Investigations on the Biosynthesis of Steroids and Terpenoids. Part VI.† The Sterols of Yeast

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All the detectable sterols of yeast (*Saccharomyces cerevisiae*) with polarities (as measured by their R_F values on silica gel) ⩽ ergosta-5,7,22,24(28)-tetraen-3β-ol have been isolated and characterised. In addition to the known fully defined sterols, lanosterol, 14-demethyl-lanosterol, 4α-methylzymosterol, 24,25-dihydro-4α-methyl-24-methylenezymosterol, zymosterol, 5,6-dihydroergosterol, ergosta-5,7,22,24(28)-tetraen-3β-ol, and ergosterol, the less well characterised sterols, fecosterol and episterol, have been reisolated and the structures confirmed. Sterols previously unreported in yeast, parkeol, ergost-7-en-3β-ol, ergosta-7,22,24(28)-trien-3β-ol, and ergosta-5,7,24(28)-trien-3β-ol have also been isolated and characterised. Ascosterol has been shown to be a mixture.

As a preliminary to studies [1] on the biosynthesis of ergosterol in *Saccharomyces cerevisiae* we have re-examined the sterols of the non-saponifiable fraction of this yeast. The composition of this fraction has been investigated a number of times [1,2] and twelve sterols have been reported previously (see Table 1), although some of these may have been mixtures (see below). Our own results are also summarised in Table 1 and represent all the sterols detectable by spectroscopic and chromatographic techniques with polarities (measured by chromatography) ⩽ ergosta-5,7,22,24(28)-tetraen-3β-ol.

The non-saponifiable lipids of yeast, after removal of the bulk of the ergosterol by crystallisation,[3] were separated on an alumina (grade III) column [2h] into five main fractions (Table 2). Fraction I was squalene, fraction II an as yet unidentified oil, fraction III the 4,4-dimethylsterols, fraction IV the 4α-methylsterols, and fraction V the 4-demethylsterols. This last fraction

† Part V, D. H. R. Barton, T. Shiori, and D. A. Widdowson, *J. Chem. Soc.* (*C*), 1971, 1968. Professor F. B. Mallory (Cornell University) has pointed out that in Part III [D. H. R. Barton, G. Mellows, and D. A. Widdowson, *J. Chem. Soc.* (*C*), 1971, 110] we cited his work incorrectly in implying that sterols are formed in *Tetrahymena pyriformis* by cyclisation of 2,3-epoxysqualene. In fact, as Professor Mallory has shown (R. L. Conner, F. B. Mallory, J. R. Landrey, and C. W. L. Iyengar, *J. Biol. Chem.*, 1969, **244**, 2325; R. L. Conner, J. R. Landrey, C. H. Burns, and F. B. Mallory, *J. Protozool.*, 1968, **15**, 600), this protozoan is incapable of synthesing sterols *de novo* though it is capable of metabolising them. Our misreading of the literature does not affect any conclusions drawn from our own experiments.

[1] D. H. R. Barton, D. M. Harrison, G. P. Moss, and D. A. Widdowson, *J. Chem. Soc.* (*C*), 1970, 775.

[2] (*a*) E. Gérard, *J. Pharm. Chim.*, 1895, **1**, 601; (*b*) I. Smedley-MacLean, *Biochem. J.*, 1928, **22**, 22; (*c*) H. Wieland and G. Coutelle, *Annalen*, 1941, **548**, 270 and the preceding papers of that series; (*d*) R. K. Callow, *Biochem. J.*, 1931, **25**, 87; (*e*) E. M. Honeywell and C. E. Bills, *J. Biol. Chem.*, 1933, **99**, 71; (*f*) O. N. Breivik, J. L. Owades, and R. F. Light, *J. Org. Chem.*, 1954, **19**, 1734; (*g*) K. Petzolt, M. Kuhne, E. Blanke, K. Kieslick, and E. Kaspar, *Annalen*, 1967, **709**, 203; (*h*) G. Ponsinet and G. Ourisson, *Bull. Soc. chim. France*, 1965, 3682; (*i*) W. Fürst, *Annalen*, 1966, **699**, 206; (*j*) D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 1948, 1354.

[3] The minor sterol concentrates were the residues of the commercial production of ergosterol from *S. cerevisiae*.

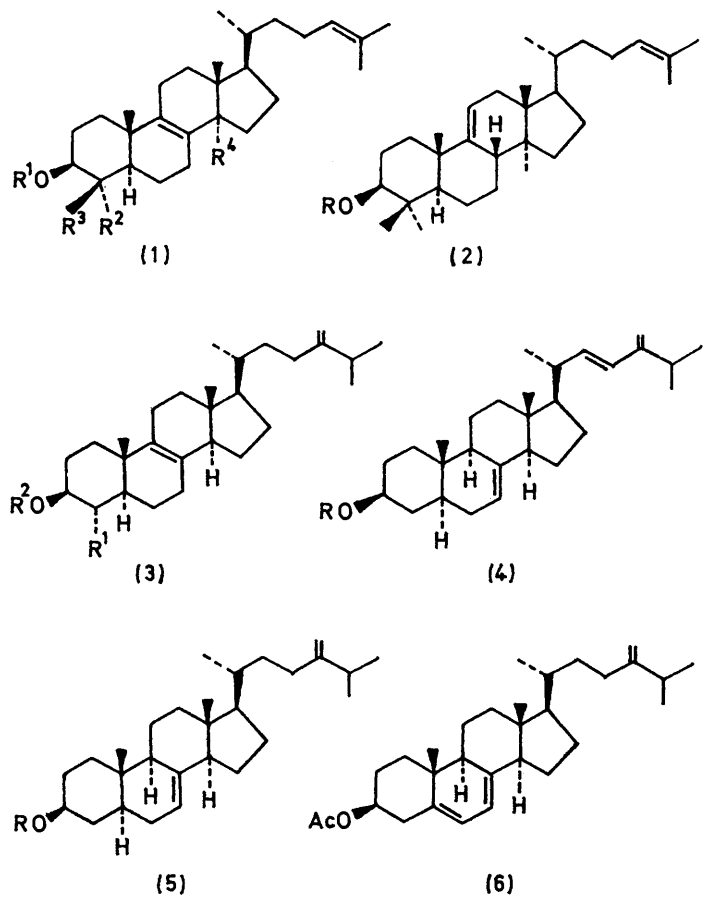


TABLE 1

Sterols of *Saccharomyces cerevisiae*

Fraction	Sterol	Reported			Isolated [a]		
		M.p. (°C)	$[\alpha]_D$ (°)	Ref.	M.p. (°C)	$[\alpha]_D$ (°)	(%) [b]
III	Lanosterol [c]	193—194	+66·9	2*c*	196—198	+78	
III	14-Demethyl-lanosterol [c]	135·5—140	+42	2*h*	147—150	+41·1	
III	Parkeol				159·5—160	+78·4	0·07
IV	4α-Methylzymosterol [c]	136—136·5	+77	2*h*	139—140	+74	
IV	4α-Methylergosta-8,24(28)-dien-3β-ol [c]	142—144	+81	1	141—142	+80·3	
V/1—5	Zymosterol [c]	126—128	+36·4	2*b*	126—128	+44·8	
V/1—5	Fecosterol	161—163	+42	2*c*	126—130	+44·9	0·9
	Ascosterol [d]	141—142	+45	2*c*			
V/8 and 9	Episterol	135—136	+6·2	2*c*	125—126	+6·5	0·09
V/7—9	5,6-Dihydroergosterol [c]	194—196	−13	2*d*	188—191	−10·4	
V/7—9	Ergosta-5,7,24(28)-trien-3β-ol [c]				128—132	−75·5	0·2
V/1—5	Ergosta-7,22,24(28)-trien-3β-ol				119—121	+36·5	0·1
V/7—9	Ergost-7-en-3β-ol [c]				174—175	−1	0·3
V/7—9	Ergosta-5,7,22,24(28)-tetraen-3β-ol	118—120	−78	2*f*, *g*			2
V/7—9	Ergosterol	165	−135	2*a*			2·5 [f]
	Cerevisterol [g]	256—259	−83	2*e*			

[a] In this study. [b] The percentage is of the concentrated residues. [c] These data refer to the benzoate. [d] This compound is shown to be a mixture (see Discussion section). [e] These data refer to the acetate. [f] The ergosterol was considered expendable in the separations and this is much below the true figure. [g] Not sought in this study.

was the most complex and was divided into subfractions V/1—10.

