

Stereochemistry of Reduction by the 5 α -Reductase Enzyme of *Penicillium decumbens* and the ^1H NMR Assignment of 5 α -Dihydrotestosterone

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Reduction of the alkenic bond of testosterone and androst-4-ene-3,17-dione by the 5 α -reductase enzyme of *Penicillium decumbens* proceeds with *trans* stereochemistry.

Although testosterone (**1**) is one of the major end products of steroid biosynthesis in the human male, a more potent natural androgenic hormone is 5 α -dihydrotestosterone (**3**), the product of reduction of (**1**) by the testosterone-NADPH oxidoreductase enzyme known as 5 α -reductase.¹ As the continued functioning of this enzyme, and consequent high levels of (**3**), are implicated in the proliferation of prostate tumour cells,² much effort has been expended in the search for specific 5 α -reductase inhibitors.^{3,4}

In spite of the attention which has been focused on this enzyme, however, several basic features of its mode of action necessary for the rational design of a mechanism based inhibitor remain obscure; notable among these is the stereochemistry of the reduction process.

We have studied the latter aspect of the 5 α -reductase enzyme using as a model for the mammalian enzyme the 5 α -reductase of *Penicillium decumbens* NRRL 742.⁵ In a

whole cell biotransformation mode this organism reduces testosterone (**1**) to the 5 α -dihydrosteroids (**3**) and (**4**) in yields of 10 and 40%, respectively. Control experiments have shown that 5 α -androstane-3,17-dione (**4**) can be produced from the corresponding alcohol (**3**) in a subsequent reaction separate from that catalysed by the 5 α -reductase enzyme. Androst-4-ene-3,17-dione (**2**) is reduced to give only (**4**) in isolated yields of 50–70%.

The stereochemistry of reduction was determined by 500 MHz ^1H NMR analysis of the products resulting from the deuterium labelled substrates (**5**) and (**6**), prepared by base catalysed exchange of (**1**) and (**2**), respectively, with deuterium oxide.⁶ No loss or scrambling of label (as detectable by mass spectral or NMR analysis) occurs at either the substrate or the product stage during the 5 α -reductase catalysed reaction.

The NMR analysis described is dependent upon the unambiguous assignment of the signals due to H-4 α and H-4 β in 5 α -dihydro steroids, and to this end we have assigned the 500 MHz ^1H NMR spectrum of unlabelled substrate (**3**), in addition to that of (**8**), the product obtained by biotransformation of (**5**) by *P. decumbens* (Figure 1a). The results (Table 1) were obtained by an analysis of the NOE difference spectra,

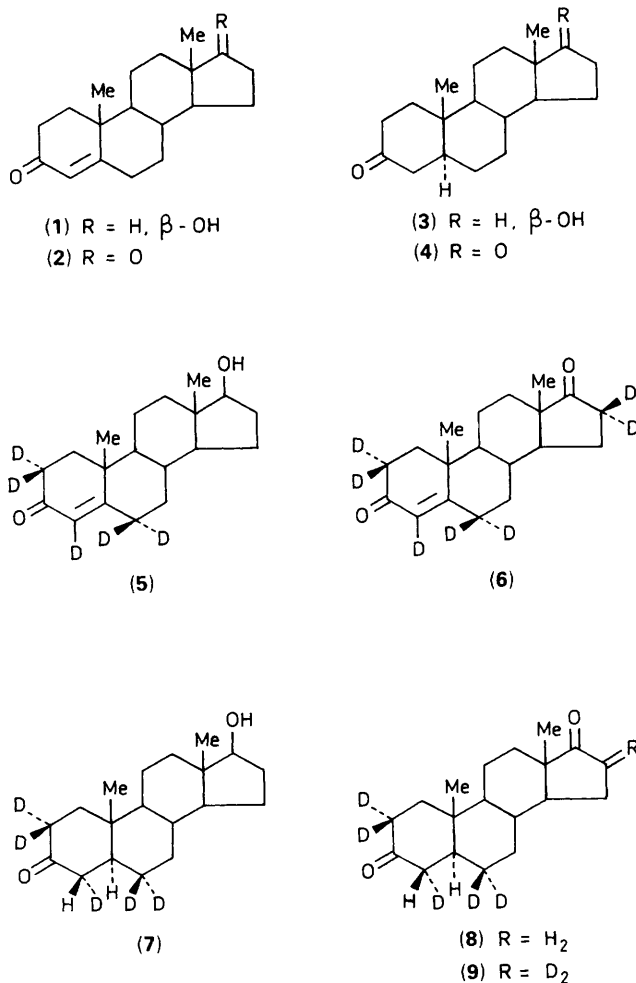


Table 1. ^1H NMR chemical shifts of 5 α -dihydrotestosterone (**3**) and (**8**).^a

