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TRANSFORMATION OF 23,24-BISNORCHOL-4-EN-3-ONE-22-OL BY *RHIZOPUS ARRHIZUS*

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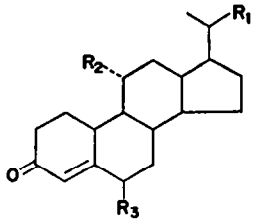
Key Word Index—*Rhizopus arrhizus*; fungus; 6β,11α,22-trihydroxy-23,24-bisnorchol-4-en-3-one transformation.

Abstract—The transformation of 23,24-bisnorchol-4-en-3-one-22-ol into 6β,11α,22-trihydroxy-23,24-bisnorchol-4-en-3-one by the fungus *Rhizopus arrhizus* has been shown to be dependent on the composition of the culture medium, with respect to yield of the triol. The transformation of the 22-alcohol to 6β,11α-dihydroxy-pregn-4-ene-3,20-dione is also reported.

DIFFICULTIES encountered in certain steroid transformations by chemical methods may be overcome by feeding the appropriate steroidal intermediate to a fungal culture, and after a suitable incubation period, isolating the modified product. One of the steps involved in the synthesis of 3β,6α-dihydroxy-5α-cholest-9(11)-en-23-one (dihydromarthasterone), a steroid isolated from the starfish *Marthasterias glacialis*, required functionalization of positions 6 and 11 of 23,24-bisnorchol-4-en-3-one-22-al (I). Microbial transformations of C_{22} steroids using members of the fungal genus *Rhizopus* have been reported by Meister *et al.*[1] The effect of cultural conditions on this functionalization, using *Rhizopus arrhizus*, was investigated with a view to increasing the yield of the principal product, 6β,11α,22-trihydroxy-23,24-bisnorchol-4-en-3-one (II).

Using a malt extract medium, yields of (II) superior to those reported by Meister *et al.*[1]



(I) R_1 = CHO; R_2 = R_3 = H
(II) R_1 = CH_2OH; R_2 = R_3 = OH
(III) R_1 = CH_2OH; R_2 = R_3 = H

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[1] P. D. MEISTER, D. H. PETERSON, S. H. EPPSTEIN, H. C. MURRAY, C. M. REINEKE, A. WEINTRAUB and H. M. LEIGH OSBORNE, *J. Am. Chem. Soc.* **76**, 5679 (1954).

were obtained when (I) was initially reduced to the 22-alcohol (III) with $NaBH_4$ (1 equivalent) in ethanol before incorporation into the medium. The optimum incubation period was determined as 7 days under the conditions used.

TABLE 1. COMPOSITION OF MEDIA AND ITS EFFECT UPON MYCELIAL DRY WT AND YIELD OF 6β,11α,22-TRIHYDROXY-23,24-BISNORCHOL-4-EN-3-ONE FROM 23,24-BISNORCHOL-4-EN-3-ONE-22-OL

Principal carbon and nitrogen source*	Mycelial dry wt (g)	% Yield of II
Glucose (10 g/l.), peptone (mycological, oxoid, 5·0 g/l.)	1·200	44
Glucose (10 g/l.), malt extract (Oxoid, 30 g/l.)	1·136	27
Glucose (10 g/l.), asparagine (1·0 g/l.)	0·807	11
Glucose (10 g/l.), $(NH_4)_2SO_4$ (0·5 g/l.)	0·612	9
Glucose (10 g/l.), potato extract (Oxoid, 4 g/l.)	0·423	0
Sucrose (10 g/l.), $NaNO_3$ (2·0 g/l.)	0·013	0

* All media contained KCl (0·5 g/l.), K_2HPO_4 (1·0 g/l.), $MgSO_4.7HO$ (0·3 g/l.), $FeSO_4$ (0·01 g/l.) dist. H_2O 1 l. and pH adjusted to 6·0.

The composition of the medium was found to have a marked effect upon the production of (II) from (III) as well as on the mycelial dry wt (Table 1). Media containing peptone, with malt extract or glucose as a supplement, gave the highest mycelial dry wt; the greatest yield of (II), however, was obtained when glucose, rather than malt extract, was used as the supplement. The yields of (II) from the other media were relatively low, as were the mycelial dry wts.

In experiments designed to produce larger quantities of (II), samples of (III) (120 mg) were incorporated into the glucose–peptone medium and this then inoculated and incubated for 7 days. Yields of 46–56% were recorded under these conditions.

Also isolated from these incubations was a second, less polar compound, identified by its IR and NMR spectra, and the IR, NMR, GLC and MS of its diacetate as 6β,11α-dihydroxypregn-4-ene-3,20-dione. This was present in only trace amounts (< 3%), but was shown to arise from (III) and not to be an artifact of the organism. The microbial transformation of (III) to the above dione is, to the knowledge of the authors, a hitherto unreported transformation.

EXPERIMENTAL

6β,11α,22-Trihydroxy-23,24-bisnorchol-4-en-3-one (II). Each of the media (Table 1) (250 ml) were dispersed into 1 l. flasks and sterilized by autoclaving at 103 kN/m² for 15 min. 23,24-Bisnorchol-4-en-3-one-22-ol (III) (50 mg) in acetone (3 ml) was added to each medium before autoclaving; preliminary experiments had shown the steroid to be stable under the autoclaving conditions. The medium was now inoculated with a loopful of spores and mycelia from a 4-day-old culture of *Rhizopus arrhizus* grown on malt extract agar (Oxoid). The culture of *R. arrhizus* was maintained on malt extract agar slopes at 4°. The inoculated solutions were incubated at 25° on an orbital incubator (Gallenkamp, 150 rpm) for 7 days. Each solution was then filtered through tared glass-fibre paper (Whatman GF/C) and the mycelial mat washed 2 × with acetone (10 ml). The mycelial dry wt was determined by drying, at 80°, the mycelial mat and filter paper to constant wt. Each filtrate was extracted with $CHCl_3$–MeOH (5:1, 3 × 300 ml). The organic extracts were evaporated to dryness under reduced pressure and each residue examined by GLC. The triol (II) was isolated in each case by TLC ($CHCl_3$–Me_2CO, 2:1, two developments) and yields based on the amount of TLC-pure material isolated in each case (purity confirmed by GLC). Crystallization from acetone–CCl_4 gave analytically-pure (II), m.p. 237–239° (lit.[1] m.p. 238–240°). (Found: C, 72·7; H, 9·4. Calcd. for $C_{22}H_{34}O_4$: C, 72·9; H, 9·4%), ν_{max} (KBr) 3410–3280, 1663, 1613 cm^{-1}, τ(CD_3OD) 9·17 (*s*, 3H, C_{18}Me), 8·85 (*d*, *J* 6 Hz, 3H, C_{21}Me), 8·49 (*s*, 3H, C_{19}Me), 6·42 (*m*, 2H, C_{22}–H_2), 5·96 (*m*, 1H, 11β–H), 5·74 (*m*, 1H, 6α–H), 4·22 (*s*, 1H, C_4–H).

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