4α-Methyl-24-methylene-24,25-dihydrozymosterol, a New Sterol of *Saccharomyces cerevisiae* of Possible Importance in the Biosynthesis of Ergosterol

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THE sterol fraction of yeast has afforded a variety of nortriterpenoids[1,2] all conceivably derived from lanosterol. We report here the discovery of a new sterol to which we assign the structure (I; R=H) on the basis of physical evidence and chemical correlation with the known 4α-methylzymosterol (III; R=H).[2]

The new sterol (I; R=H) was isolated from yeast sterol residues† by column chromatography on grade V alumina. This yielded a mixture of (I; R=H) and (III; R=H). Preparative thin-layer chromatography of the derived acetates on silver nitrate–silica gel G plates gave 4α-methylzymosterol acetate (III; R=Ac) in 0·94% yield, identical with an authentic specimen.‡ There was also obtained the new acetate (I; R=Ac) in 0·052% yield as plates, m.p. 110—111° (from acetone–methanol), $[\alpha]_D^{28}$ + 61° (*c*, 1; all rotations in $CHCl_3$). Alkaline hydrolysis gave the sterol (I; R=H) as needles, m.p. 143—145° (from acetone–methanol), $[\alpha]_D^{29}$ + 55° (*c*, 3). Benzoylation of this gave the benzoate (I; R=CO·Ph), as needles, m.p. 142—144° (from chloroform–methanol), $[\alpha]_D$ +81° (*c*, 2), and oxidation in benzene with chromic oxide in acetic acid gave the ketone (II), m.p. 123—125° (from acetone–methanol), $[\alpha]_D$ + 47° (*c*, 1). Reduction of (II) with lithium aluminium hydride regenerated the sterol, confirming the β-assignment of the 3-hydroxy-group. Both the sterol (I; R=H) and its acetate (I; R=Ac) were transparent above 220 mμ in the u.v. region.

The mass spectrum of the acetate (I; R=Ac)

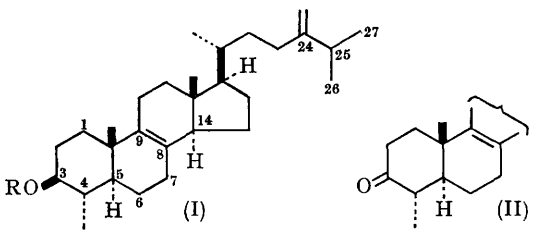
† Kindly supplied by Koninklijke Nederlandsche Gist en Spiritus Farbriek N.V., Holland.
‡ The comparison was kindly carried out by Professor G. Ourisson and Dr. G. Ponsinet.

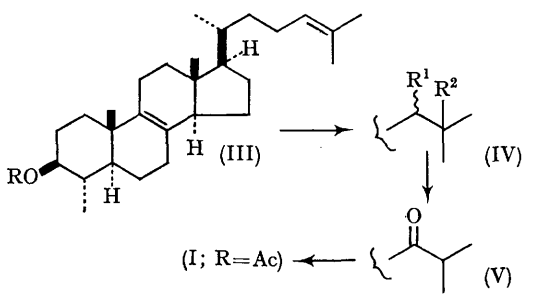
TABLE

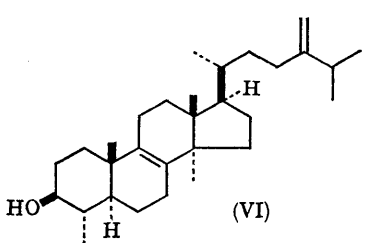
N.m.r. spectra in $CDCl_3$ (*τ values*)

	Methyl groups					Other protons		
	4α	10β	13β	26, 27	3β–OAc	3α	24	28
(I); R=H	8·96[a]	9·02	9·38	8·91, 9·02	—	7·0	—	5·31
(I); R=Ac	9·14[b]	9·01	9·38	8·93, 9·01	7·96	5·65	—	5·33
(I); R=COPh	9·12[a]	9·05	9·39	8·93, 8·98	—	5·50	—	5·35
(II)	(9·00)[c]	8·82	9·36	8·94, 9·04	—	—	—	5·32
(III); R=Ac	9·15[b]	9·03	9·40	8·34, 8·41	7·97	5·65	4·93	—
24-Methylenedihydrolanosteryl acetate[13c]	9·12[d]	9·03	9·30	8·92, 8·99	7·96	5·46	—	5·29
(IV); R^1=Br, R^2=OH	9·15[b]	9·02	9·38	8·66, 8·66	7·97	5·67	6·07[e]	—
(IV); R^1, R^2=O	9·15[b]	9·01	9·38	8·70, 8·75	7·97	5·61	7·32[f]	—
(V)	9·15[b]	9·02	9·39	8·86, 8·98	7·97	5·65	—	—

[a] One half of a doublet; the other is obscured by other methyl groups; [b] Doublet, *J* 6 c./sec.; [c] Estimated position; [d] 4α-,4β-, and 14α-methyl groups; [e] Doublet, *J* 7 c./sec.; [f] Triplet, *J* 5 c./sec.







