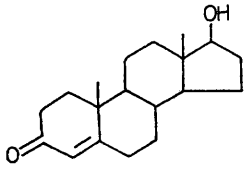
Direct Determination of the Stereospecificity of the Δ^1-Dehydrogenation of Testosterone using Tritium Nuclear Magnetic Resonance

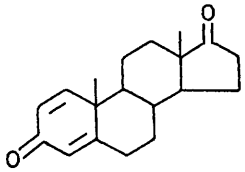
By JASMI M. A. AL-RAWI, JOHN A. ELVIDGE, ROBERT THOMAS,* and BRIAN J. WRIGHT
(*Department of Chemistry, University of Surrey, Guildford, Surrey* GU2 5XH)

Summary ^{3}H-N.m.r. spectroscopic studies of the steric course of the Δ^1-dehydrogenation of testosterone show a highly stereospecific microbial 1α,2β-*trans* elimination, in contrast to the corresponding chemical transformation by DDQ, in which both *cis*- and *trans*-elimination processes occur.

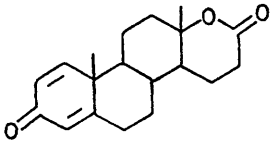
TRITIUM-n.m.r. spectroscopy has recently been applied to the direct determination of the labelling pattern of the mould metabolite penicillic acid, derived from [^{3}H]acetate.[1] In addition to its utility as a biosynthetic probe, this technique also has considerable potential for examining the biotransformation of foreign substrates, as described herein.



(I)



(III)



(II)

Conventional isotopic approaches to the determination of the stereochemistry of the Δ^1-dehydrogenation of 3-keto-steroids by bacteria have demonstrated the removal of the 1α-hydrogen atom, together with the concomitant loss of the 2β-hydrogen, consistent with a *trans*-diaxial elimination mechanism.[2] An analogous fungal Δ^1-dehydrogenation process is also known, whereby *Cyclindrocarpon radicicola* can convert testosterone (I) into Δ^1-dehydrotestololactone (II) *via* androsta-1,4-diene-3,17-dione (III),[3] but the steric course of the fungal C-1,2 elimination has not been investigated.

We have used the ^{3}H-n.m.r. technique to examine this system. Commercially available [1,2-^{3}H]testosterone (0·25 mg; 45 mCi) containing predominantly the two *cis*-labelled species (characterised by ^{3}H-n.m.r.),[4] namely 1α,2α and 1β,2β, in the ratio of *ca.* 5:1, was diluted with unlabelled testosterone (25 mg) in acetone (1 ml) and incubated (24 h at 25°) with a shake culture of *C. radicicola* (ATCC strain 1011), previously grown for 48 h on the prescribed medium[3] (50 ml). $CHCl_3$ extraction of the culture filtrate followed by preparative t.l.c. on silica ($CHCl_3$–Me_2CO 9:1) yielded (III)† (23 mg; 14·5 mCi). The ^{3}H n.m.r. spectrum showed that the ^{3}H signals at C-1 and C-2 had an intensity ratio of 1:5, thus directly demonstrating a 1α,2β-*trans* elimination pathway, as previously reported for analogous dehydrogenations of 3-keto-steroids by the bacterium *Bacillus sphaericus*.[2]

In a parallel experiment using the bacterium *Pseudomomas testosteroni* (ATCC strain 11,996) grown on a yeast medium[5] (24 h at 30°), the conversion (I) → (III) was effected in 45% yield in 8·5 h. Again, a 1α,2β-*trans* elimination was observed.

By contrast with these Δ^1-biodehydrogenation systems, the corresponding selective dehydrogenation with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) has been reported to be less specific,[6] involving some attack at the 1β-position. We have been able to confirm this finding using DDQ to convert [1α,2α-^{3}H]androst-4-ene-3,17-dione (29 mg; 26·5 mCi) (recovered as a by-product of the above *P. testosteroni* experiment) into (III) (12·6 mg; 4·2 mCi). The ^{3}H n.m.r. spectrum contained a major signal due to ^{3}H on C-2 of (III), consistent with a predominant 1α,2β-*trans* elimination, but in contrast to the spectra obtained from the biodehydrogenation experiments, the broad-band proton-decoupled spectrum exhibited tritium–tritium spin coupled doublets of moderate intensity, due to ^{3}H atoms on C-1 and C-2. These can only have arisen as a consequence of a 1β,2β-*cis* elimination. A corresponding experiment with [1β,2β-^{3}H]-testosterone, yielded 17β-hydroxyandrosta-1,4-dien-3-one in which the major signal was due to ^{3}H on C-1, as required by the 1α,2β-*trans* elimination. As before, proton decoupling gave a spectrum clearly showing doublets due to tritium–tritium coupling, thus indicating the simultaneous operation of a 1α,2α-*cis* elimination reaction.

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† Correct microanalytical and spectroscopic data were obtained for all products.

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