

SHORT COMMUNICATION

PROGESTERONE SIDE-CHAIN DEGRADATION BESIDE HYDROXYLATION WITH *RHIZOPUS NIGRICANS* DEPENDS ON THE PRESENCE OF NUTRIENTS

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Summary—In the absence of nutrients, *Rhizopus nigricans* transforms progesterone into a mixture of 11 α -hydroxy-4-androstene-3,17-dione and 11 α -hydroxy-1,4-androstadiene-3,17-dione. The same mixture is obtained by the transformation of testosterone and its acetate.

The fungus *Rhizopus nigricans* is now widely accepted [1] as an 11 α -hydroxylator of progesterone (1) though 6 β , 7 β , 17 α - and 21-hydroxylated products have also been found among products of its action on progesterone [2-4]. However, its ability to degrade the side chain of the pregnane skeleton of progesterone leading to a 17-oxo androstane derivative or dehydrogenate 3-oxo steroids in Δ^1 -position has not been described.

We now report on this degradation which under certain conditions accompanies the usual 11 α -hydroxylation.

When progesterone (30 mg in 0.5 ml of DMSO) was incubated with *Rhizopus nigricans* in 100 ml of culture medium (0.5 g of corn-steep liquor, 1.1 g of glucose, 0.04 g of dibasic potassium phosphate, 0.04 g of magnesium sulfate heptahydrate) for 48 h at 25°C in an orbital shaker, we obtained 11 α -hydroxyprogesterone (2) along with 6 β ,11 α -dihydroxyprogesterone (3) (see Table 1, entry 1). When, however, the fungus was grown in the absence of progesterone as substrate or initiator, and after 2 days the culture medium was substituted for phosphate buffer only (pH 7.6) with progesterone, it transformed the substrate (entry 2) into 11 α -hydroxyandrost-4-ene-3,17-dione (4) and 11 α -hydroxyandrost-1,4-diene-3,17-dione (5). The structures of the products were deduced from their respective IR spectra and were consistent with the MS, [¹H] and [¹³C]NMR spectra. All parameters including melting points and specific rotations of the products were in good accord with data published in the literature [5-7].

The mechanism of the degradation might formally involve the Baeyer-Villiger oxidation of the 20-ketone to a 17 β -acetoxy derivative, followed by its hydrolysis to a 17 β -alcohol and oxidation to the ketone (4). Each of these reactions has already been reported [1, 8] in microbial transformation of steroids. Equally well the formation of the 17-ketones (4) and (5) might be explained by 17 α -hydroxylation of the 20-ketone followed by the cleavage of the C₍₁₇₎-C₍₂₀₎ bond.

In order to decide between these two alternatives we carried out the following experiments: compounds (6) to (8) were added to the fungus in the culture medium; we found that the first two substrates were converted to the 17-ketones (4) and (5), while the transformation of the 17 α -hydroxy 20-ketone (8) gave rise to products of 11 α -hydroxylation only (9). These results appear to indicate that the former mechanism or one that is analogous operated in the system.

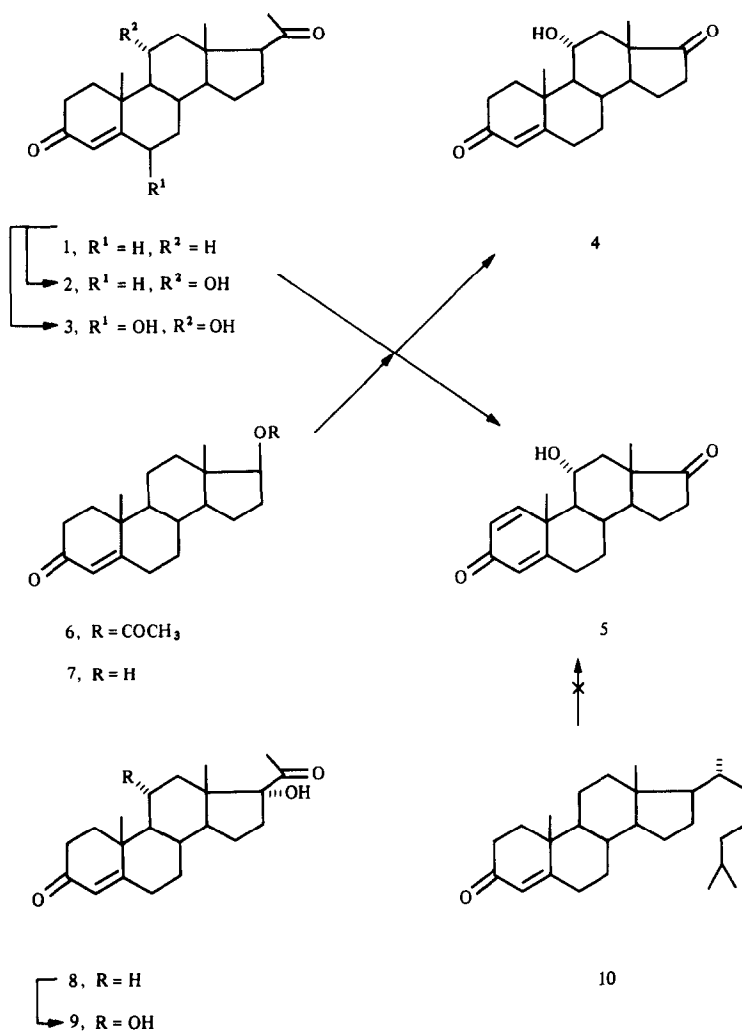
We also became interested in the likely cause of the difference observed in the transformation course: was it due to the different age of the culture used or the different concentration of nutrient at the stage of steroid transformation? Additional experiments (see entries 3 and 4) disclosed that the side chain degradation was typical of the "hungry" fungus only. Apparently a well nourished fungus hydroxylates the substrate in order to get rid of a lipophilic compound which presents itself as a ballast but a "hungry" fungus uses it also as food, a source of carbon for organism growth. A compound with the cholestane side chain (10) might be considered an even richer food but in spite of that it was not utilized: its very low solubility in the medium may have made it unavailable for a biochemical reaction.

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Table 1. Yields (%) of transformation products on the treatment of progesterone with *Rhizopus nigricans*

Entry	Age of the fungus (days)	Medium	Hydroxylation to		Oxidation of the side chain	Dehydrogenation to $\Delta^{(2)}$
			6 β -OH	11 α -OH		
1	0-2	N ^a	5	73	0	0
2	2-4	B ^b	0	50	43	22
3	2-4	N ^c	0	75	0	0
4	2-4	N ^d	0	71	0	0

N^a (nutrient): corn-steep (0.5 g), glucose (1.1 g), dibasic potassium phosphate (0.04 g), magnesium sulfate heptahydrate (0.04 g) in water (100 ml); B^b (buffer): Sørensen, pH 7.6; N^c the original medium was used in which the fungus was grown, 9% of progesterone was recovered unchanged; N^d the original medium was replaced by a fresh one, 6% of progesterone was recovered.



Scheme 1

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