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Biotransformation of corticosteroids by *Penicillium decumbens* ATCC 10436

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The biotransformation of a series of corticosteroids by the fungus Penicillium decumbens *ATCC 10436 has been investigated. Conversion to the corresponding 5α-dihydrosteroid was observed for all the Δ^4-3-ketosteroids studied with the exception of deoxycorticosterone, which was converted to a $\Delta^{1,4}$-diene. Deoxycorticosterone acetate was, however, converted to a 5α-dihydro product concomitant with ester hydrolysis. Other substrates carrying a C-21 acetoxy group were also hydrolyzed to the alcohol. In two cases (resulting from deoxycorticosterone acetate and 11-deoxycortisone) the 5α-3-keto product was further reduced to the 3β-alcohol. No reduction of $\Delta^{1,4}$-dienes was observed. (Steroids* **60**:646–649, 1995)

Keywords: biotransformation; bioconversion; *Penicillium decumbens;* corticosteroid

Introduction

Biotransformations by the fungus *Penicillium decumbens* have been employed in the steroid area for the reduction of double bonds, particularly the conversion of Δ^4-3-ketosteroids to 5α-3-ones,[1,2] and in other areas for the asymmetric reduction of conjugated ketones.[3] The first report of the use of this microorganism for steroid biotransformation dealt with progesterone and androst-4-ene-3,17-dione as substrates.[2] More recently, we have determined that the reduction of testosterone and androst-4-ene-3,17-dione to the corresponding 5α-3-ketosteroids by *P. decumbens* proceeds with *trans* addition of the elements of hydrogen at C-4 and C-5[4], and have systematically examined the ability of this fungus to reduce the olefinic bond of a series of androst-4-en-3-ones and pregn-4-en-3-ones, with a view to defining the substrate specificity of the 5α-reductase enzyme(s) concerned.[1]

The mammalian steroid 5α-reductase enzymes, which carry out the conversion of Δ^4-3-ketosteroids to 5α-3-ketosteroids, occupy a central role in steroid metabolism,[5] and their cofactor requirements, kinetics and inhibition have been thoroughly examined (see reference 1 for extensive citations). In view of the fact that *P. decumbens* is one of the few microorganisms known to perform this biotransformation (the others being, in low yield, *Nocardia corallina*,[6,7] *Rhizopus nigricans*,[8] and the pathogenic *Aspergillus fumigatus*[9]) we have extended our investigations of Δ^4-3-ketosteroid biotransformation by *P. decumbens* to include a series of corticosteroid substrates with the dual goals of (a) examining their biotransformation from a preparative standpoint; and (b) facilitating a role for the 5α-reductase of *P. decumbens* in future mechanistic studies of the 5α-reductase–catalyzed reaction.

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Experimental

Apparatus, materials, and methods

Melting points were determined on a Kofler heating stage. Infrared spectra were recorded with a Bomem FTIR spectrometer. The NMR spectra were recorded at 200 mHz (^{1}H) or 50 mHz (^{13}C) with a Bruker AC200 spectrometer using $CDCl_3$ as solvent and $CHCl_3$ as internal standard. Mass spectra were obtained with a Kratos 1S instrument operating in EI mode. Thin-layer chromatography was performed on Merck silica gel 60F-254 and flash column chromatography used silica gel, 230–400 mesh, eluting with benzene-ether in a 10% stepwise gradient followed by ether-methanol in a 5% stepwise gradient.

Maintenance and growth of Penicillium decumbens *ATCC 10436*

This microorganism was maintained and grown as described in our earlier paper.[1]

Biotransformations and product isolation and analysis

The substrates used in this study were commercial samples from Sigma (St. Louis, MO, USA). Biotransformations listed as "nor-

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mal" in the table were performed as described[1]: *P. decumbens* was grown in sterilized potato dextrose broth (Difco, Toronto, Canada; 200 mL in a 1 L Erlenmeyer flask) at 24–26°C, shaken at 180 rpm. Substrate (75 mg) in 95% ethanol (2 mL) was added after 24 h growth, and the incubation allowed to proceed for a further 4 days. Those listed as "replacement" were carried out in the following manner: a 3 L culture of *P. decumbens* distributed in 200 mL aliquots in 15 1-L Erlenmeyer flasks was grown for a period of 5 days on a rotary shaker at 180 rpm, 24–26°C. The fungal material was then harvested by centrifugation and redistributed in 3 L of distilled water in 15 1-L Erlenmeyer flasks as before. Substrate (1 g) in 95% ethanol (30 mL) was added at the rate of 2 mL per flask, and the flasks returned to the shaker, 180 rpm, 24–26°C, for a period of 48 h. Subsequent extractions of product and chromatography was performed as previously described.[1] In all cases products were identified by chromatographic (TLC) and spectral (NMR and MS) comparison with authentic samples. The mass balance of the substrate was accounted for as unchanged starting material.

