Biosynthesis of Terpenes and Steroids. Part VIII.[1] The Synthesis and Metabolism in *Saccharomyces cerevisiae* of Ergosta-7,22,24(28)-trien-3β-ol

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A partial synthesis of ergosta-7,22,24(28)-trien-3β-ol has confirmed the structure of this yeast sterol. Feeding experiments with the tritium-labelled sterol have shown its intermediacy in ergosterol and 5-dihydroergosterol biosynthesis in *Saccharomyces cerevisiae* and added further evidence for a proposed scheme for the later stages of ergosterol biosynthesis.

THE isolation of ergosta-7,22,24(28)-trien-3β-ol (I; R = H) from the non-saponifiable residues of yeast has recently been reported.[1] As a final proof of structure and a source of radioactively labelled material for metabolic studies, a partial synthesis from 5-dihydroergosterol has been developed.

The selective ozonolysis of 5,6-dihydroergosterol acetate at the 22,23-double bond has been reported.[2] In an attempt to optimise the yield of the 22-aldehyde (II), the reaction has been examined in some detail. At −70° in methylene chloride solution, the selectivity is greatly enhanced by the presence of ⩾1% pyridine.[3] In the absence of pyridine, the further ozonolysis of the aldehyde (II) occurred at a rate comparable to that of its formation. Ozone was best added as a saturated solution in methylene chloride at −70°. Under these conditions no over-oxidation was apparent until the reaction was 40—50% complete. Work-up at this point gave 50% unchanged starting material and 40% of the 22-aldehyde (II) (80% allowing for recovered starting material). Acid work-up gave a product which showed, in the n.m.r. spectrum, the aldehyde proton as two doublets at τ 0·45 and 0·40 in the ratio of 4 : 1. The epimerisation at C-20 thus apparent[4] could be avoided by use of a reductive (zinc–acetic acid) work-up.

Wittig reaction of the pure (20*S*)-aldehyde (II) with the 2-isopropylallylphosphorane (III)[4a] gave an 85% yield of the required sterol acetate (I; R = Ac). The n.m.r. spectrum showed a doublet (*J* 16 Hz) at τ 4·10 assignable to H-23 and a double doublet at 4·18 (J_1 16, J_2 8 Hz) assignable to H-22. The large coupling constant (16 Hz) indicated the *trans*-configuration of the 22,23-double bond. Although the Wittig reaction might be expected to give a mixture of *cis*- and *trans*-isomers,[5a] no *cis*-isomer was detectable, as has been noted in previous similar syntheses.[4a,5]

The synthetic sterol acetate was identical with the natural material.

For biosynthetic experiments, the synthesis was repeated starting with [2,2,4,4-$^3H_4$]-5,6-dihydroergosterol, prepared by base-catalysed exchange from the corresponding ketone.[6] The susceptibility of the side-chain diene system to oxidation made it unwise to

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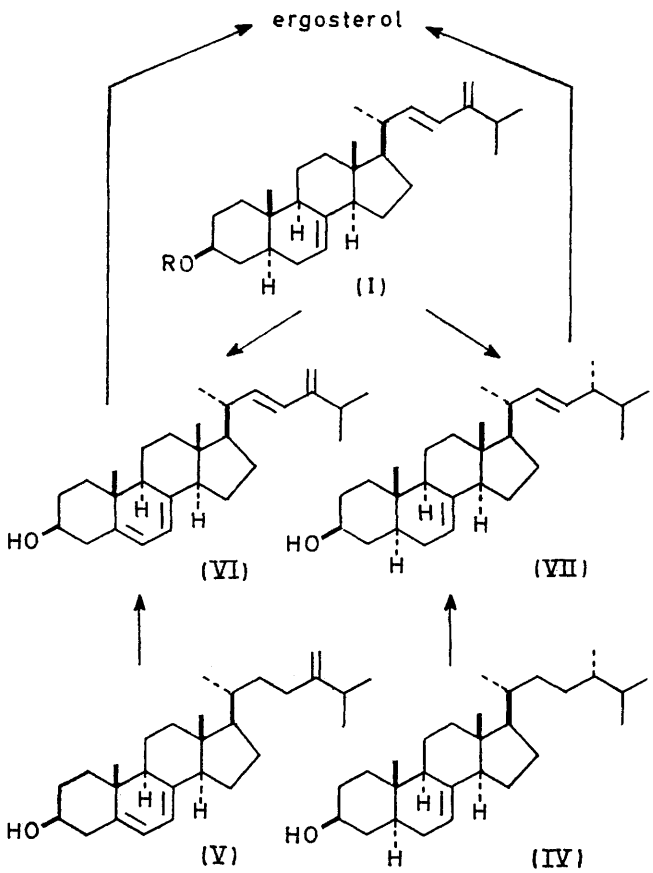
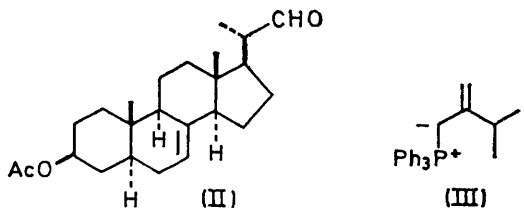
attempt such exchange with the trienone corresponding to (I).

