β-zymostenyl acetate and alternatively, by acetylation of zymostanol. No depressions in melting point were observed on mixing the two specimens or on admixture of either with cholestanyl acetate, and no explanation, other than the possibility of partial racemisation at one of the asymmetric centres, can be offered to explain the discrepancy. The rather lower melting points, compared with the cholestane derivatives, of zymostane and the dimethyl ester of zymostane-C_2||C_3-dicarboxylic acid are to be attributed to experimental difficulties in the purification of the small amounts of these low-melting substances available.

The carbon skeleton of zymosterol having been established, the position of the two ethylenic bonds was next investigated. Our observation that zymosterol dibromide is only very slowly debrominated by treatment with sodium iodide in acetone or alcohol (Schoenheimer, *J. Biol. Chem.*, 1935, **110**, 461) suggested that one of the double bonds was located in the side chain. Fernholz and Stavely (*J. Amer. Chem. Soc.*, 1939, **61**, 2956) have recently taken advantage of a similar observation in order partly to debrominate

stigmasteryl acetate tetrabromide. Accordingly the ozonolysis of the sterol was studied and a 50% yield of acetone (estimated as its 2 : 4-dinitrophenylhydrazone) was readily obtained together with a small quantity of formaldehyde. The isolation of the latter is of no consequence, since a rather greater yield was obtained on ozonolysis of cholesterol. It now became evident that zymosterol contains an *iso*propylidene group in the side chain, which must be represented by $-CHMe\cdot CH_2\cdot CH_2\cdot CH{:}CMe_2$ (I), and further, since α-zymostenol fails to yield any acetone on ozonolysis, it is this side-chain ethylenic linkage which is readily hydrogenated.

In a study of the constitution of the diethenoid, α_1-sitosterol, isolated from wheat germ oil, Bernstein and Wallis (*J. Amer. Chem. Soc.*, 1939, **61**, 2308), having obtained evidence for the location of one ethylenic linkage, assumed the position of the other by analogy, since " all natural sterols which are unsaturated have been found to have one double bond in the 5 : 6-position ". The location of the second ethylenic linkage of zymosterol in this position is improbable for several reasons. Sterols with 5 : 6-unsaturation are usually readily hydrognated, and the high dextrorotatory power of zymosterol (and some of the other yeast sterols) is a strong indication of the absence of the familiar 5 : 6-ethenoid linkage (cf. α-spinasterol; Simpson, J., 1937, 730). We have been unable to obtain any evidence (absorption spectrum) of the formation of an αβ-unsaturated ketone on oxidation of zymosterol by the Oppenauer method (*Rec. Trav. chim.*, 1937, **56**, 137), which can now be regarded as a characteristic test for 5 : 6-unsaturation, and distillation of zymosterol with anhydrous copper sulphate fails to produce a hydrocarbon containing a conjugated system (cf. Mauthner and Suida, *Monatsh.*, 1896, **17**, 29).

The behaviour of α-zymostenol in its isomerisation to β-zymostenol, which no longer resists hydrogenation, is very reminiscent of the conversion of α-ergostenol (II, R = C_9H_{17}) (Heilbron and Wilkinson, J., 1932, 1708) and α-cholestenol (II, R = C_8H_{17}) (Schenck, Buchholz, and Wiese, *Ber.*, 1936, **69**, 2696) into the corresponding β-stenols (III). Both



γ-cholestenol (IV) (Schenck, Buchholz, and Wiese, *loc. cit.*) and δ-cholestenol (V) (Windaus, Linsert, and Eckhardt, *Annalen*, 1938, **534**, 22) contain inert ethylenic linkages and the

possibility that zymosterol has an inert ethenoid linkage in one or other of these positions cannot immediately be excluded. It has been found, however, that zymosterol is quite unaffected by shaking with platinum-black, whereas, under these conditions, both the γ- and δ-cholestenols are isomerised to the stable α-cholestenol, and a careful comparison of the properties of the latter with α-zymostenol reveals that these two compounds and their derivatives are almost certainly identical (Table III), although a direct comparison has not been possible.

