BIOSYNTHETIC ROUTES TO ERGOSTEROL IN YEAST

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Received June 26,1972

Summary

The routes from episterol (10) to ergosterol (2) in aerobically grown Saccharomyces cerevisiae have been evaluated by systematically feeding and trapping with suspected intermediates (12-16) which were independently synthesized. In this manner three sterols (13-15) not previously reported were discovered to be present in this organism. Based on relative incorporation efficiencies the major route from episterol to ergosterol involves the introduction of unsaturation at C_{22} then at C_5 and finally reduction of the 24-methylene.

The conversion of lanosterol (1) to ergosterol (2) in yeast (Saccharomyces cerevisiae) involves loss of three nuclear methyl groups, alkylation at C_{24} and introduction of C_{22}, C_7 and C_5 unsaturation. Gaylor[1] has recently demonstrated that zymosterol (3) is superior to 4,4-dimethyl zymosterol (4), 4α-methyl zymosterol (5), 7,24-cholestadien-3β-ol (6), 5,24-cholestadien-3β-ol (7) and 5,7,24-cholestatrien-3β-ol (8) as a substrate for a soluble Δ^{24}-sterol methyl-transferase isolated from yeast. Assuming the relative rates observed in this system to be indicative of the ability of whole cells to alkylate these substrates, C_{24}-alkylation most likely occurs after partial or complete nuclear demethylation, but prior to the introduction of the C_{22}, C_7 and C_5 unsaturation. Consistent with this sequence is the

apparent absence in yeast sterol mixtures of 6-8 and the presence of 4[2], 5[2], 24-methylene 5[3], 3[4], fecosterol (9)[5], episterol (10)[5], 5-dihydroergosterol (11)[6] and ergosta-5,7,22,24(28)-tetraen-3β-ol (12)[7]. Since only 24-methylene sterols were produced by the sterol methyl-transferase system, 9 and its Δ^7-isomer, 10, are likely intermediates in the lanosterol to ergosterol conversion. In order to investigate the alternative pathways from 10 to ergosterol, we have synthesized sterols 10, and 12-16 and studied their interconversions in yeast. Sterols 13-16 have not previously been reported in S. cerevisiae but trapping experiments and subsequent isolation have revealed the occurrence of 13-15. A search for 16 indicated its absence.

Episterol (10) was synthesized from 3β-acetoxy-ergosta-7,22-dien-24-one (17)[8] by reduction with 5% $Pd/BaSO_4$, reaction with methylene triphenyl phosphorane (18) and deacetylation (2% K_2CO_3 in 10% water-ethanol)[9,10]. Ergosta-7,22,24(28)-trien-3β-ol (14, mp 121-124) was prepared by reaction of 17 and 18 followed by deacetylation. Ergosta-5,7,24(28)-trien-3β-ol (13, mp 129-130.5[11]) was obtained from 3β-acetoxy-ergosta-5,22-dien-24-one[9] by ketalization, conversion of the acetate to a benzoate, reaction with NBS and triphenylphosphite, deketalization and reaction with 18[10]. Ergosta-5,7,22,24(28)-tetraen-3β-ol (12, mp 149.5-150.5[9]) was prepared by reaction of the corresponding 3β-benzoyl-24-ketone with 18[9]. Utilization of [^{14}C]-18 in the above produced [28-^{14}C]-10 (4.52 x 10^5 cpm/mg), [28-^{14}C]-13 (7.1 x 10^5 cpm/mg) and [28-^{14}C]-12 (7.2 x 10^5 cpm/mg). Ergost-7-en-3β-ol (15, mp 145-146[12]) was prepared by reduction of ergosterol over Raney-Ni[13] and was labelled (3.2 x 10^5 cpm/mg) by base catalyzed exchange of the corresponding 3-ketone in the presence of ^3H_2O followed by

$NaBH_4$ reduction. Ergosta-5,7-dien-3β-ol (16, mp 152-153[14]) was prepared via reduction of the ergosterol-maleic anhydride adduct.[14] The labelled sterol (2.9 x 10^4 cpm/mg) was obtained using ^{14}C labelled ergosterol obtained from yeast incubated with [1-^{14}C]-acetate.

In separate experiments each of the labelled sterols (10, 12-16) was fed to aerobically growing yeast which had been depleted of sterols by anaerobic growth. After each incubation suspected unlabelled metabolites (usually differing by one structural change) were added, the sterol fraction chromatographically separated and the amount of label in individual sterols determined.[15]

Since feeding labelled 10 and trapping with 13, 14 and 15 produced radioactivity in the latter a search was made for these sterols in yeast sterol concentrate.[16] All three of these sterols were isolated after extensive column and thin layer chromatography.[15] Although 16 was rather efficiently converted to ergosterol it was not produced from 15. G.l.p.c./m.s. of the t.l.c. fraction corresponding to 16 revealed its apparent absence in the sterol mixture. The results recorded in Table 1 indicate that the major route to ergosterol involves the sequence 10→14→12→2 corresponding to introduction of unsaturation at C_{22} prior to C_5 which is introduced prior to reduction of the 24-methylene. The sequences 10→13→12→2 and 10→15→11→2 also operate to a lesser extent in this organism.