Fraction III was converted into the benzoates and fractionally crystallised (Table 3) to give pure lanosteryl

TABLE 2

Separation of the crude sterol mixture

Sterol residues

↓

Column chromatography on aluminium oxide (grade III).

↓

five fractions

Fraction I. Squalene
Fraction II. Oil (unidentified)
Fraction III. 4,4-Dimethylsterols
Fraction IV. 4α-Methylsterols
Fraction V. 4-Demethylsterols obtained in ten subfractions V/1—10

TABLE 3

4,4-Dimethylsterols

Fraction III

(1) Mixed benzoates
(2) Fractional crystallisation
(3) Separation on silver nitrate plates

(3a) (3b) (3c)

(3a) Lanosteryl benzoate
(3b) 14-Demethyl-lanosteryl benzoate
(3c) Parkeyl benzoate

benzoate (1; R^1 = Bz, $R^2 = R^3 = R^4$ = Me) [2c] and 14-demethyl-lanosteryl benzoate (1; R^1 = Bz, $R^2 = R^3$ = Me, R^4 = H).[2h] The mother liquors were shown, by silver nitrate–silica gel t.l.c., to contain a third component. Preparative t.l.c. on silver nitrate–silica gel allowed the isolation of this hitherto unreported sterol benzoate, m.p. 200—203° $[\alpha]_D$ +93°, in 0·07% yield from the enriched residues.

The mass spectrum of the benzoate, which was isomeric with lanosteryl benzoate, had M^+ at *m/e* 530 and showed a strong peak at *m/e* 417, indicative of an unsaturated C_8 side chain. A corresponding fragment was observed in the mass spectrum of the free sterol at *m/e* 313. The n.m.r. spectrum of the benzoate showed a triplet at τ 4·96 (*J* 6 Hz) and two singlets at τ 8·35 (3H) and 8·44 (3H) (*cf.* lanosteryl benzoate, τ 4·92, 8·32, and 8·36, respectively), indicative of a lanosterol-type side chain. In addition there was a broad multiplet at τ 4·80 and the 10β- and 13β-methyl groups absorbed at τ 8·91 and 9·35, respectively. On biogenetic grounds, the trisubstituted double bond giving rise to the τ 4·80 signal could be a Δ^5-, a Δ^7-, or a $\Delta^{9(11)}$-system. The 10β- and 13β-methyl resonances are unlike those of ergost-7-en-3β-ol (τ 9·16 and 9·48, respectively) or of cholesterol (τ 9·00 and 9·33, respectively), and the compound was tentatively assigned the parkeyl benzoate structure (2; R = Bz). Authentic parkeol prepared from cycloartenol [4] was identical with the natural material (see Experimental section).

[4] D. H. R. Barton, *J. Chem. Soc.*, 1951, 1444; H. R. Bentley, J. A. Henry, D. S. Irvine, and F. S. Spring, *ibid.*, 1953, 3673.

TABLE 4

4α-Methylsterols

Fraction IV

(1) Mixed benzoates
(2) Separation on silver nitrate plates

(4a) (4b)

(4a) 4α-Methyl-5α-cholesta-8,24-dien-3β-yl benzoate
(4b) 4α-Methyl-5α-ergosta-8,24(28)-dien-3β-yl benzoate

The 4α-methyl fraction IV had been examined previously.[1,2h] A brief re-examination (Table 4) by silver nitrate–silica gel t.l.c. of the benzoates showed only the previously reported sterols, 4α-methylzymosterol (1; $R^1 = R^3 = R^4$ = H, R^2 = Me) and 4α-methylergosta-8,24(28)-dien-3β-ol(3; R^1 = H, R^2 = Me).

The subfractions of the complex fraction V were assayed by u.v. spectroscopy and optical rotation. Nos. V/1—5 were identical and were combined. Fractional crystallisation of the benzoates. (Table 5) gave

TABLE 5

4-Demethylsterols

Fraction V/1—5

(1) Mixed benzoates
(2) Fractional crystallisation

Oil Middle fractions (5a)

(3) Separation on silver nitrate plates

(5a) (5c) (5b)

(5a) Zymosteryl benzoate
(5b) Fecosteryl benzoate
(5c) 5α-Ergosta-7,22,24(28)-trien-3β-yl benzoate

essentially pure zymosteryl benzoate (1; R^1 = Bz, $R^2 = R^3 = R^4$ = H).[2b,5] The middle fractions were subjected to preparative silver nitrate–silica gel t.l.c. and a second component with R_F 0·55 (relative to zymosteryl benzoate), m.p. 127—129°, $[\alpha]_D$ +39·9°, was isolated in 0·9% yield from the concentrated residues. Microanalysis agreed with a molecular formula $C_{35}H_{50}O_2$. The i.r. spectrum showed bands at 890 and 1640 cm^{-1}, characteristic of a 1,1-disubstituted ethylene group. The n.m.r. spectrum showed singlet methyl absorptions at τ 9·02 and 9·38 as in zymosteryl benzoate (τ 9·00 and 9·38) but the side chain terminal methyl groups now appeared as a doublet at τ 8·96 (*J* 7 Hz) [1] and the 24-H signal of zymosteryl benzoate (τ 4·92) was replaced by a broad two-proton absorption at τ 5·35.[1] The 24-methylene group thus indicated was confirmed by synthesis of 24,25-dihydro-24-methylenezymosterol from zymosterol by a route similar to one previously described.[1] 24,25-Epoxyzymosteryl benzoate [6] was isomerised with Kenner's reagent [7] to the 24-ketone, m.p. 152—156°, $[\alpha]_D^{24}$ +50°. A Wittig reaction with methylenetriphenylphosphorane gave 24,25-dihydro-24-methylenezymosteryl benzoate, m.p. 127—130°, $[\alpha]_D^{25.5}$ +39·5°, identical with the benzoate of the natural material. The identity was confirmed by appropriate comparisons of the derived free sterol, m.p. 126—136°, $[\alpha]_D^{25}$ +46·6° and acetate, m.p. 136—138°, $[\alpha]_D^{24}$ +34·9°. This sterol has the structure assigned to fecosterol,[2c,5] isolated from yeast by Wieland. The physical constants differ considerably from those reported by Wieland. These discrepancies will be discussed later, but it is convenient to retain the name as fecosterol. This compound has also been isolated during studies on the methylation of zymosterol by a yeast enzyme. No physical constants were given and the compound was not rigorously characterised.[8]

The preparative t.l.c. also gave a third component (Table 5) of fraction V/1—5, R_F 0·64 (relative to zymosteryl benzoate), m.p. 139—141°, $[\alpha]_D^{26}$ +13·5°, in 0·1% yield. Microanalysis agreed with a molecular formula $C_{35}H_{48}O_2$. The u.v. spectrum showed an absorption at 232 nm (ε 33,050), analogous [2f,2g,9] to the side-chain diene absorption of ergosta-5,7,22,24(28)-tetraen-3β-ol. The i.r. spectrum again showed bands corresponding to a 1,1-disubstituted ethylene (890 and 1640 cm^{-1}). The benzoate was converted into the free sterol, m.p. 119—121°, $[\alpha]_D^{25}$ +36·5°, by reduction with lithium aluminium hydride. The sterol was acetylated with acetic anhydride–pyridine to give the acetate, m.p. 133—136°, $[\alpha]_D^{25}$ +6·4°. Both the sterol and its acetate showed diene and 1,1-disubstituted ethylene absorptions. The n.m.r. spectrum showed resonances at τ 9·18 and 9·46 for the 10- and 13-methyl groups, indicating a Δ^7-sterol nucleus.[10] The mass spectrum of the acetate had a peak at *m/e* 313, corresponding to the loss of (side chain +2).[11] Thus the side chain contained an 'extra carbon' and the diene system indicated by the u.v. spectrum. A high resolution n.m.r. spectrum enabled the structure of the side chain to be determined unambiguously. The *exo*-methylene protons at C-28 gave a broad signal with peaks centred at τ 5·24 (fecosterol, τ 5·3). A septet appeared at τ 7·52 (*J* 7 Hz), irradiation of which caused the doublet centred on τ 8·96 (*J* 7 Hz) to collapse to a singlet and a doublet of considerably reduced intensity. This indicated an isopropyl end group to the side chain. A multiplet at τ 7·91 could be assigned to the C-20 proton, since on irradiation of this the smaller part of the doublet at τ 8·96 (21-Me) collapsed to a singlet, and the signal at τ 4·50 (C-22) collapsed to a doublet (*J* 16 Hz), which had the same coupling constant as the doublet at τ 2·14 (C-23). This irradiation did not effect the septet at τ 7·52 (C-25). The coupling constant (16 Hz) indicated a *trans*-configuration of the 22,23-double bond.