On isomerisation of α-zymostenol with hydrogen chloride in chloroform we have obtained a β-isomer, identical in properties with the β-zymostenol described by Reindel and Weickmann (*loc. cit.*, 1930). We believe, however, that, like the β-ergostenol prepared in a similar manner, this almost certainly contains some unchanged α-isomer. By employing α-zymostenyl benzoate (cf. Heilbron and Wilkinson, *loc. cit.*) in place of the free sterol, it has been possible to complete the isomerisation and obtain a pure β-*zymostenyl benzoate*, yielding a β-*zymostenol* on hydrolysis, both of which in properties closely resemble β-cholestenol and its benzoate (Table III). Whereas there would appear to be little doubt

TABLE III.

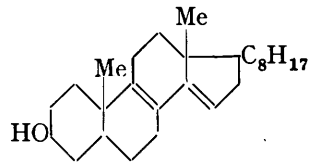
Compound.	M. p.	$[\alpha]_D^{20°}$.	Lit. m. p.	Lit. $[\alpha]_D^{20°}$.	Ref.
α-Zymostenol	119—120°	+20·8°	120—122°	+20·7°	(1)
			120—121	+28·7	(12)
			115—116	+28·9 (Hg)	(3)
α-Cholestenol	—	—	119—120	+20·4	(7)
α-Zymostenyl acetate	77—78	+ 7·6	81—84	+ 9·7	(1)
α-Cholestenyl acetate	—	—	77—78	+ 9·5	(7)
α-Zymostenyl benzoate	109—111	+ 6·4	—	—	—
α-Cholestenyl benzoate	—	—	115	+ 8·5	(7)
β-Zymostenol	128	+30·5	—	—	—
β-Cholestenol	—	—	130	+34	(7)
β-Zymostenyl acetate	76—77	—	—	—	—
β-Cholestenyl acetate	—	—	91—92	—	(7)
β-Zymostenyl benzoate	165—166	+31·9	—	—	—
β-Cholestenyl benzoate	—	—	168	+32·5	(7)

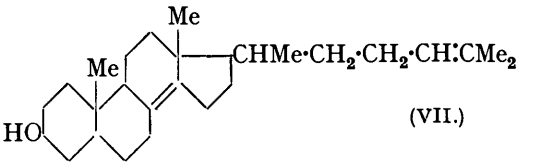
(12) Wieland and Kanaoka, *loc. cit.*

that β-zymostenol is identical with β-cholestenol, we have so far been unable to raise the melting point of β-zymostenyl acetate to that recorded in the literature for β-cholestenyl acetate. While both zymosterol and β-zymostenol give approximately theoretical values on titration with perbenzoic acid, we find that α-zymostenol, in contradiction to Reindel and Weickmann (*loc. cit.*), takes up twice the expected amount of oxygen. Such an anomaly is not without parallel in the sterol series (Fernholz and Moore, *J. Amer. Chem. Soc.*, 1939, **61**, 2467; Windaus and Lüttringhaus, *Annalen*, 1930, **481**, 119).

A further analogy between α-zymostenol and α-ergostenol has been revealed by the oxidation of the former with selenium dioxide (cf. Callow, J., 1936, 462) to *dehydro-α-*

(VI.)





(VII.)

zymostenol (VI), m. p. 98—99°, exhibiting light absorption (max. at 2475 A.) similar to that of dehydro-α-ergostenol (Windaus and Lüttringhaus, *loc. cit.*).

From the evidence which has been offered it is clear that zymosterol has the constitution (VII) (*i.e.*, a $\Delta^{8:14,\ 24:25}$-cholestadienol) and hence represents the first authenticated example of a natural sterol devoid of the 5 : 6-ethenoid linkage.

EXPERIMENTAL.

M. p.'s are uncorrected and rotations were measured in chloroform in a 1 dcm. tube. All specimens for analysis were dried in a high vacuum (10^{-3} mm.) for several hours at temperatures about 30° below the m. p. The ergosterol residues had m. p. about 100°, $[\alpha]_D^{20°}$ + 12° and $E_{1\ cm.}^{1\%}$ at 2800 A. = 50, equivalent to about 15% of residual ergosterol.