The [2,2,4,4-$^3H_4$]trienol thus obtained was added to a growing culture[7] of *Saccharomyces cerevisiae* (strain



SCHEME Later stages of ergosterol biosynthesis

NRRL-y-2250). The harvested cells were diluted with carrier ergosterol prior to a previously described work-up procedure[1] which gives very pure ergosterol. Incorporation into ergosterol was 0·79% of the activity fed.



As an intermediate in ergosterol biosynthesis, ergosta-7,22,24(28)-trien-3β-ol (I; R = H) thus occupies a position parallel to and independent of ergost-7-en-3β-ol (IV) * and ergosta-5,7,24(28)-trien-3β-ol (V) * (see Scheme), which have recently been isolated from yeast sterols.

The relationship of ergosta-5,7,22,24(28)-tetraen-3β-ol (VI) to ergosterol has already been defined.[4a] Ergosta-7,22-dien-3β-ol (VII),* a known constituent of yeast sterols,[8] is an alternative possible intermediate in the conversion of (I; R = H) into ergosterol (see Scheme).

In order to test this, a feeding experiment was carried out as above, but the harvested cells were diluted with ergosta-7,22-dien-3β-ol (VII) before saponification. Work-up as previously defined[1] gave reisolated dienol (VII) containing 0·32% of the activity fed.

The later stages of ergosterol biosynthesis may be, at this stage, tentatively defined by the Scheme, which is limited to known yeast sterols. The relative importance and inter-relationships of the various pathways invoked have yet to be elucidated.

EXPERIMENTAL

Unless otherwise stated m.p.s were determined with a Kofler hot-stage apparatus and are corrected. I.r. spectra were run for solutions in chloroform, u.v. spectra for solutions in ethanol, and n.m.r. spectra for solutions in deuteriochloroform at 60 or 100 MHz. Radioactivity was assayed with Nuclear Enterprises type NE 213 scintillator at an efficiency of 45% for ^3H. Total activities are based on the amount of added carrier and endogenous material is ignored. P.l.c. refers to preparative layer chromatography on 1 mm silica GF plates.

3β-*Acetoxy*-23,24-*dinor*-5α-*chol*-7-*en*-22-*al* (II).—5,6-Dihydroergosterol acetate[9] (1·4 g, 3·2 mmol) was dissolved in 1% pyridine–dichloromethane. To this solution, stirred rapidly at −70°, was added a saturated solution of ozone in 1% pyridine–dichloromethane (700 ml) at 20 ml s^{-1}. Zinc powder (3 g) and acetic acid (25 ml) were added and the mixture was allowed to warm to room temperature with continuous stirring. After filtration, the solution was washed with water (200 ml), dried (Na_2SO_4), and evaporated. The residue was diluted with methanol and the starting material (0·7 g, 50%) separated as fine plates. Addition of water to the methanolic solution precipitated the aldehyde (II) (0·47 g, 40%) as needles, m.p. (from acetone) 144—147°, $[\alpha]_D^{20}$ −14·5° (*c* 0·71) (lit.,[10] m.p. 139—141°, $[\alpha]_D$ −18·7°).

3β-*Acetoxy*-5α-*ergosta*-7,22,24(28)-*triene* (I; R = Ac).—The 2-isopropylallyltriphenylphosphorane (III)[4a] [generated from the phosphonium salt (1·31 g) with methyllithium in ether (1·10N; 2·2 ml)] in tetrahydrofuran (50 ml) was treated with the aldehyde (II) (1·00 g) in tetrahydrofuran (5 ml). After 16 h water (50 ml) was added and the mixture was extracted with ether (3 × 150 ml). The extract was washed with water (100 ml), dried (Na_2SO_4), and evaporated. The residue, in pyridine (5 ml), was acetylated with acetic anhydride (2·5 ml) during 16 h. Ice–water (20 ml) was added and the mixture extracted with ether (3 × 20 ml). The extract was washed with water (20 ml), dried (Na_2SO_4), and evaporated to give 3β-acetoxy-5α-ergosta-7,22,24(28)-triene (1·03 g, 85%), m.p. (from chloroform–methanol) 132—134°, $[\alpha]_D^{25}$ +7·3° (*c* 0·1), $\nu_{max.}$ 1720 and 890 cm^{-1}, $\lambda_{max.}$ 227 (ε 21,500) and 241 nm (ε 16,250), τ 9·5 (3H, s, 18-H_3), 9·21 (3H, s, 19-H_3), 8·96 (9H, d, *J* 6·5 Hz, 21-, 26-, and 27-H_3), 8·08 (3H, s,