These data were consistent with structure (4; R = H) for the sterol. This was confirmed and the stereochemistry at C-20 defined by showing the identity of the acetate with the synthetic ester (4; R = Ac) prepared from 5,6-dihydroergosterol (to be reported later).

The mass spectra of the sterol fractions V/7—9 showed a molecular ion peak at *m/e* 400, corresponding to an ergostenol. The much greater abundance of diene sterols however masked this component on the t.l.c. plates and it was necessary to resort to a chemical separation of these fractions. We had previously shown [9] that 4-phenyl-1,2,4-triazoline-3,5-dione adds efficiently to ring B dienes at −70°. This compound is also a powerful enophile [12,13] but, nevertheless, selective Diels–Alder addition could be readily accomplished at −70°.

The benzoates of fractions V/7—9 were titrated at −70° with the azo-reagent in methylene chloride. When rapid decolourisation ceased, the products were crudely separated on alumina to give the non-conjugated sterol olefins, as the benzoates. These were separated by silver nitrate–silica gel t.l.c. into two new components, together with the benzoates of zymosterol and fecosterol (Table 6). The first, m.p. 174—175° (0·3% of concentrated residues), showed M^+ 400 with a strong peak at *m/e* 231 (side chain +42), indicative of a saturated C_9 side chain.[14] The n.m.r. spectrum showed absorptions for the 10β- and 13β-methyl groups at τ 9·16 and 9·48, and these together with an olefinic proton signal at τ 4·9,

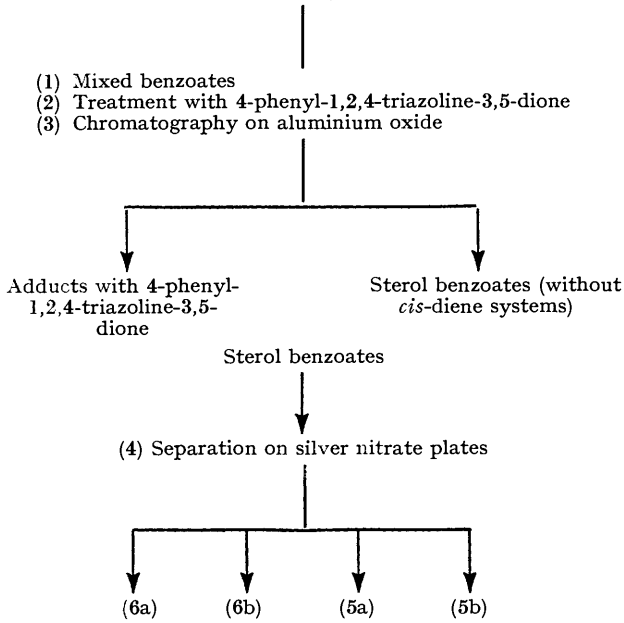
[5] D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 1949, 214.
[6] Prepared by the method of E. E. van Tamelen and T. J. Curphey, *Tetrahedron Letters*, 1962, 121.
[7] D. Bethell, G. W. Kenner, and P. J. Powers, *Chem. Comm.*, 1968, 227.
[8] J. T. Moore and J. L. Gaylor, *J. Biol. Chem.*, 1969, **244**, 6334.
[9] D. H. R. Barton, T. Shiori, and D. A. Widdowson, *J. Chem. Soc.*, 1971, 1968.
[10] N. S. Bhacca and D. H. Williams, 'Applications of N.M.R. Spectroscopy in Organic Chemistry,' Holden-Day, San Francisco, 1964.
[11] S. G. Wyllie and C. Djerassi, *J. Org. Chem.*, 1968, **33**, 305.
[12] H. M. R. Hoffmann, *Angew. Chem. Internat. Edn.*, 1969, **10**, 556.
[13] D. H. R. Barton, U. M. Kempe, J. N. Shah, and D. A. Widdowson, unpublished work.
[14] H. Budzikiewicz, C. Djerassi, and D. H. Williams, 'Structure Elucidation of Natural Products by Mass Spectrometry,' Holden-Day, San Francisco, 1964.

the mass spectral data, and the lack of absorption in the u.v. region identified the compound as ergost-7-en-3β-yl benzoate. The identity of the natural sample with an authentic specimen confirmed the assignment. The

TABLE 6

4-Demethylsterols

Fraction V/6—8



(6a) Erogst-7-en-3β-yl benzoate
(6b) 5,6-Dihydroergosteryl benzoate

other compound, isolated in 0·3% yield, was shown by comparison to be 5,6-dihydroergosterol, a known component [2d] of yeast sterols.

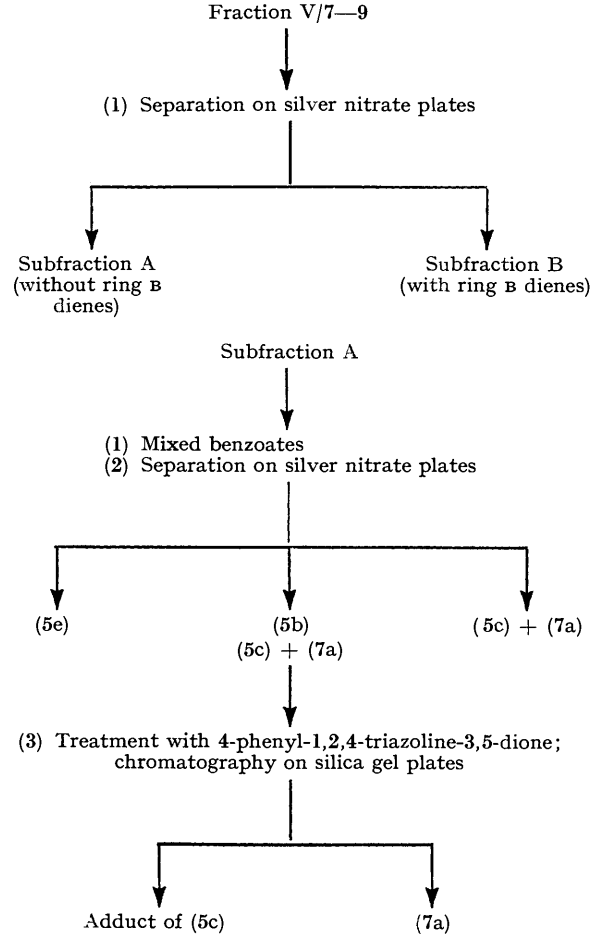
Some samples of fecosterol (3; $R^1 = R^2 = H$) showed a weak absorption in the n.m.r. spectrum at τ 9·46, indicative of a Δ^7-sterol. The u.v. spectra of these samples were however very low in ergosta-7,22,24(28)-trienol absorption and, by t.l.c., ergost-7-en-3β-ol and 5,6-dihydroergosterol were shown not to be present. Thus the possibility remained that the compound corresponding to the structure (5; R = H) assigned to episterol [2c] was present in these mixtures. Accordingly, fractions V/8 and 9 were separated by silver nitrate–silica gel t.l.c. of the free sterols, into two subfractions A and B, differing in the presence (B) or absence (A) of ring B dienes (Table 7). Subfraction A was shown by its u.v. spectrum to contain *ca.* 30% of side-chain diene sterols ($\lambda_{max.}$ 232 nm) but the n.m.r. spectrum indicated the presence of *ca.* 75% Δ^7-sterols. These fractions were combined, benzoylated, and separated on silver nitrate plates into zymosteryl benzoate, ergosta-7,22,24(28)-trien-3β-yl benzoate (4; R = Bz), and a mixture of the latter ester and a new compound. The benzoate (4; R = Bz) was removed from the mixture by titration at −30° with 4-phenyl-1,2,4-triazoline-3,5-dione, with which it formed a side-chain Diels–Alder adduct. The residual sterol benzoates were separated on silver nitrate plates to give a new component (0·09% of sterol concentrates), m.p. 175—177°, $[\alpha]_D$ +7·3° (free sterol, m.p. 125—126°, $[\alpha]_D$ +6·5°, and acetate, m.p. 136—138°, $[\alpha]_D$ +4·7°). The parent compound was formulated as episterol (5; R = H) on the basis of the following evidence.