Zymosterol Dibromide.—To an ice-cold solution of the yeast sterols (5 g.) in dry ether (100 c.c.), bromine in acetic acid (30 c.c.; 10%) was added over about a minute with vigorous shaking, the separated dibromide being filtered off after standing for a further 10 minutes at 0°, washed with a small quantity of alcohol, and dried in air. Yield, 1·3—1·5 g. The crude bromide was crystallised once from alcohol–chloroform (3 : 1) and then twice from ethyl acetate, from which zymosterol dibromide separated in needles, m. p. 157°, $[\alpha]_D^{20°}$ + 7·4° (*c* = 3·3) (Found: C, 59·3; H, 8·45; Br, 27·3. Calc. for $C_{27}H_{44}OBr_2$: C, 59·5; H, 8·2; Br, 29·4%). Treatment of the dibromide with sodium iodide in alcohol for 2 hours under reflux effected only partial debromination.

Zymosterol.—(a) The pure dibromide (4 g.), suspended in acetic acid (400 c.c.; 95%) and shaken for 18 hours with zinc dust (8 g.), still contained halogen, but after two additions of fresh zinc dust and shaking for a further 36 hours, a halogen-free product was obtained by filtration and precipitation with water. The zymosterol crystallised from methyl alcohol in plates, m. p. 107—109°, $[\alpha]_D^{20°}$ + 47·6° (*c* = 1·7).

(b) The pure dibromide (10 g.) in alcohol (500 c.c.) was treated with activated (ammonium

chloride) zinc dust (14 g.), and the mixture heated under reflux for 1 hour. The major portion of the solvent was distilled off, and the filtered solution diluted with water and extracted with ether, the ethereal solution being washed with water and dried. The halogen-free residue, on crystallisation from methyl alcohol or acetone, yielded zymosterol (5·4 g.), m. p. 107—109°, undepressed on admixture with a specimen prepared by method (a), $[\alpha]_D^{20°}$ + 49° (c = 1·4). After sublimation at 100°/10^{-3} mm., it had m. p. 107—109° and $[\alpha]_D^{20°}$ + 50° (c = 1·6) (Found: C, 84·1; H, 11·8. Calc. for $C_{27}H_{44}O$: C, 84·3; H, 11·6%). After shaking with platinum-black in an atmosphere of nitrogen for 18 hours, the recovered zymosterol had m. p. 107—109° and $[\alpha]_D^{20°}$ + 51° (c = 1·2). Debromination of the crude dibromide by this method, followed by acetylation and fractional crystallisation of the product, resulted in a partial separation into a fraction corresponding to zymosteryl acetate, m. p. 95—102°, and a second fraction, m. p. 115—120°.

(c) Ergosterol residues (150 g.) were heated on the steam-bath for 2 hours with pyridine (600 c.c.) and benzoyl chloride (150 g.) and poured on ice. The solid obtained was washed with water and alcohol, dried, and dissolved in hot ethyl acetate (1500 c.c.). The crystalline benzoate separating on cooling was removed, and the solution concentrated, the process being repeated several times until no more solid benzoate could be obtained. The last solid fraction (21 g.) was hydrolysed with alcoholic potassium hydroxide (210 c.c.; 10%) under reflux for 1 hour, and the sterol mixture isolated by means of ether and twice crystallised from methyl alcohol–acetone, giving impure zymosterol, m. p. 96°. A solution of this sterol in dry benzene (150 c.c.) was thrice percolated through a column of alumina and the residual solid, after removal of the benzene, on crystallisation from acetone gave zymosterol in flat needles, m. p. 105—108°, undepressed on admixture with authentic material, $[\alpha]_D^{20°}$ + 46° (c = 1·3). This product, in spite of the repeated adsorption, still gave a faint pink coloration with antimony trichloride in chloroform.

Pure zymosterol as prepared by methods (a) and (b) gives no coloration with the antimony trichloride reagent. With the Salkowski test an orange-yellow acid layer is observed, a pale green colour is produced by the Tortelli–Jaffé reaction, and the Liebermann–Burchard test gives an immediate red colour, transformed to green within a few minutes.

