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

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

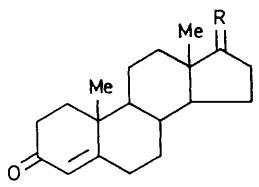
Although testosterone (**1**) is one of the major end products of steroid biosynthesis in the human male, a more potent natural androgenic hormone is 5α-dihydrotestosterone (**3**), the product of reduction of (**1**) by the testosterone–NADPH oxidoreductase enzyme known as 5α-reductase.[1] As the continued 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 5α-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.

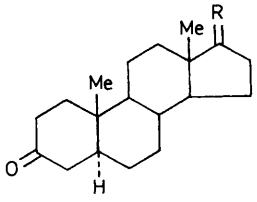
We have studied the latter aspect of the 5α-reductase enzyme using as a model for the mammalian enzyme the 5α-reductase of *Penicillium decumbens* NRRL 742.[5] In a whole cell biotransformation mode this organism reduces testosterone (**1**) to the 5α-dihydrosteroids (**3**) and (**4**) in yields of 10 and 40%, respectively. Control experiments have shown that 5α-androstane-3,17-dione (**4**) can be produced from the corresponding alcohol (**3**) in a subsequent reaction separate from that catalysed by the 5α-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 ^{1}H 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 5α-reductase catalysed reaction.

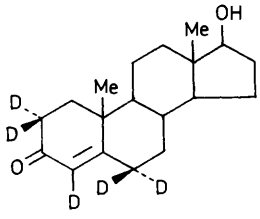
The NMR analysis described is dependent upon the unambiguous assignment of the signals due to H-4α and H-4β in 5α-dihydro steroids, and to this end we have assigned the 500 MHz ^{1}H NMR spectrum of unlabelled substrate (**3**), in addition to that of (**8**), the product obtained by biotransformation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



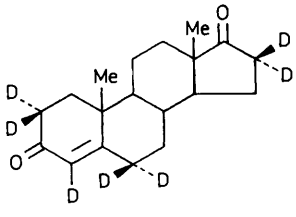
(**1**) R = H, β-OH
(**2**) R = O



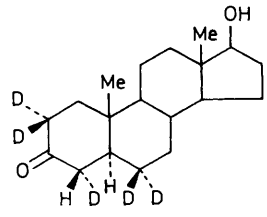
(**3**) R = H, β-OH
(**4**) R = O



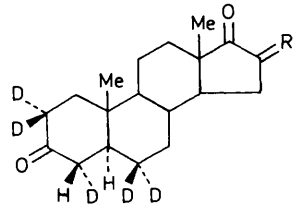
(**5**)



(**6**)



(**7**)



(**8**) R = H_2
(**9**) R = D_2

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

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

[a] In $CDCl_3$ relative to $CHCl_3$ at δ 7.240.

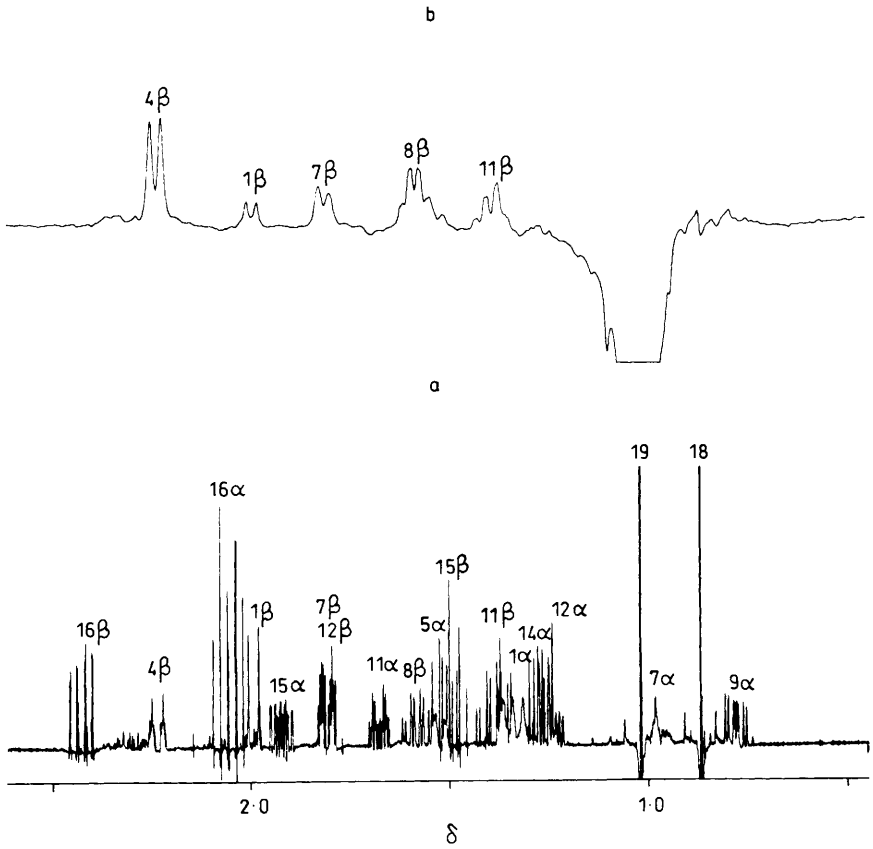


Figure 1. (a) 500 MHz ^{1}H NMR spectrum of (**8**) recorded in $CDCl_3$. (b) NOE difference spectrum obtained by saturation of the C-19 methyl resonance.

double-quantum filtered phase sensitive COSY 2-D spectra, and ^{13}C–^{1}H 2-D shift correlation spectra of both (**3**) and (**8**). These assignments are consistent with the few ^{1}H NMR data which are currently available for ring *A* saturated steroids[7] 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 4β and 5α. Using the assignments of Table 1, the ^{1}H NMR spectra of the other products of enzymic 5α reduction [(**7**) from (**5**) and (**9**)] also show clearly the presence of hydrogen at C-4β and its absence at C-4α, 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-4β 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-5α, which must result from a *trans* di-axial orientation of these two hydrogens.

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

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