It is noteworthy that once the C_{22} and C_5 double bonds are introduced they are not removed (i.e. Δ^{22} ↛ 22H,23H: 14 ↛ 10, 12 ↛ 13 and Δ^5 ↛ 5H,6H: 12 ↛ 14). Nor does it seem likely that reintroduction of a methylene at $C_{24(28)}$ occurs once it is

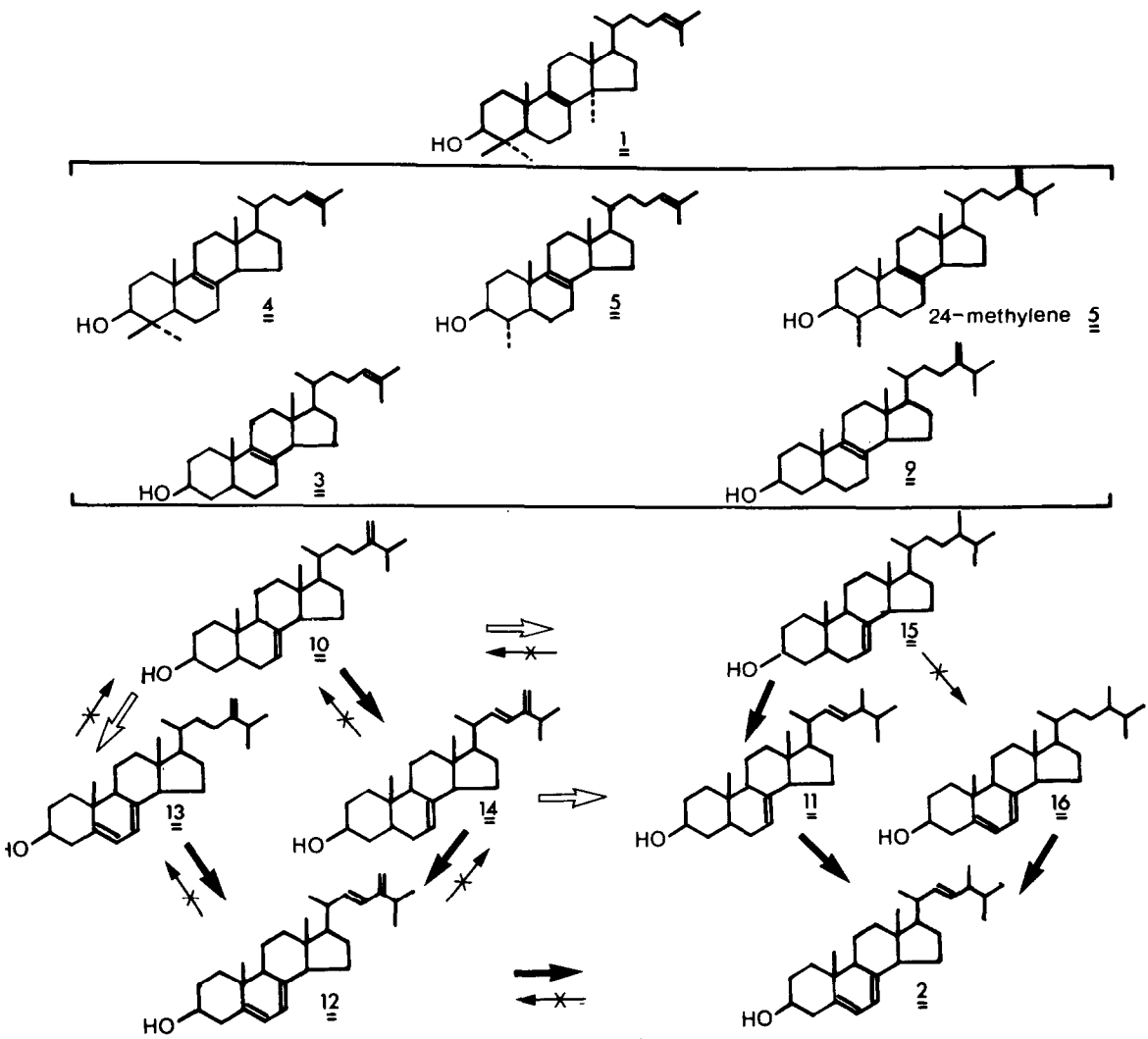

TABLE 1

INCUBATION EXPERIMENTS

ACTIVITY IN METABOLITES‡

LABELLED STEROL ADDED	10	15	13	14	11	16	12	2
10		0.32 0.21	0.4	6.1			5.4 17.6	2.9 5.1
15	0				11.6 10.45	0	0 >0.001	15.2 16.4
13							7.1	3.5
14	>0.001				0.23 0.4		9.7 10.0	3.7 4.1
16		0	0					19.2
12			0	0				17.8

‡% incorporation based on total activity recovered from nonsaponifiable fraction.

reduced (24-Me↔ $\Delta^{24(28)}$: 15↔ 10, 2↔ 12[17]). Extensions of these studies are in progress and will be reported shortly.

Acknowledgements

We wish to thank the N.R.C. of Canada and S.F.U. Research Fund for continued support and the Upjohn Company for a generous supply of stigmasterol.[9]

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