Zymosterol (94 mg.) was set aside at 0° with a solution of perbenzoic acid in chloroform (10 c.c.; 0·14N). At intervals samples of the solution were treated with an excess of potassium iodide solution and titrated against 0·06N-sodium thiosulphate, all measurements being standardised against a blank consisting of the same volume of perbenzoic acid in chloroform. After 24 hours one mole of the sterol had absorbed 2·1 atoms of oxygen.

Zymosteryl Acetate.—Prepared in the usual manner, the acetate, after two crystallisations from methyl alcohol, had m. p. 107—108°, mixed with zymosterol, m. p. 84°; $[\alpha]_D^{20°}$ + 35° (c = 1·3) (Found: C, 81·3, 81·5; H, 10·9, 10·7. Calc. for $C_{29}H_{46}O_2$: C, 81·6; H, 10·9%).

α-Zymostenol.—Zymosterol (12·5 g.) in ether (65 c.c.) and acetic acid (125 c.c.) was shaken with hydrogen at atmospheric pressure in the presence of platinic oxide (Adams) (0·5 g.) for 1 hour, after which time no further absorption took place. The filtered solution was diluted with water and extracted with ether, the extract being washed with water and sodium bicarbonate solution, dried, and evaporated. On crystallising the residue three times from methyl alcohol, α-zymostenol (10·5 g.) was obtained in flat needles, m. p. 119—120°, $[\alpha]_D^{20°}$ + 20·8° (c = 2·1). α-Zymostenol (103 mg.) was treated with perbenzoic acid in chloroform (10 c.c.; 0·12N) in the manner already described. After 24 hours one mole of the sterol had taken up 1·9 atoms of oxygen.

α-Zymostenyl Acetate.—Prepared with acetic anhydride and pyridine, the acetate formed plates from aqueous methyl alcohol, m. p. 77—78°, $[\alpha]_D^{20°}$ + 7·6° (c = 2·0).

α-Zymostenyl Benzoate.—The *benzoate*, prepared with benzoyl chloride and pyridine, was crystallised from both acetone and methyl alcohol–ethyl acetate, separating from these solvents in felted needles, m. p. 109—111°, $[\alpha]_D^{20°}$ + 6·4° (c = 2·0) (Found: C, 83·05; H, 10·4. $C_{34}H_{50}O_2$ requires C, 83·2; H, 10·3%).

Isomerisation of α-Zymostenol with Hydrogen Chloride.—A rapid stream of dry hydrogen chloride was passed into a solution of α-zymostenol (8 g.) in dry chloroform (100 c.c.) at 20° for 1 hour; the yellow solution was then evaporated, and the residue crystallised from aqueous alcohol, from which the α + β-zymostenol complex separated in plates of constant m. p. 98—99°, $[\alpha]_D^{20°}$ + 26·9° (c = 1·6). The complex (99 mg.) was treated with perbenzoic acid in chloroform (10 c.c.; 0·15N) in the manner already described. After 24 hours one mole of the sterol had taken up 1·1 atoms of oxygen. The acetate formed microscopic flat needles from acetone–methyl alcohol, m. p. 70—71°, $[\alpha]_D^{20°}$ + 11·0° (c = 1·5).

β-*Zymostenyl Benzoate*.—Dry hydrogen chloride was passed for 3 hours into a solution of α-zymostenyl benzoate (1 g.) in dry chloroform (50 c.c.) at 0°. β-*Zymostenyl benzoate* was crystallised alternately from acetone and methyl alcohol–ethyl acetate, separating in needles, m. p. 165—166°, $[\alpha]_D^{20°}$ + 31·9° (*c* = 1·0) (Found: C, 83·25; H, 10·4. $C_{34}H_{50}O_2$ requires C, 83·2; H, 10·3%).

β-*Zymostenol*.—A solution of the benzoate (130 mg.) in alcoholic potassium hydroxide (10 c.c.; 3%) was heated under reflux for 2 hours. The product, isolated in the usual manner, was percolated in benzene solution through a column of alumina, and after evaporation the residue was crystallised from acetone–methyl alcohol, yielding β-*zymostenol* in felted needles, m. p. 128°, $[\alpha]_D^{20°}$ + 30·5° (*c* = 0·9) (Found: C, 84·3, 84·2; H, 12·1, 12·2. $C_{27}H_{46}O$ requires C, 83·9; H, 12·0%). The *acetate*, crystallised from methyl alcohol, had m. p. 76—77° (Found: C, 81·1; H, 11·2. $C_{29}H_{48}O_2$ requires C, 81·2; H, 11·3%).