Microanalysis and mass spectral data of the sterol acetate indicated the formula $C_{30}H_{48}O_2$. The base peak in the mass spectrum occurred at *m/e* 313, corresponding

TABLE 7

4-Demethylsterols

Fraction V/7—9



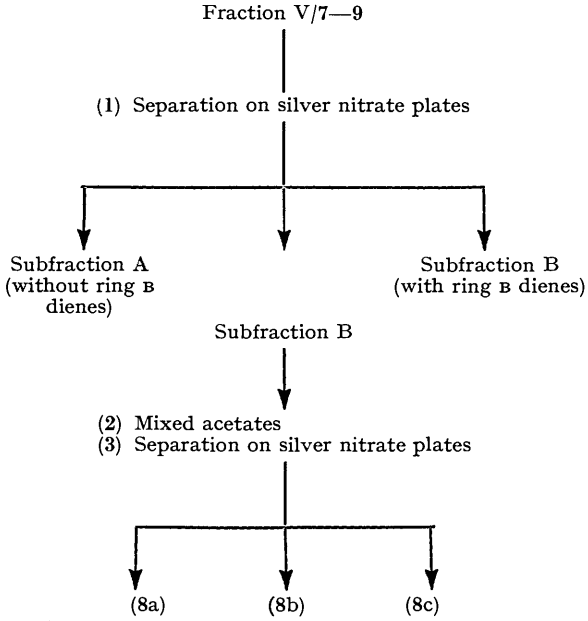
(7a) Episteryl benzoate

to loss of an unsaturated C_9 side chain +2.[11] The i.r. absorptions at 890 and 1640 cm^{-1}, and the n.m.r. signal at τ 5·27 (2H) indicated the presence of a 1,1-disubstituted ethylene group. A 25,26-double bond was shown to be lacking by the absence of a vinylic methyl resonance in the n.m.r. spectrum. Thus on biogenetic grounds, the methylene group was placed at C-24. The methyl resonances at τ 9·18 and 9·44 were in good agreement with those of the 10β- and 13β-methyl groups of Δ^7-sterols. In addition, a signal at τ 4·78 was assignable to the C-7 olefinic proton.

The subfraction B from fraction V/7—9 was acetylated and the acetates were separated by silver nitrate–silica gel t.l.c. into three components (Table 8). The

TABLE 8

4-Demethylsterols



(8a) Ergosteryl acetate
(8b) Ergosta-5,7,22,24(28)-tetraen-3β-yl acetate
(8c) Ergosta-5,7,24(28)-trien-3β-yl acetate

first two were identified as ergosteryl acetate and ergosta-5,7,22,24(28)-tetraenyl acetate.[2f] The third was a new component, isolated in 0·2% yield, m.p. 128—132°, $[\alpha]_D$ −75·5°. Microanalytical and mass spectral data indicated the formula $C_{30}H_{40}O_2$. The u.v. spectrum ($\lambda_{max.}$ 263, 272, 282, and 294 nm) indicated a ring B diene and the i.r. spectrum ($\nu_{max.}$ 1640 and 890 cm^{-1}) a 1,1-disubstituted ethylene group. This was confirmed by the broad two-proton absorption at τ 5·30 in the n.m.r. spectrum. These data suggested the compound to be ergosta-5,7,24(28)-trien-3β-yl acetate (6), the parent sterol of which was recently isolated from *Phycomyces blakesleanus*.[15] Lack of a reference sample precluded a direct comparison, but a comparison of the published spectroscopic data of the free sterol with data observed for the isolated material showed their identity.

Other more polar sterols are present in the yeast residues, but these have not yet been exhaustively examined. The situation is complicated by the presence of autoxidation products of the less polar sterols.

The fractions III—V have been examined by Dr. C. J. W. Brooks (University of Glasgow) using g.l.c.–mass spectroscopic techniques [16] to check our isolation procedures. No sterols other than those reported here were detected.

The identity of the sterols reported by Wieland, fecosterol, ascosterol, and episterol,[2c] remains in doubt. These were isolated by fractional crystallisation procedures and before modern spectroscopic and chromatographic techniques were available to check purity. Our experience is that many of these sterols co-crystallise and could not be isolated pure by such methods. The physical constants reported for the three sterols are very different from those of the pure materials, and they were probably mixtures of varying amounts of zymosterol, fecosterol, episterol, 5,6-dihydroergosterol, and ergost-7-en-3β-ol. No compound corresponding to the structure of ascosterol was detectable, and the occurrence of this compound in yeast must be considered doubtful. Recently a sterol with the physical properties of ascosterol has been reisolated in a manner analogous to Wieland's methods by Fürst.[2i] We have examined the benzoate of a sample, supplied by Dr. Fürst, on silver nitrate plates and found it to consist of a mixture of mainly fecosterol, with minor amounts of zymosterol, 5,6-dihydroergosterol, and ergost-7-en-3β-ol. At levels undetectable by t.l.c., but evident from the u.v. spectrum were contaminants with ring B dienes, ($\lambda_{max.}$ 282 nm). This again suggests that Wieland's original ascosterol was a mixture.

Of the other sterols reported from yeast, neosterol [2c] has already been shown to be a mixture.[17] Similarly, the mixed nature of anasterol and hyposterol [2c] on re-examination, has been demonstrated.[18]

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus. Optical rotations and i.r. spectra were recorded for chloroform solutions. U.v. spectra were recorded for ethanol, and n.m.r. spectra for deuteriochloroform solutions at 60 or 100 MHz, with tetramethylsilane as internal standard.

T.l.c. refers to thin layer chromatography on silica gel GF 254 (Merck). Silver nitrate plates refer to silica gel plates impregnated (20%) with silver nitrate. Unless otherwise stated, a solvent system of 20% benzene–light petroleum (b.p. 60—80°) was used with these plates, which were developed four times.

Alumina was Brockmann grade III unless otherwise stated. LAH refers to lithium aluminium hydride and THF to tetrahydrofuran.

Column Chromatography of the Sterol Residues of Saccharomyces cerevisiae (Tables 2 and 9).—Sterol residues (50 g) from which the bulk of the ergosterol had been removed by the suppliers were dissolved in benzene (200 ml), applied to an alumina (1 kg) column, and eluted (for solvents see Table 9). Fractions (25 ml) were collected automatically. The fractions were analysed by t.l.c. (fractions I—IV) or by silver nitrate t.l.c. (V) (solvent system, 20% ethyl acetate–benzene) and combined accordingly. The results are expressed in Table 9.

Fraction I. This was shown to be squalene by chromatographic and mass spectral comparison. It has been reported previously [19] in yeast.