showed the molecular ion at *m/e* 454 and a typical sterol fragmentation pattern. Significant peaks occurred at *m/e* 439 (loss of CH_3), 394 (loss of $CH_3{\cdot}CO_2H$), 379 (loss of CH_3 and $CH_3{\cdot}CO_2H$), 287 (loss of side chain +42), 227 (loss of side chain +42 and $CH_3{\cdot}CO_2H$). The side-chain fragmentation indicated the presence of an additional methylene group in this part of the molecule together with one double bond. It followed that an additional methyl group was present in the nucleus, which also must have one double bond. A comparison with the mass spectrum of 4α-methylzymosterol acetate (III; R=Ac) showed that after side-chain loss, the spectra were virtually identical. In particular, a 7 or 9(11) double bond could be ruled out by the lack of the ready reverse Diels–Alder fragmentation leading to cleavage of ring B or C.

The infrared spectra of (I) and its derivatives all showed characteristic strong absorptions at 1642 and 898 $cm.^{-1}$, assignable to a terminal methylene group. This conclusion was confirmed by the n.m.r. spectra (see Table) which, for the acetate (I;R=Ac) showed a broad doublet at τ 5·33 integrating for two protons. This was 0·4 p.p.m. upfield from the C-24 proton absorption in the 4α-methylzymosterol side-chain, but was identical to that observed for 24-methylenedihydrolanosterol (see Table). There were no other vinylic proton resonances in the spectrum. Thus the second double bond must be tetrasubstituted and at 8(9) or 8(14), since the only other alternative was ruled out by the appearance of the 4-methyl group as a doublet at τ 9·14 (*J* 6 c./sec.). The methyl resonances at C-10 and C-13 (τ 9·01 and 9·38 respectively) indicated an 8(9) position by comparison with 4α-methylzymosterol (III; R=H) and the calculated values of τ 9·04 and 9·41,[3] rather than the $\Delta^{8(14)}$ alternative (calculated τ 9·28 and 9·16). The resonances due to the 26,27 methyl groups were shifted upfield compared with those of 4α-methylzymosterol, to τ 8·93 and 9·01, but were still 0·1—0·2 p.p.m. downfield from those of a saturated side-chain.[4] This was explicable if

the methylene group was at C-24, a position which is strongly favoured on biogenetic grounds.[5]

Biogenetically, an additional methyl group in the nucleus would be expected to be at C-4 or C-14. The latter was ruled out by the n.m.r. spectrum and the C-4 assignment was confirmed by the o.r.d. curve of the ketone (II), which showed a positive Cotton effect at 286 mμ of amplitude +43, which is in accordance with a 4α-methyl configuration.[6] The molecular rotation differences[7] of the sterol (I; R=H) and its acetate (I; R=Ac) and benzoate (I; R=COPh) (Δ_1, +51; Δ_2, +192) gave further confirmation of this assignment.[8]

Finally the structure was proved by synthesis from 4α-methylzymosterol acetate as follows. Addition of hypobromous acid to (III; R=Ac) with *N*-bromosuccinimide in 20% aqueous Glyme[9] gave quantitatively a mixture of bromohydrins (IV; R^1=Br, R^2=OH), which on repeated crystallisation from actone had m.p. 146—149°, $[\alpha]_D$ +54° (*c*, 2) with the expected n.m.r. spectrum (see Table). Chromatography of the mixture over alumina grade III gave the mixed epoxides (IV; R_1,R_2, = O) in quantitative yield. Repeated crystallisation from acetone gave, m.p. 160—163° $[\alpha]_D$ +55° (*c*, 1), with the appropriate n.m.r. spectrum (see Table). Rearrangement of the expoxide mixture with boron trifluoride etherate at 25° gave the ketone (V), m.p. 102·5—104·5° (from acetone–methanol), $[\alpha]_D$ +61° (*c*, 1), which on reaction[10] with the methylene phosphorane gave the 24-methylene derivative, m.p. 109·5—110·5°, mixed m.p. 109·5—110·5° with the natural sterol acetate, $[\alpha]_D$ +61° (*c*, 0·5), i.r., n.m.r., and mass spectra were all identical with those of the natural material.

The isolation of the 24-methylene derivative of a yeast sterol still containing a 4-methyl group has important biogenetic implications concerning the point at which side-chain modification occurs.[5,11] Accordingly the new sterol (I; R=H), 4α-methylzymosterol (III; R=H) and obtusifoliol (VI)[12] were tritiated at C-2 (and C-4) by base-catalysed exchange of the corresponding ketones. The sterols were fed in parallel to growing cultures of yeast according to the method previously described.[11c] They were incorporated into ergosterol to extents of 12, 15, and 8%, respectively. The isolation of (I; R=H) and (III; R=H) from yeast and their ready incorporation into ergosterol means that they must lie on one or more biosynthetic routes to ergosterol. The incorporations of obtusifoliol and 24-methylenedihydrolanosterol,[11b,c] which have not yet been detected in yeast, are incompatible with a route involving 4α-methylzymosterol. The possibilities of two or more biosynthetic pathways to ergosterol, or nonspecific enzyme systems which accept unnatural precursors, are under investigation.

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