Zymostanol.—β-Zymostenol (4·5 g.), obtained by isomerisation of α-zymostenol, was hydrogenated in acetic acid solution with platinic oxide (0·2 g.). The crude product, m. p. 118°, was re-treated with hydrogen chloride and hydrogenated again, yielding a product which after crystallisation from aqueous methyl alcohol had m. p. 140°, $[\alpha]_D^{20°}$ + 23·4° (*c* = 2·0). In order completely to remove the last traces of unsaturated sterol, this material was treated according to the method of Anderson and Nabenhauer (*J. Amer. Chem. Soc.*, 1924, **46**, 1957). A mixture of this zymostanol (7 g.), acetic anhydride (35 c.c.), and carbon tetrachloride (70 c.c.) was shaken with sulphuric acid (1·75 c.c.); after 15 minutes, water (3·5 c.c.) was added, and the carbon tetrachloride layer washed with small quantities of water until colourless. This process was continued until the addition of sulphuric acid produced no coloration. The residue obtained on removal of the carbon tetrachloride was heated under reflux with alcoholic potassium hydroxide (230 c.c.; 3%), and the product, isolated by means of ether (charcoal), crystallised from aqueous alcohol, yielding zymostanol in plates, m. p. 140—141°, $[\alpha]_D^{20°}$ + 24·8° (*c* = 0·6) (Found: C, 83·65, 83·4; H, 12·6, 12·4. Calc. for $C_{27}H_{48}O$: C, 83·4; H, 12·5%).

A specimen of cholestanol, prepared by the reduction of cholesterol, and purified in the same manner as zymostanol, had m. p. 140—141°, undepressed on admixture with zymostanol; $[\alpha]_D^{20°}$ + 24·1° (*c* = 0·6).

Zymostanyl Acetate.—(a) Prepared in the usual manner by acetylation of zymostanol, the acetate was crystallised first from acetone–methyl alcohol and then repeatedly from aqueous methyl alcohol, separating in flat needles of constant m. p. 114—116°, $[\alpha]_D^{20°}$ + 10·9° (*c* = 1·2) (Found: C, 80·9; H, 11·5. Calc. for $C_{29}H_{50}O_2$: C, 80·8; H, 11·7%). Cholestanyl acetate produced under the same conditions had m. p. 109—110°, and when mixed with the zymostanyl acetate, m. p. 110—112°; $[\alpha]_D^{20°}$ + 11·5° (*c* = 1·8).

(b) β-Zymostenyl acetate (800 mg.), containing some of the α-isomer, was hydrogenated in acetic acid (50 c.c.) in the normal manner with platinic oxide. The product was treated with acetic anhydride and sulphuric acid as described above, except that the subsequent hydrolysis was omitted, and repeated crystallisation from aqueous methyl alcohol gave zymostanyl acetate of constant m. p. 109—110°, undepressed on admixture with cholestanyl acetate and having m. p. 110—113° on admixture with the zymostanyl acetate prepared under (a). Hydrolysis gave zymostanol, which on crystallisation from aqueous alcohol had m. p. 140—141°, undepressed on admixture with a specimen obtained by the alternative method.

Zymostanyl Benzoate.—This was prepared with benzoyl chloride and pyridine in the cold and after repeated crystallisation alternately from acetone and methyl alcohol–ethyl acetate, *zymostanyl benzoate* was obtained in feathery needles, m. p. 131—133°, $[\alpha]_D^{20°}$ + 17·8° (*c* = 1·3) (Found: C, 82·9; H, 10·7. $C_{34}H_{52}O_2$ requires C, 82·85; H, 10·7%). Cholestanyl benzoate prepared under identical conditions had m. p. 131—133°, undepressed on admixture with zymostanyl benzoate; $[\alpha]_D^{20°}$ + 18·5° (*c* = 0·7).