Fraction II. This consisted of an oil, homogeneous on

[15] G. Goulston and E. I. Mercer, *Phytochemistry*, 1969, **8**, 1945.
[16] Dr. Brooks will publish this data elsewhere.
[17] D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 1948, 1357.
[18] T. G. Halsall and C. C. Sayer, *J. Chem. Soc.*, 1959, 2031.
[19] E. Schwenk, G. J. Alexander, T. H. Staudt, and C. A. Fish, *Arch. Biochem. Biophys.*, 1955, **55**, 274.

t.l.c. with R_F slightly greater than that of lanosterol. The structure is unknown but it is possibly an artefact.[20]

Fraction III (Table 3). Fraction III (9·7 g) was treated with benzoyl chloride (5 ml) in pyridine (20 ml) for 12 h at room temperature. The solution was poured into water

TABLE 9

Fraction	Solvent Type *	Solvent Ratio (v/v)	Solvent Amount (l)	Amount of material (g)
	b–p	30 : 70	5·0	
I		50 : 50	2·25	
II			4·0	0·66
II + III			1·75	1·48
III			3·0	4·55
III + IV			2·5	1·30
IV			2·5	1·27
IV + V			3·0	2·86
V/1			2·0	3·51
V/2			2·0	1·55
V/3			2·0	1·52
V/4		75 : 25	2·5	1·64
V/5			2·5	2·94
V/6		90 : 10	1·0	2·38
V/7	b	100	2·5	2·79
V/8	b	100	2·5	1·65
V/9	a–b	50 : 50	2·5	5·40
V/10	a–b	50 : 50	5·0	0·6
end	m–b	50 : 50	3·0	
			Recovered	36·18
			Lost	13·82 (27·6%)

* Abbreviations used: a = acetone, b = benzene, p = light petroleum, m = methanol.

and extracted with benzene. The extract was washed with 0·5N-sulphuric acid, aqueous sodium hydrogen carbonate, and water until neutral. It was then dried (Na_2SO_4) and evaporated, and the residues were filtered through alumina in 1 : 1 benzene–light petroleum (b.p. 40—60°) to give the mixed benzoates of fraction III (10 g). These were fractionated by crystallisation from acetone, chloroform, and methanol over five stages of a triangulation procedure. Three major fractions were obtained: (i) 1·862 g $[\alpha]_D^{24}$ +69·5° (*c* 1·13); (ii) 1·933 g, $[\alpha]_D^{24}$ +77° (*c* 1·07); and (iii) 1·969 g, $[\alpha]_D^{24}$ +74·5° (*c* 1·45). Analytical t.l.c. on silver nitrate plates showed (i) to be mainly lanosteryl benzoate, (ii) to be 14-demethyl-lanosteryl benzoate,[2a] and (iii) to be mainly a mixture of lanosteryl and a new sterol benzoate.

Each fraction was separated on silver nitrate thick plates, at a loading of 1 : 200 initially and 1 : 500 for final purification.

Bands were cut at regular intervals and tested on analytical silver nitrate plates. Appropriate bands were combined and extracted with hot chloroform (×5). The chloroform solutions were washed with aqueous ammonium chloride and water, dried (Na_2SO_4), and evaporated. The residue was filtered through alumina (5 g) and the whole separation process was repeated until the analytical plates indicated one component.

By this means there were obtained three fractions. Fraction (i) was lanosteryl benzoate (I; R^1 = Bz, R^2 = R^3 = R^4 = Me) [Table 3, (3a)], needles, m.p. (from chloroform–methanol) 196—198°, $[\alpha]_D^{24}$ +78° (*c* 1·026) (lit.,[21] m.p. 193—194°, $[\alpha]_D$ +66·9°), identical with authentic lanosteryl benzoate, spectroscopically and chromatographically.

Fraction (ii) was 14-demethyl-lanosteryl benzoate (1; R^1 = Bz, R^2 = R^3 = Me, R^4 = H) [Table 3, (3b)], needles, m.p. (from chloroform–methanol), 152—155°, $[\alpha]_D^{21}$ +67° (*c* 0·54) (lit.,[2h] m.p. 138—139°, $[\alpha]_D$ +61°), τ 4·92 (1H, m, 24-H), 5·25 (1H, m, 3α-H), 8·32, [3H, s, 26(or 27)-Me], 8·39 [3H, s, 27(or 26)-Me], 8·94 (3H, s, 4α-Me), 9·04 (6H, s, 4β-Me, 10-Me), and 9·39 (3H, s, 13-Me).

Because of discrepancy in the m.p., a sample (220 mg) of the benzoate was treated with LAH in ether to give a crude sterol (210 mg), which was purified by t.l.c. and recrystallisation to give 14-demethyl-lanosterol (1; R^1 = R^4 = H, R^2 = R^3 = Me), m.p. (from chloroform–methanol) 147—150°, $[\alpha]_D^{25}$ +41·1° (*c* 1·11) (lit.,[22] m.p. 139·5—140°, $[\alpha]_D$ +42°).

The sterol (40 mg) was acetylated with acetic anhydride–pyridine. Normal work up and t.l.c. purification gave 14-demethyl-lanosteryl acetate, m.p. (from chloroform–methanol) 135—137°, $[\alpha]_D^{27}$ +40·7° (*c* 1·55) (lit.,[2h] m.p. 133—134°, $[\alpha]_D$ +39°).

The sterol (1; R^1 = R^4 = H, R^2 = R^3 = Me) (112 mg) in benzene (10 ml) was shaken at room temperature with chromic oxide (148 mg) in water (2 ml) and glacial acetic acid (4 ml) for 1·5 h. The mixture was worked up in the usual way and purified by t.l.c. (20% ethyl acetate–benzene) to give an oil which crystallised on low temperature evaporation of a methanol–ether solution to give needles of *14-demethyl-lanosta-8,24-dien-3-one* (64 mg), m.p. 73—75°, $[\alpha]_D^{24}$ +66·7° (*c* 0·78), $\nu_{max.}$ 1700 cm^{-1} (Found: C, 74·7; H, 11·2. $C_{29}H_{46}O$ requires C, 84·8; H, 11·3%).

Fraction (iii) was parkeyl benzoate (2; R = Bz) [Table 3, (3c)] [78 mg from 1·814 g of (III/iii)], needles, m.p. (from chloroform–methanol) 200—203°, $[\alpha]_D^{33}$ +93° (*c* 0·57) (lit.,[22] m.p. 200·5—201°, $[\alpha]_D$ +95·4°), τ 4·80 (1H, m, 11-H), 4·96 (1H, t, *J* 6 Hz, 24-H), 5·35 (1H, m, 3α-H), 8·36 [3H, s, 26(or 27)-Me], 8·44 [3H, s, 27(or 26)-Me], 8·91 (3H, s, 10-Me), 8·98 (3H, s, 4α-Me), 9·09 (3H, s, 4β-Me), 9·27 (3H, s, 14-Me), and 9·35 (3H, s, 13-Me), M^+ 530 with significant peaks at *m/e* 515, 417, 408, and 393 (100%), identical with an authentic specimen prepared from cycloartenol,[4] and with undepressed mixed m.p. (Found: C, 83·6; H, 10·4. Calc. for $C_{37}H_{54}O_2$: C, 83·7; H, 10·3%).

Parkeyl benzoate (74 mg; naturally derived material) was refluxed with LAH (200 mg) in anhydrous ether (200 ml). After 1 h the solution was cooled and the excess of LAH destroyed with water and 0·5N-sulphuric acid. The mixture was extracted with ether (×5) and the extract was washed with aqueous sodium hydrogen carbonate and water until the washings were neutral. The dried (Na_2SO_4) solution was evaporated and the residue purified by t.l.c. (solvent 10% ethyl acetate–benzene) to give parkeol (2; R = H) (53 mg, 89%) as needles, m.p. (from chloroform–methanol) 159·5—160°, $[\alpha]_D^{23}$ +78·4 (*c* 1·49) (lit.,[22] m.p. 159·5—160°, $[\alpha]_D$ +76·8°), M^+ 426, with significant peaks at *m/e* 411 (100%) 393, and 313.

Parkeol (53 mg) was acetylated in pyridine (2 ml) with acetic anhydride (1 ml) for 16 h at room temperature. The excess of reagent was removed under vacuum and the residue was purified by t.l.c. (solvent 10% ethyl acetate, 45% benzene, 45% light petroleum) to give parkeyl acetate (2; R = Ac) (53 mg, 91%), as plates, m.p. (from chloroform–methanol) 162—166°, $[\alpha]_D^{25}$ +90° (*c* 1·82) (lit.,[22] m.p. 159·5—160°, $[\alpha]_D$ +86°).

Fraction IV (Table 4). Fraction (IV) was benzoylated as

[20] D. H. R. Barton, J. McGarrity, and D. A. Widdowson, unpublished work.