Results

The results of the biotransformation by *P. decumbens* of substrates **2** to **15** (Figure 1) used in this study are presented in Table 1, which also includes, for comparative purposes, the results of the biotransformation of progesterone (**1**). The products were all identified by comparison of their physical, chromatographic, and spectral data with those obtained from authentic commercial samples. The yields listed in the table refer to combinations of homogenous chromatographic fractions prior to crystallization.

Discussion

Although progesterone (**1**) is efficiently converted to the 5α-dihydrosteroid **16** by *P. decumbens*,[1] deoxycorticosterone (**2**) was not transformed under the usual biotransforma-

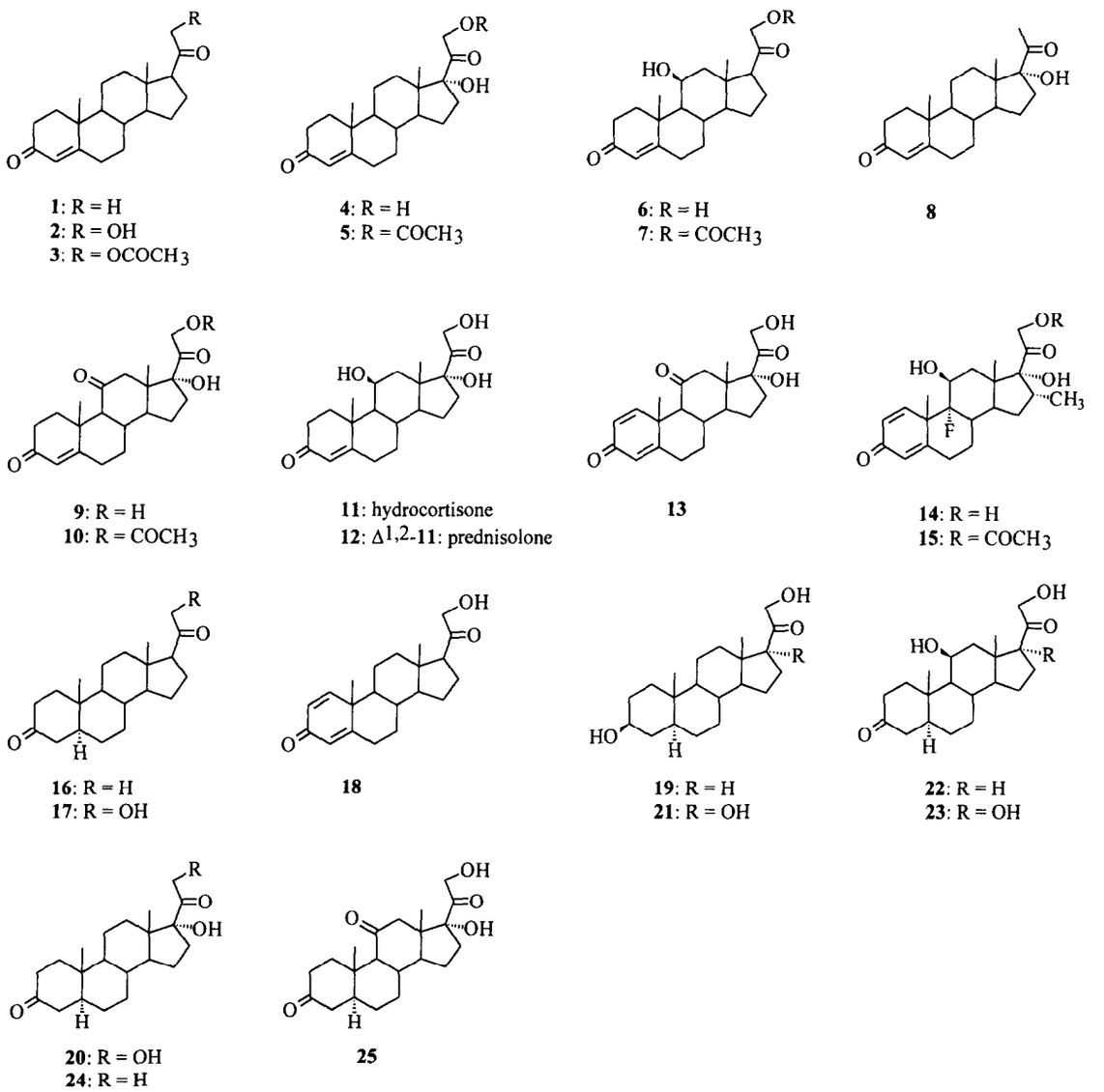

Figure 1 Substrates and products of their biotransformation by *P. decumbens*.