Zymostanyl Phenylurethane.—A solution of zymostanol (100 mg.) in dry benzene (2 c.c.) was heated under reflux for 4 hours with phenyl *iso*cyanate (200 mg.). The reaction mixture was evaporated under diminished pressure and repeated crystallisation of the residue from aqueous alcohol gave *zymostanyl phenylurethane* in plates, m. p. 155—156°, $[\alpha]_D^{20°}$ + 11·3° (*c* = 1·9) (Found: C, 80·5; H, 10·4. $C_{34}H_{53}O_2N$ requires C, 80·4; H, 10·5%). Cholestanol under the same conditions gave *cholestanyl phenylurethane* in plates, the m. p. of which could not be raised above 151°, but a mixture with the zymostanol derivative had m. p. 151—153°; $[\alpha]_D^{20°}$ + 11·8° (*c* = 1·5) (Found: C, 80·4; H, 10·5. $C_{34}H_{53}O_2N$ requires C, 80·4; H, 10·5%).

Zymostanone.—To a suspension of zymostanol (1 g.) in acetic acid (360 c.c.; 95%), a solution of chromic anhydride (250 mg.) in water (2 c.c.) and acetic acid (60 c.c.) was added during 30 minutes with stirring and the mixture was then kept for 20 hours at 20°. The reaction mixture

was evaporated under diminished pressure, the residue treated with sulphuric acid (100 c.c.; 6N), and the neutral portion isolated by means of ether, acidic products being removed by washing with sodium hydroxide solution (7%). After several crystallisations, *zymostanone* (620 mg.) separated from methyl alcohol in small needles, m. p. 125—126°, $[\alpha]_D^{20°}$ + 40° (c = 1·0) (Found: C, 83·7, 83·5; H, 12·3, 12·25. $C_{27}H_{46}O$ requires C, 83·8; H, 12·0%). A specimen of cholestanone obtained in the same manner had m. p. 127—128°, undepressed on admixture with zymostanone; $[\alpha]_D^{20°}$ + 40° (c = 1·1).

Bromozymostanone.—To a solution of zymostanone (100 mg.) in acetic acid (4 c.c.), a drop of a solution of hydrogen bromide in acetic acid (50%) and a solution of bromine in acetic acid (0·24 c.c.; 16·5%) were added. Decoloration was rapid and crystals began to separate. After leaving overnight, the *bromozymostanone* was separated and crystallised from acetic acid and aqueous alcohol, separating from the latter solvent in needles, m. p. 166—167° (Found: C, 69·5; H, 10·0. $C_{27}H_{45}OBr$ requires C, 69·6; H, 9·8%). It gave no depression in m. p. on admixture with a specimen of 2-bromocholestanone, m. p. 167—168°, prepared under identical conditions.

Zymostane-C_2‖C_3-dicarboxylic Acid.—A solution of chromic anhydride (1 g.) in acetic acid (10 c.c.; 90%) was added to zymostanol (1 g.) in acetic acid (30 c.c.; 90%), and the mixture kept at 60° for 2 hours. After dilution with sulphuric acid (100 c.c.; 6N) the precipitated solid was taken up in ether, the acid extracted with sodium hydroxide solution (7%) and isolated, after acidification, by means of ether. *Zymostane-C_2‖C_3-dicarboxylic acid* was crystallised twice from acetic acid and finally from ethyl acetate–light petroleum (b. p. 40—60°), from which it separated in plates, m. p. 196—197°, $[\alpha]_D^{20°}$ + 33·4° (c = 1·0) (Found: C, 74·8; H, 10·9. $C_{27}H_{46}O_4$ requires C, 74·6; H, 10·7%). The acid produced by a similar oxidation of cholestanol had m. p. 195—196°, undepressed on admixture with the acid from zymostanol; $[\alpha]_D^{20°}$ + 35·7° (c = 1·0).