[21] D. A. Lewis and J. F. McGhie, *Chem. and Ind.*, 1956, 550.

[22] W. Learie, F. S. Spring, and H. S. Watson, *Chem. and Ind.* 1956, 1458.

above. After the usual work-up, the mixed benzoates (3·354 g), m.p. 132—138°, were separated on silver nitrate plates to give 4α-methyl-5α-ergosta-8,24(28)-dien-3β-yl benzoate (3; R^1 = Bz, R^2 = Me) [Table 4, (4b)], as needles, m.p. (from chloroform–acetone) 141—142°, $[\alpha]_D^{24}$ +80·3° (*c* 0·83) {lit.,[1] m.p. 142—144°, $[\alpha]_D^{27}$ +81° (*c* 2)} and 4α-methylzymosteryl benzoate (1; R^1 = Bz, R^2 = Me, R^3 = R^4 = H) [Table 4, (4a)], as needles, m.p. (from chloroform–methanol) 139—140·5°, $[\alpha]_D$ +74° (*c* 1·10) (lit.,[2h] m.p. 136—136·5°, $[\alpha]_D$ +77°). These compounds were spectroscopically and chromatographically identical to authentic specimens.

Fraction V (Table 5). Fraction (V) was obtained in 10 subfractions. These were assayed by u.v. spectroscopy and by optical rotation (Table 10). Fractions V/1—5 showed no u.v. absorption at 282 nm. They were combined and worked-up together.

TABLE 10

Subfraction	$[\alpha]_D$ (°)	$\lambda_{max.}$/nm (ε)	Diene (%) [a]
1	+47·9	End absorption	
2	+44·8		
3	+39·8		
4	+39·6		
5	+35·0		
6	+32·8	232(7040);	30—33
		282(396)	3
7	−11·2	232(7850);	34—37
		282(2840)	24
8	−58·0	232(14,000);	61—56
		282(7560)	63
9	−91·5	232(14,850);	64—70
		282(10,880)	91
10			

[a] $\lambda_{max.}$ 232 nm, side-chain diene; $\lambda_{max.}$ 282 nm, ring B diene; % calculated per chromophore.

Zymosteryl Benzoate (1; R^1 = Bz, R^2 = R^3 = R^4 = H) [Table 5, (5a)].—The subfractions V/1—5 (23 g) were benzoylated as above. The usual work up gave the crude sterol benzoates which were partially separated by a triangulation over four stages, as above.

The fraction consisting largely of zymosteryl benzoate (5·8 g), m.p. 126—128° (final clarification at 138°), was purified on silver nitrate plates to give the pure benzoate as prisms, m.p. (from chloroform–methanol) 126—128°, $[\alpha]_D^{27}$ +44·8° (*c* 1·30) (lit.,[2c] m.p. 126—128°, $[\alpha]_D$ +36·4°).

A sample of zymosteryl benzoate was saponified with 10% potassium hydroxide in methanol. After t.l.c. purification, plates of zymosterol (1; R^1 = R^2 = R^3 = R^4 = H), m.p. (from chloroform–methanol and acetone–methanol) 110—113°, $[\alpha]_D^{25}$ +53·7° (*c* 1·55) (lit.,[2c] m.p. 108·5—111°, $[\alpha]_D$ +47·3°), were obtained.

Fecosteryl Benzoate (3; R^1 = Bz, R^2 = H) [Table 5, (5b)].—The middle fractions of the triangulation of the mixed benzoates V/1—5 were separated on silver nitrate plates to give *fecosteryl benzoate* (1·132 g), m.p. (from chloroform–methanol) 127—129°, $[\alpha]_D^{26}$ +39·9° (*c* 0·38) (lit.,[2c] m.p. 144—146°, $[\alpha]_D$ +34°), $\nu_{max.}$ 1710, 1640, and 890 cm^{-1}, τ 5·1 (1H, m, 3α-H), 5·30 (2H, m, 28-H), 9·00 (6H, d, *J* 8 Hz, 26 and 27-Me), 9·02 (3H, s, 10-Me), 9·38 (3H, s, 13-Me), and 2·0 and 2·5 (5H, m, ArH) (Found: C, 83·5; H, 10·1. $C_{35}H_{50}O_2$ requires C, 83·6; H, 10·0%).

Fecosterol (3; R^1 = R^2 = H).—Fecosteryl benzoate (272 mg) was dissolved in methanol (100 ml) and heated with potassium hydroxide (2 g) for 4 h under reflux. The usual work-up and t.l.c. purification gave *fecosterol* (221 mg) as plates, m.p. (from chloroform–methanol) 126—130°, $[\alpha]_D^{24}$ +44·9° (*c* 0·64) (lit.,[2c] m.p. 161—163°, $[\alpha]_D$ +42°). The sterol was transparent above 220 nm in the u.v. spectrum and showed $\nu_{max.}$ 3600, 1640 and 890 cm^{-1}, τ 5·35 (2H, m, 28-H), 6·4 (1H, m, 3α-H), 8·99 (3H, d, *J* 7 Hz, 26 and 27-Me), 9·05 (3H, s, 10-Me), and 9·39 (3H, s, 13-Me) (Found: C, 82·0; H, 11·5. $C_{28}H_{46}O$,0·5H_2O requires C, 82·5; H, 11·4%).

Fecosteryl Acetate (3; R^1 = Ac, R^2 = H).—Fecosterol (50 mg) was acetylated with acetic anhydride (1 ml) in pyridine (5 ml). The usual work-up and t.l.c. purification gave *fecosteryl acetate* as plates, m.p. (from acetone–methanol) 136—138°, $[\alpha]_D$ +34·7° (*c* 0·42) (lit.,[2c] m.p. 159—161°, $[\alpha]_D$ +20°), $\nu_{max.}$ 1710, 1640, and 890 cm^{-1}, τ 5·31 (1H, m, 28-H), 5·35 (1H, m, 3α-H), 8·0 (3H, s, OAc), 8·98 (6H, d, *J* 7 Hz, 26 and 27-Me), 9·03 (3H, s, 10-Me), and 9·38 (3H, s, 13-Me), M^+ 440 (100%) with significant peaks at *m/e* 425, 380, 365, 356, 341, and 313 (Found: C, 81·7; H, 10·9. $C_{30}H_{48}O_2$ requires C, 81·8; H, 11·0%).

5α-Ergosta-8,24(28)-dien-3-one.—Fecosterol (87 mg) in benzene (5 ml) was oxidised with chromium trioxide (130 mg) in water (2 ml) and glacial acetic acid (3 ml). The usual work-up gave the *ketone* (3; 3-keto, R^2 = H) (71 mg, 82%) as plates, m.p. (from methanol) 131—135°, $[\alpha]_D^{24}$ +67° (*c* 1·0), $\nu_{max.}$ 1710, 1640, and 890 cm^{-1}, τ 5·32 (2H, m, 28-H), 8·85 (3H, s, 10-Me), 8·96 (6H, d, *J* 7 Hz, 26 and 27-Me), 9·03 (3H, d, *J* 5 Hz, 20-Me), 9·36 (3H, s, 13-Me) (Found: C, 84·8; H, 11·2. $C_{28}H_{44}O$ requires C, 84·8; H, 11·2%).

24-Oxo-5α-cholest-8-en-3β-yl Benzoate.—Crude 24,25-epoxyzymosteryl benzoate [6] (833 mg) was heated in dimethyl sulphoxide (40 ml) with sodium iodide (1·1 g) and n-propyl iodide (1·2 g) at 80° for 3 h.[7] The mixture was cooled and poured into water. The solution was extracted thoroughly with chloroform and the extract washed with aqueous sodium thiosulphate and water. The chloroform solution was dried (Na_2SO_4) and evaporated. T.l.c. purification (solvent 10% ethyl acetate–benzene) gave the *keto-ester* (605 mg), m.p. (from acetone–methanol) 152—156°, $[\alpha]_D^{24}$ +50·0° (*c* 0·48), τ 5·2 (1H, m, 3α-H), 8·92 (6H, d, *J* 7 Hz, 26 and 27-Me), 8·98 (3H, s, 10-Me), 9·05 (3H, d, 20-Me), 9·38 (3H, s, 13-Me), and 2·0 and 2·5 (5H, m, ArH) (Found: C, 80·8; H, 9·4. $C_{34}H_{48}O_3$ requires C, 80·9; H, 9·6%).