* The biosynthetic experiments on these sterols will be reported elsewhere.

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OAc), 5·22 (2H, d, J 2 Hz, 28-H_2), 4·90 (1H, m, 7-H), 4·48 (1H, dd, J_1 16, J_2 8 Hz, 22-H), and 4·10 (1H, d, J 16 Hz, 23-H).

The spectral data were essentially identical with those of the natural material, and a mixed m.p. determination showed no change.

[2,2,4,4-$^3H_4$]Ergosta-7,22,24(28)-trien-3β-ol of activity $1·943 \times 10^5$ decomp. s^{-1} mg^{-1} was prepared analogously from 5,6-dihydro[2,2,4,4-$^3H_4$]ergosterol, which had been tritiated by a method previously described.[6]

Feeding of [2,2,4,4-$^3H_4$]*Ergosta-7,22,24(28)-trienol to Yeast.*—(*a*) A modification of our previous technique [6] was used. A medium made up of malt (150 g), mycological peptone (30 g), and ammonium dihydrogen phosphate (12 g) in water (1·5 l) was sterilised in three 1 l flasks (10 min at 15 lb in^{-2}). The medium was filtered through Celite and resterilised. The cooled medium was inoculated with *Saccharomyces cerevisiae* (strain NRRL-y-2250) and the [2,2,4,4-$^3H_4$]ergosta-7,22,24(28)-trien-3β-ol (activity $2·87 \times 10^5$ decomp. s^{-1} mg^{-1}; 0·567 mg) added as a dispersion in 50% aqueous acetone (5 ml) containing Tween 80 (150 mg). The flasks were incubated at 28° for 48 h then aerated (120 l h^{-1}) at 28° for 48 h. The yeast cells were harvested by centrifugation and saponified, after addition of carrier ergosterol (240 mg), with 10% potassium hydroxide in methanol (200 ml). The solution was reduced to half its original volume and poured into water (2 l). The aqueous suspension was adjusted to pH 8 and extracted with ether (3×250 ml) with the use of centrifugation to break the emulsions formed. Evaporation of the dried (Na_2SO_4) ethereal solutions gave the crude sterol mixtures. These were worked up for ergosterol by the previously described method [1,4a] to give pure ergosterol (206·1 mg). Over four recrystallisations this showed a constant activity of 5·35 decomp. s^{-1} mg^{-1}, equivalent to $1·29 \times 10^3$ decomp. s^{-1} total activity (based on carrier ergosterol only), equivalent to 0·79% incorporation.

(*b*) A medium of glucose (8 g), mycological peptone (8 g), and malt extract (4 g) in water (400 ml) was sterilised as in (*a*). [2,2,4,4-$^3H_4$]Ergosta-7,22,24(28)-trien-3β-ol (2·0 mg; activity $1·943 \times 10^5$ decomp. s^{-1} mg^{-1}) was added in 50% aqueous acetone (5 ml) containing Tween 80 (150 mg). The medium was inoculated with the yeast strain as before and incubated aerobically for 4 days at 29°. After harvesting the cells by centrifugation, carrier 5,6-dihydroergosterol (200 mg) was added and the whole mixture was saponified with 10% potassium hydroxide in ethanol (100 ml). Work-up as before gave pure unchanged 5,6-dihydroergosterol as the benzoate (141 mg) with activity constant over four recrystallisations at 226 counts min^{-1} mg^{-1} (*i.e.* $1·25 \times 10^3$ decomp. s^{-1} total activity, equivalent to 0·32% incorporation).

We thank Dr. E. Kaspar and Schering A.G. Berlin for the supply of the yeast strain and for information about growth conditions. The financial support of the German Academic Exchange service (to U. M. K.) and the awards of a Northern Ireland Scholarship (to J. F. M.) and an S.R.C. studentship (to P. J. D.) are gratefully acknowledged.

[1/2455 *Received*, 22*nd December*, 1971]