Table 1 Biotransformation of corticosteroids by *Penicillium decumbens*

Substrate	Biotransformation conditions	Product	% yield
1 (Progesterone)	Normal	**16**	45
	Replacement	**16**	10
2 (Deoxycorticosterone)	Normal	None	
	Replacement	**18**	42
3 (Deoxycorticosterone acetate)	Normal	**17**	44
		19	4
4 (11-Deoxycortisone)	Normal	**20**	34
		21	10
5 (11-Deoxycortisone acetate)	Normal	**20**	8
6 (Corticosterone)	Normal	**22**	68
7 (Corticosterone acetate)	Normal	**22**	40
		6	12
8 (17-Hydroxyprogesterone)	Normal	**24**	30
9 (Cortisone)	Normal	**25**	40
10 (Cortisone acetate)	Normal	None	
11 (Hydrocortisone)	Normal	**23**	62
12 (Prednisolone)	Normal	None	
13 (Prednisone)	Normal	None	
14 (Dexamethasone)	Normal	None	
15 (Dexamethasone acetate)	Normal	**14**	5

tion conditions, the bulk of the substrate being recovered unchanged. In light of this unpromising result, we examined the biotransformation of both **1** and **2** using pre-grown *P. decumbens*. Although progesterone still gave rise to the reduction product **16** (albeit in much reduced yield), deoxycorticosterone was converted in moderate yield to **18,** the product of C-1(2) dehydrogenation, a mode of biotransformation which has not been previously reported for *P. decumbens*. This change in the dominant mode of biotransformation of a steroid with the maturity of a culture is not commonly reported, but has been observed previously with *Mucor griseocyanus*, where 7α-hydroxylation becomes dominant over the more usual 14α-hydroxylation as the age of the culture increases, and *Bacillus cereus*, where reduction of a C-20 carbonyl to the 20β-alcohol is observed only during the initial stages of growth of the organism.[10]

The failure of *P. decumbens* to reduce deoxycorticosterone may be due to the inability of this substrate to induce the necessary 5α-reductase enzyme. To examine this possibility, we grew *P. decumbens* for 24 h in the presence of a small amount (10 mg per L) of progesterone as an inducer, followed by the addition of deoxycorticosterone as usual, but again no conversion of **2** was observed.

In contrast to these observations, however, the use of deoxycorticosterone acetate (**3**) as substrate gave rise to the formation of the 21-hydroxy-5α-3-ketosteroid **17** in 44% yield, together with a lesser amount of the corresponding product **19** in which the C-3 carbonyl group had been reduced to the 3β-alcohol. These results taken together indicate that the acetate **3** is an acceptable substrate for 5α-reduction, even though the corresponding alcohol **2** is not, and therefore suggest that reduction of the C-4(5) olefinic bond precedes hydrolysis of the C-21 acetoxy group. At the present time we have no explanation of the apparent failure of *P. decumbens* to metabolize **2** under normal biotransformation conditions, other than to suggest that, perhaps for reasons of permeability, this substrate is not accessible to the appropriate intracellular enzymes.

The presence of the C-21 hydroxyl group is not a general barrier to successful 5α-reduction by *P. decumbens*, however, as evidenced by the successful reduction of 11-deoxycortisone (**4**) to **20,** corticosterone (**6**) to **22,** hydrocortisone (**11**) to **23,** and cortisone (**9**) to **25.** Furthermore, the corresponding 21-acetates **5** and **7** also gave rise to 5α-reduction products concomitant with hydrolysis to the 21-alcohol, although the yields were somewhat lower than those obtained by the use of the hydroxysteroid as substrate. Reduction was also observed for 17α-hydroxyprogesterone (**8**), which gave the corresponding 5α-3-ketosteroid **24** in 30% isolated yield.

In contrast to the earlier report of the reduction of $\Delta^{1,4}$-dien-3-ones to saturated 5α-3-keto products by *P. decumbens*,[2] no reduction of the substrates **12** to **15** was observed: dexamethasone acetate (**15**) was converted to the corresponding alcohol **14** only in low yield.

These results are consistent with our earlier observation[1] that Δ^1-testosterone gave only Δ^1-androstenedione on incubation with *P. decumbens* and suggest that, at least under our experimental conditions, this organism does not reduce the olefinic bonds of $\Delta^{1,4}$-dien-3-ones. The preparative value of *P. decumbens* for the production of 5α-3-ketosteroids from the corticosteroids used in this study has therefore been established—in particular, the reductions of corticosterone (**6**) and hydrocortisone (**11**) in isolated yields of 68% and 62%, respectively, represent efficient methods for the preparation of the corresponding 5α-steroids 5α-pregnane-11β,21-diol-3,20-dione (**22**) and hydrallostane (**23**).

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