Synthesis of Fecosteryl Benzoate (3; R^1 = Bz, R^2 = H)—Methyltriphenylphosphonium bromide (1·784 g, 5 mmol) in THF (20 ml) was added to an ethanolic solution of methyllithium (20 ml; 0·24N). The solution was stirred under dry nitrogen for 2 h at room temperature. 24-Oxo-5α-cholest-8-3β-yl benzoate (298 mg, 0·59 mmol) was added and the mixture was kept at reflux for 24 h. The resulting solution was worked up with water and extracted with benzene. The dried (Na_2SO_4) benzene solution was evaporated and the residue treated with benzoyl chloride (1·4 g) in pyridine (10 ml). After the usual work-up, the crude benzoate was purified by t.l.c. (solvent benzene). The band with R_F 0·6 was extracted to give fecosteryl benzoate (304 mg) as plates, m.p. (from chloroform–methanol) 127—130°, $[\alpha]_D^{25}$ +39·5 (*c* 0·59). The physical data were identical with those of the natural benzoate and the compounds showed identical chromatographic properties (Found: C, 83·6; H, 10·0. Calc. for $C_{35}H_{50}O_2$: C, 83·7; H, 10·0%).

Synthetic fecosteryl benzoate (110 mg) was saponified with potassium hydroxide (2 g) in methanol (60 ml) under nitrogen at reflux for 2 h. The usual work-up gave fecosterol (3; R^1 = R^2 = H) (40 mg, 46%) as plates, m.p. (from acetone–methanol) 126—136° (133—136° in a sealed

tube), $[\alpha]_D^{25}$ +46·6° (*c* 0·79), $\nu_{max.}$ 3500, 1640, and 890 cm^{-1} (Found: C, 81·9; H, 11·5. Calc. for $C_{28}H_{46}O,0{\cdot}5H_2O$: C, 82·5; H, 11·4%). The free sterol was identical with the natural material.

Fecosterol was acetylated as above. After the usual work-up and t.l.c. purification, fecosteryl acetate (3; R^1 = Ac, R^2 = H), m.p. (from acetone–methanol) 136—138°, $[\alpha]_D^{24}$ +34·9° (*c* 0·37), was obtained (Found: C, 81·7; H, 11·1. Calc. for $C_{30}H_{48}O_2$: C, 81·8; H, 11·0%).

5α-Ergosta-7,22,24(28)-trien-3β-yl Benzoate (4; R = Bz) [Table 5, (5c)].—Some middle fractions of the triangulation of fraction V/1—5 contained mainly a component with R_F 0·64 (relative to zymosteryl benzoate) on silver nitrate plates. Three successive separations on silver nitrate plates gave pure *ester*, m.p. (from chloroform–methanol) 139—141°, $[\alpha]_D^{26}$ +13·5° (*c* 1·56), $\lambda_{max.}$ 232 nm (ε 33,000), $\nu_{max.}$ 1710, 1640, 1600, and 890 cm^{-1}, τ 4·50 (1H, dd, J_1 8, J_2 16 Hz, 22-H), 4·12 (1H, d, *J* 16 Hz, 23-H), 4·90 (1H, m, 7-H), 5·2 (1H, m, 3α-H), 5·25 (2H, m, 28-H), 7·52 (1H, septet, *J* 7 Hz, 25-H), 8·98 (6H, d, *J* 7 Hz, 26 and 27-Me), 9·18 (3H, s, 10-Me), and 9·46 (3H, s, 13-Me) (Found: C, 83·7; H, 9·4. $C_{35}H_{48}O_2$ requires C, 83·9; H, 9·7%).

5α-Ergosta-7,22,24(28)-trien-3β-ol (4; R = H).—The benzoate (4; R = Bz) (355 mg) was heated under reflux with an excess of LAH in anhydrous ether. Neutral work-up gave, after t.l.c. purification, the *alcohol*, (220 mg, 78%), m.p. (from acetone–methanol) 119—121°, $[\alpha]_D^{25}$ +36·5° (*c* 0·85), $\nu_{max.}$ 3400, 1640, 1600, and 890 cm^{-1}, $\lambda_{max.}$ 232 (ε 18,600), 226sh (18,300), and 240sh nm (12,800), τ 4·12 (1H, d, *J* 16 Hz, 23-H), 4·50 (1H, dd, J_1 8, J_2 16 Hz, 22-H), 4·90 (1H, m, 7-H), 5·25 (2H, m, 28-H), 6·46 (1H, m, 3α-H), 7·52 (1H, septet, *J* 7 Hz, 25-H), 8·98 (6H, d, *J* 7 Hz, 26 and 27-Me), 9·23 (3H, s, 10-Me), and 9·46 (3H, s, 13-Me) (Found: C, 84·5; H, 11·0. $C_{28}H_{44}O$ requires C, 84·8; H, 11·2%).

5α-Ergosta-7,22,24(28)-trien-3β-yl Acetate (4; R = Ac).—The sterol (4; R = H) (135 mg) was acetylated as above. The usual work-up and t.l.c. purification gave the *acetate* (135 mg, 90%) as plates, m.p. (from chloroform–methanol) 133—136°, $[\alpha]_D^{24}$ +6·4° (*c* 0·85), $\lambda_{max.}$ 227sh (ε 22,100), 232 (23,200), and 240sh nm (15,700), $\nu_{max.}$ 1700, 1640, 1600, and 890 cm^{-1}, τ 4·12 (1H, d, *J* 16 Hz, 23-H), 4·50 (1H, dd, J_1 16, J_2 8 Hz, 22-H), 4·90 (1H, m, 7-H) 5·24 (2H, m, 28-H), 5·30 (1H, m, 3α-H), 7·52 (1H, septet, *J* 7 Hz, 25-H), 8·98 (6H, d, *J* 7 Hz, 26 and 27-Me), 9·22 (3H, s, 10-Me), and 9·46 (3H, s, 13-Me), M^+ 438 with significant peaks at *m/e* 423, 395, 378, 363, 354, 315, and 313 (100%) (Found: C, 82·0; H, 10·6. $C_{30}H_{46}O_2$ requires C, 82·1; H, 10·6%).

Ergost-7-en-3β-yl Benzoate and 5,6-Dihydroergosteryl Benzoate [Table 6, (6a) *and* (6b)].—A sample of fraction V was chosen which showed in the mass spectrum a molecular ion at *m/e* 400 and which, after conversion into the benzoates did not show significant amounts of zymosteryl benzoate (fractions V/7—9).

4-Phenylurazole (6·36 g) was added to *N*-bromosuccinimide (5·34 g) in chloroform (100 ml) at −70°. After 1 h the benzoates of fractions V/7—9 (2·99 g) were added. After a further 1 h excess of azo-compound was destroyed by addition of a few drops of thioglycolic acid. The solution was washed with aqueous sodium hydrogen carbonate and water and dried (Na_2SO_4). After evaporation of the solvent, the residue (2·22 g) was chromatographed on alumina [solvent 1 : 1 benzene–light petroleum (b.p. 40—60°)] to give mixed benzoates (416 mg) free of dienes.

The mixed benzoates were separated by thick-layer chromatography on silver nitrate plates. The band with R_F 1·32 (relative to zymosteryl benzoate) was 5,6-dihydroergosterol benzoate (50 mg) m.p. (from chloroform–methanol and acetone–methanol) 188—191°, $[\alpha]_D^{22}$ −10·4° (*c* 0·91), mixed m.p. 188·5—191·5° with an authentic specimen. The spectra of both samples were identical.

The band with R_F 1·6 (relative to zymosteryl benzoate) was ergost-7-en-3β-yl benzoate (50 mg), m.p. 174—175°, $[\alpha]_D^{25}$ −1° (*c* 0·95) (lit.,[23] m.p. 180·5°, $[\alpha]_D$ +2°), and mixed m.p. 173—175° with an authentic specimen of m.p. 172—174°. The i.r. and n.m.r. spectra and chromatographic behaviour were identical with those of the reference sample.