Proton	(3)	(8)
1 α	1.316	1.327
1 β	1.991	1.991
2 α	2.266	
2 β	2.350	
4 α	2.052	
4 β	2.232	2.234
5 α	1.483	1.522
6 α	1.306	
6 β	1.281	
7 α	0.857	0.978
7 β	1.677	1.806
8 β	1.413	1.580
9 α	0.703	0.776
11 α	1.575	1.675
11 β	1.347	1.388
12 α	1.041	1.244
12 β	1.790	1.805
14 α	0.936	1.268
15 α	1.560	1.922
15 β	1.233	1.499
16 α	2.021	2.056
16 β	1.417	2.425
17 α	3.605	
18	0.728	0.865
19	0.989	1.016

^a In CDCl₃ relative to CHCl₃ at δ 7.240.

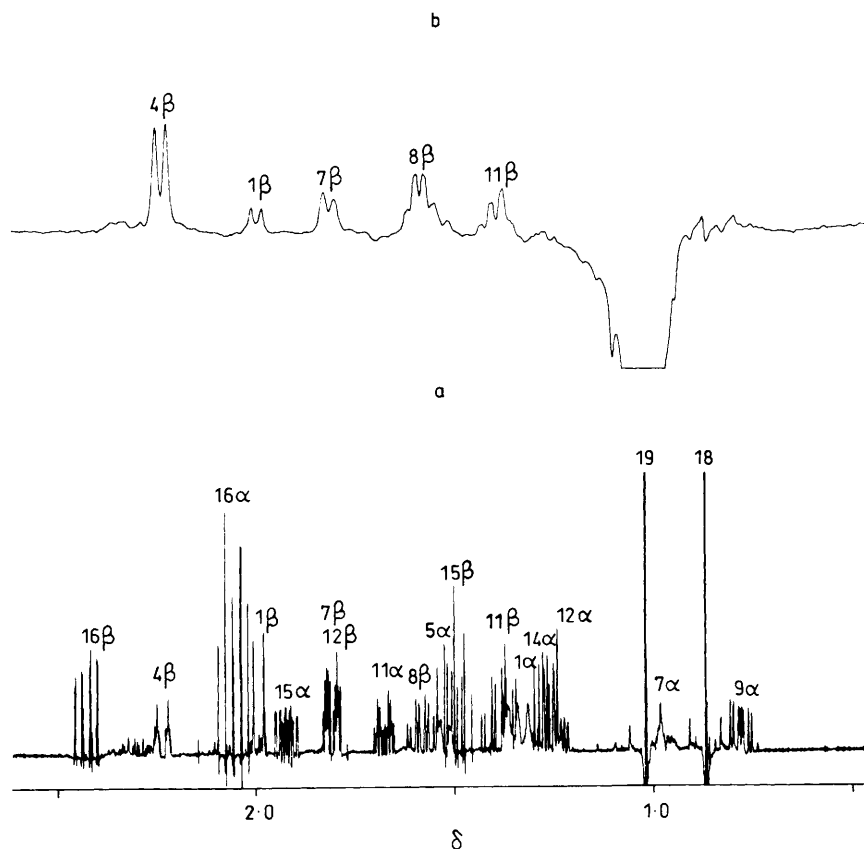


Figure 1. (a) 500 MHz ^1H NMR spectrum of (8) recorded in CDCl_3 . (b) NOE difference spectrum obtained by saturation of the C-19 methyl resonance.

double-quantum filtered phase sensitive COSY 2-D spectra, and ^{13}C - ^1H 2-D shift correlation spectra of both (3) and (8). These assignments are consistent with the few ^1H NMR data which are currently available for ring A saturated steroids⁷ and, for rings C and D, with other published androstane assignments.^{8,9}

The data in Table 1 show clearly that addition of hydrogen to the 4(5) π bond has occurred in a *trans* manner at positions 4 β and 5 α . Using the assignments of Table 1, the ^1H NMR spectra of the other products of enzymic 5 α reduction [(7) from (5) and (9)] also show clearly the presence of hydrogen at C-4 β and its absence at C-4 α , confirming that reduction has occurred with *trans* stereochemistry. Figure 1b shows the NOE difference spectrum of (8) obtained by irradiation of the C-19 methyl hydrogens, in which the signal at δ 2.234, assigned to the C-4 β hydrogen, is clearly enhanced. Additional evidence for the β stereochemistry of the C-4 hydrogen was the observation of a 14.0 Hz vicinal coupling with H-5 α , which must result from a *trans* di-axial orientation of these two hydrogens.

We conclude that the 5 α -reductase enzyme of *P. decumbens* functions with *trans* addition to the double bond.

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