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MICROBIAL HYDROXYLATION OF STEROIDAL $\Delta^{3,5}$ ENOL ACETATES (1)

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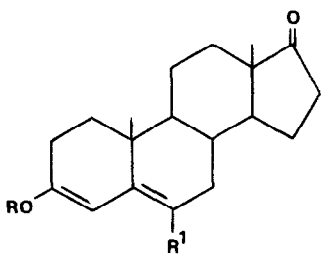
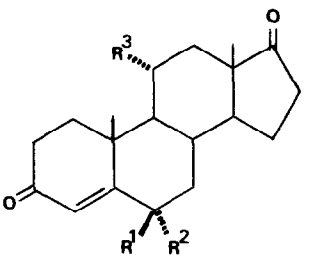
It has been proposed (2) that microbial C-6β hydroxylation of Δ^4-3-ketosteroids involves intermediacy of the $\Delta^{3,5}$ enol; we report here results of incubations of $\Delta^{3,5}$ enols (1a-1c) with Rhizopus arrhizus ATCC 11145, which support this hypothesis.



1a; R = Ac, R^1 = H
b; R = Et, R^1 = H
c; R = Ac, R^1 = Me



2a; $R^1 = R^2 = R^3$ = H
b; R^1 = OH, $R^2 = R^3$ = H
c; $R^1 = R^2$ = H, R^3 = OH
d; $R^1 = R^3$ = H, R^2 = Me
e; R^1 =H, R^2 = Me, R^3 = OH
f;, R^1 = OH, R^2 = Me, R^3 = H
g; R^1 = Me, $R^2 = R^3$ = H
h; R^1 = Me, R^2 = H, R^3 = OH

R. arrhizus converts androst-4-ene-3,17-dione (2a) into 6β-hydroxyandrost-4-ene-3,17-dione (2b)(3): the corresponding $\Delta^{3,5}$ enol acetate 1a gave , upon incubation with R. arrhizus, 2a (40%), 11α-hydroxyandrost-4-ene-3,17-dione (2c, 5%), and 2b (20%). Incubation of 1a with autoclaved mycelium results in a quantitative recovery of starting material. Co-incubation of equimolar amounts of 1a and 16-d-androst-4-ene-3,17-dione (50% d), Table 1, gave 6β-hydroxy-Δ^4-3-ketone whose deuterium content (18% d) differed substantially from the value of ≥ 25% predicted if the pathway 1a→2a→2b were mandatory.

Control experiments confirm that 16-d-androst-4-ene-3,17-dione is hydroxylated at C-6β

without loss of label. Assuming that factors such as differential solubility and transport of the substrates are small, this suggests that 1a is hydroxylated at C-6β without prior conversion to 2a, and that this hydroxylation is faster than that of 2a. The enol ether 1b is recovered unchanged from incubation with R. arrhizus: C-6β hydroxylation is therefore dependent on hydrolysis at C-3, but the order in which oxidation and hydrolysis occur cannot be inferred from the available data.

TABLE 1

Starting Material		% d Products	
Substrate	% d Ketone	Δ^4-3-Ketone	6β-hydroxy-Δ^4-3-ketone
16-d-2a	50	50*	50
16-d-2a + 1a	50	38	18

* Recovered substrate

Further evidence for the intermediacy of the $\Delta^{3,5}$ enol was obtained by incubation of the 6-methyl-$\Delta^{3,5}$ enol acetate 1c; 6α-methylandrost-4-ene-3,17-dione (2d, 40%), 11α-hydroxy-6α-methylandrost-4-ene-3,17-dione (2e, 16%), and 6β-hydroxy-6α-methylandrost-4-ene-3,17-dione (2f, 14%) were obtained. Incubation of 2d or 6β-methylandrost-4-ene-3,17-dione (2g) gave only the corresponding C-11α hydroxylated products 2e (17%) and 2h (19%) respectively. The ability of R. arrhizus to hydroxylate 1c but neither 2d nor 2g at C-6β may be interpreted as follows: C-6β hydroxylation occurs via the $\Delta^{3,5}$ enol, and the fungus is able to hydrolyse and oxidise 1c to 2f, but lacks the ability to enolise 2d or 2g.

Satisfactory spectral and analytical data were obtained for all compounds.

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