Episteryl Benzoate (5; R = Bz) [Table 7, (7a)].—The sterol fractions V/7—9 were separated by preparative t.l.c. on silver nitrate plates (solvent 5% ethyl acetate–benzene; run twice) to give fraction A, without the ring B diene system, and fraction B, with the ring B diene system. The combined fractions V/8 and A/9 (706 mg) were converted into the benzoates as above and separated on silver nitrate plates to give three fractions: zymosteryl benzoate (144 mg; R_F 1·0, reference), 5α-ergosta-7,22,24(28)-trien-3β-yl benzoate (129 mg; R_F 0·69), and a mixture of bands with R_F 0·67 and 0·55 (262 mg) (118 mg, 18% was lost by oxidation). 4-Phenyl-1,2-4-triazoline-3,5-dione (260 mg; prepared as above) in chloroform (10 ml) was added dropwise at −30° to the mixed benzoates (R_F 0·69, and 0·55) (118 mg) in chloroform until the red colour persisted. After 15 min at −30°, methanol (2 ml) and pyridine (0·5 ml) were added to destroy excess of azo-reagent. The solution was evaporated at room temperature under reduced pressure and the residue fractionated by t.l.c. (solvent 10% ethyl acetate, 45% benzene, 45% light petroleum) to give three bands (R_F 0·73, 0·27, and 0·16 relative to zymosteryl benzoate). The band of R_F 0·73 gave *episteryl benzoate* (224 mg) as a chloroform solvate, m.p. (from chloroform–methanol) 175—177°, $[\alpha]_D^{24}$ +7·35° (*c* 1·4) (lit.,[2c] m.p. 161—163°, $[\alpha]_D$ +11·8°), $\nu_{max.}$ 1710, 1640, and 890 cm^{-1}, τ 1·9 and 2·55 (5H, m, ArH), 4·78 (1H, m, 7-H), 5·2 (1H, m, 3α-H), 5·27 (2H, m, 28-H), 8·96 (9H, d, *J* 7 Hz, 21-, 26-, and 27-Me), 9·14 (3H, s, 10-Me), and 9·44 (3H, s, 13-Me) (Found: C, 73·5; H, 8·8. $C_{35}H_{50}O_2,0{\cdot}67CHCl_3$ requires C, 73·5; H, 8·8%).

The band of R_F 0·27 consisted of the side-chain adduct of 5α-ergosta-7,22,24(28)-trien-3β-yl benzoate (77 mg) and that of R_F 0·16 was an azo–ene adduct[12] and was not further identified.

Episterol (5; R = H).—The benzoate (5; R = Bz) (224 mg) was heated with excess of LAH in anhydrous ether for 1 h under reflux. Mild acid work-up and t.l.c. purification gave *episterol* (131 mg) as needles, m.p. (from chloroform–methanol) 125—126°, $[\alpha]_D^{24}$ +6·5° (*c* 1·63) (lit.,[2c] m.p. 135—136°, $[\alpha]_D$ +6·2°) $\nu_{max.}$ 3500, 1640, and 890 cm^{-1}, τ 4·79 (1H, m, 7-H), 5·27 (2H, m, 28-H), 6·4 (1H, m, 3α-H), 8·97 (9H, d, *J* 7 Hz, 21-, 26-, and 27-Me), 9·18 (3H, s, 10-Me), and 9·44 (3H, s, 13-Me) (Found: C, 84·6; H, 11·5. $C_{28}H_{46}O$ requires C, 84·4; H, 11·6%).

Episteryl Acetate (5; R = Ac).—The sterol (5; R = H) (71 mg) was acetylated as above. The usual work-up gave *episteryl acetate* (52 mg), m.p. (from acetone–methanol) 136—138°, $[\alpha]_D^{20}$ +4·7° (*c* 0·4) (lit.,[2c] m.p. 161—162°, $[\alpha]_D$ −3·8°) τ 4·81 (1H, m, 7-H), 5·2 (1H, m, 3α-H), 5·25 (2H, m, 28-H), 7·95 (3H, s, OAc), 8·96 (9H, d, *J* 7 Hz, 21-, 26-, and 27-Me), 9·18 (3H, s, 10-Me), and 9·45 (3H, s, 13-Me) (Found: C, 81·6; H, 10·9. $C_{30}H_{48}O_2$ requires C, 81·8; H, 11·0%).

[23] D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 1948, 783.

Ergosta-5,7,24(28)-*trien*-3β-*yl* (6), *Ergosta*-5,7,22,24(28)-*tetraen*-3β-*yl, and Ergosteryl Acetates* [Table 8, (8a,b,c)].—Analytical t.l.c. on silver nitrate plates showed the acetates of fractions V/7—9B (see above) to consist of five components, with R_F 1·0, 0·89, 0·78, 0·67, and 0·44 (relative to ergosteryl acetate). The band of R_F 0·89 was isolated and shown to be ergosta-5,7,22,24(28)-tetraen-3β-yl acetate by comparison with an authentic sample. This and ergosterol (the band of R_F 1·0) were the major components, but fraction V/9B showed a moderate quantity of the third component, R_F 0·78.

Thus a sample of fraction V/9B (1·9 g) was acetylated as above to give the crude acetates (2·14 g). A sample of these (1·05 g) was separated on silver nitrate plates (solvent benzene; twice developed), to give three fractions: (i) ergosteryl acetate (321 mg) (R_F 1·0, reference), (ii) ergosta-5,7,22,24(28)-tetraenyl acetate (262 mg) (R_F 0·89), and (iii) a still unresolved mixture of the tetraenyl acetate (R_F 0·89) and the unidentified acetate (R_F 0·78) (173 mg). Repeated separation of the third fraction as above (loading 1 : 500) gave essentially pure *ester* (6) (26 mg) as plates, m.p. (from acetone–methanol) 128—132°, $[\alpha]_D^{24}$ −75·5° (*c* 0·17), $\nu_{max.}$ 1720, 1640, and 890 cm^{-1}, $\lambda_{max.}$ 263 (ε 7200), 272 (10,700), 282 (11,200), and 294 nm (6290), τ 4·5 (2H, m, 6- and 7-H), 5·2 (1H, m, 3α-H), 5·30 (2H, m, 28-H), 8·97 (9H, d, *J* 7 Hz, 21-, 26-, and 27-Me), 7·85 (3H, s, OAc), 9·05 (3H, s, 10-Me), and 9·39 (3H, s, 13-Me), M^+ 438 with significant peaks at *m/e* 378 (100%), 363, 337, 317, 294, 279, 253, and 251 (Found: C, 82·1; H, 10·5. $C_{30}H_{46}O_2$ requires C, 82·1; H, 10·6%).

The reported free sterol [15] had $\nu_{max.}$ 1650, 887, 843, and 800 cm^{-1}, $\lambda_{max.}$ 263 (ε 9750), 272 (13,750), 282 (14,120), and 294 (ε 8500), τ 4·4 (6 and 7-H), 5·30 (28-H), 8·96 (26- and 27-Me), 9·05 (10-Me), and 9·35 (13-Me).

Identification of Ascosterol.—A sample of ascosterol (supplied by Dr. W. Fürst) which had been isolated according to Wieland's methods [2c] had m.p. 128—139°, $[\alpha]_D^{28}$ +34° (*c* 1·13), $\nu_{max.}$ 3500, 1640, and 890 cm^{-1}, $\lambda_{max.}$ 282 nm (ε 240). In the mass spectrum, molecular ion peaks occurred at *m/e* 400, 398, and 396.

A sample (5 mg) was benzoylated as above and qualitatively analysed on a silver nitrate plate. Four spots were observable with R_F 0·5, 1·0, 1·32, and 1·60 relative the zymosteryl benzoate. The major component, R_F 0·5, was isolated and shown to be identical with fecosteryl benzoate. The minor components were chromatographically identical with zymosteryl benzoate, 5,6-dihydroergosteryl benzoate, and ergost-7-en-3β-yl benzoate, respectively. The low u.v. absorption of the sterols also indicates the presence of *ca.* 2% of components with ring B dienes.

The n.m.r. spectrum showed the mixed nature of the material but exhibited predominantly fecosterol absorptions.

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