Prepared by treatment of the acid with diazomethane, *methyl zymostane-C_2‖C_3-dicarboxylate* crystallised with difficulty from aqueous methyl alcohol and had m. p. 50°, $[\alpha]_D^{20°}$ + 23·7° (c = 0·9) (Found: C, 75·7; H, 11·1. $C_{29}H_{50}O_4$ requires C, 75·25; H, 10·9%). The ester of the cholestane di-acid had m. p. 58—60°, a mixture with zymostane ester having m. p. 55—56°; $[\alpha]_D^{20°}$ + 23·3° (c = 1·0).

Zymostane.—A mixture of zymostanone (200 mg.), amalgamated zinc (2 g.), and acetic acid (50 c.c.) was heated under reflux for 7 hours with hydrochloric acid (5 c.c.). The filtered solution, after cooling, was diluted with water; *zymostane*, isolated by means of ether, crystallised from aqueous alcohol in plates, m. p. 74—76°, $[\alpha]_D^{20°}$ + 20·9° (c = 1·0) (Found: C, 87·2; H, 13·0. $C_{27}H_{48}$ requires C, 87·0; H, 13·0%). Cholestane prepared under the same conditions had m. p. 79—80°, mixed with zymostane, m. p. 77—78°; $[\alpha]_D^{20°}$ + 22·1° (c = 1·2).

Ozonolysis of Zymosterol.—The sterol (1 g.) was suspended in purified acetic acid (10 c.c.; Orton and Bradfield, J., 1924, **125**, 960), and ozonised oxygen (3%) passed in for 3 hours, the issuing gases being led into water. The reaction mixture, combined with the wash-water, was diluted and distilled in steam until the distillate no longer gave a precipitate with dinitrophenylhydrazine sulphate solution. The whole distillate was treated with an excess of the latter reagent, the derivative being filtered off and dried (322 mg.; 52% as acetone derivative) (see, however, Coulson and Holt, *Chem. and Ind.*, 1939, **58**, 267). It was purified by percolation of a benzene solution through alumina (Strain, *J. Amer. Chem. Soc.*, 1935, **57**, 758); evaporation of the pale yellow filtrate and crystallisation of the residue from alcohol then gave the dinitrophenylhydrazone in yellow needles, m. p. 125°, undepressed on admixture with a specimen of the acetone derivative of m. p. 126° (Found: N, 23·55. Calc. for $C_9H_{10}O_4N_4$: N, 23·5%). In a later experiment the acetone was identified by conversion into the 4-phenylsemicarbazone, which after crystallisation from alcohol had m. p. 157°, either alone or in admixture with an authentic specimen. From neither of these experiments could either neutral or acidic crystalline material be isolated from the non-volatile portion.

On ozonolysis of a similar quantity of α-zymostenol, only traces of steam-volatile ketonic material were produced, the quantity of dinitrophenylhydrazone being insufficient to purify, and ozonolysis of cholesterol gave a 14% yield of impure formaldehydedinitrophenylhydrazone, which after chromatographic purification and crystallisation was indistinguishable from an authentic specimen.

Dehydro-α-zymostenol.—A solution of α-zymostenol (500 mg.) in alcohol (75 c.c.) and water (25 c.c.) was heated under reflux for 1 hour with selenium dioxide (500 mg.); the hot solution was then filtered and carefully diluted with water. The solid which separated on cooling was filtered off and dissolved in benzene, the solution percolated twice through a column of alumina,

and the residue obtained on evaporation crystallised from aqueous methyl alcohol, from which *dehydro-α-zymostenol* separated in plates, m. p. 98—99°, $[\alpha]_D^{20°} - 9{\cdot}1°$ ($c = 1{\cdot}1$). Satisfactory analytical data could not be obtained, probably owing to the presence of traces of selenium (Found: C, 83·45; H, 11·1. $C_{27}H_{44}O$ requires C, 84·3; H, 11·6%). *Light absorption in alcohol:* Maximum, 2475 A.; log $\varepsilon = 4{\cdot}16$. Dehydro-α-zymostenol gives no colour with antimony trichloride in chloroform, and with trichloroacetic acid a pink colour is observed, changing to blue-green in the presence of lead tetra-acetate (von Christiani and Auger, *Ber.*, 1939, **72**, 1124, 1482). Dehydro-α-ergostenol gives a faint pink colour with antimony trichloride and identical colours in the latter test.

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