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CONTRIBUTION TO THE STERIC COURSE OF STEROID REDUCTION AND DEHYDROGENATION[1]

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EVIDENCE has been presented in previous publications[2,3] that both the dichlorodicyanoquinone[2] and Bacillus sphaericus[3] mediated C-1(2) dehydrogenation of 3-keto steroids proceed by a similar trans-diaxial (1α, 2β) elimination mechanism. However, since the stereochemistry of double bond introduction was determined in each case by the failure or ability of various C-1 or C-2 methyl- or hydroxyl-substituted steroids to undergo dehydrogenation, the possibility existed that steric interactions due to the additional bulky substituents might have led to results and conclusions not justified for the unsubstituted steroid case. It was therefore desirable to investigate the dehydrogenation of an isotopically labeled substrate of known configuration.

Δ^1-Androstene-3,17-dione in dioxane solution was catalytically deuterated at 25° and one atmosphere pressure over 5 per cent palladium-carbon. Re-oxidation with chromic acid followed by several equilibrations with methanolic potassium hydroxide gave 1α-deuterioandrostane-3,17-dione (I), m.p. 133°, $\lambda_{max}^{CHCl_3}$ 2155 cm^{-1} (C-D stretch). (Found: 3.55. Calc. % excess

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[2] H.J. Ringold and A. Turner, Chem. & Ind. 211 (1962).

[3] M. Hayano, H.J. Ringold, V. Stefanovic, M. Gut and R.I. Dorfman, Biochem. Biophys. Res. Comm. 4, 455 (1961).

D, 3.57.)[4] Mass spectrometric[5] analysis demonstrated that I consisted of greater than 99 per cent of a mono-deuterated species. Although it was anticipated that reduction would proceed from the unhindered back side yielding, after equilibration, the 1α-deutero isomer, further proof of configuration was desirable. Bromination of I in acetic acid solution in the presence of sodium acetate gave 1α-deuterio-2α-bromoandrostane-3,17-dione (II), m.p. 211° (dec.), $\lambda_{max}^{CHCl_3}$ 1730 cm^{-1} (17-ketone and 2α-bromo-3-ketone), U.V. $\lambda_{max}^{CH_2Cl_2}$ 291 mμ, ε 100. (Found: Br, 22.0.) The nuclear magnetic resonance[6] spectrum of II exhibited for the 2β-proton a doublet centered at $\tau = 5.37$, $J = 6.7$ cps, consistent only for the coupling of a pair of vicinal axial-equatorial protons[7] and thus establishing that the 1-deuterium atom was predominantly although not necessarily exclusively 1α.

The incubation of I for 2 hr with a sonicated[8] preparation of Bacillus sphaericus (ATCC 7055) in the presence of menadione as electron acceptor gave an 80 per cent conversion to the known Δ^1-androstene-3,17-dione (III) which by mass spectrometric analysis had lost 93 per cent deuterium while the recovered I showed no deuterium loss. The dehydrogenation of I for 41 hr with a 24 hr whole cell culture of Bacillus sphaericus[9] resulted in

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4 Deuterium analysis by Mr. Josef Nemeth, Urbana, Illinois.

5 We are grateful to Dr. H. Budzikiewicz and Prof. Carl Djerassi, Stanford University, for the mass spectrometric analyses.

6 We wish to thank Mr. Tom Wittstruck and Dr. N. McNiven for the nuclear magnetic resonance determinations which were carried out in deutero-chloroform solution with tetramethylsilane as internal standard, utilizing a Varian 4302 60 Mc/s spectrometer.

7 The non-deuterated 2α-bromoandrostane-3,17-dione exhibited for the 2β-proton a quartet centered at $\tau = 5.24$, with a total spacing of 20 cps. The assignment of J_{ae} leads to a value of $J_{aa} = 13.3$ cps. This type of ABX system has been analyzed by K.L. Williamson and W.S. Johnson, J. Org. Chem. 26, 4563 (1961) and F.J. Schmitz and W.S. Johnson, Tetrahedron Letters 647 (1962). We are grateful to Prof. Johnson for a pre-publication copy of this latter manuscript which describes the nuclear magnetic resonance spectrum of 1α-deuterio-2α-bromocholestan-3-one.

8 Cf. H.R. Levy and P. Talalay, J. Biol. Chem. 234, 2014 (1959) for a description of the preparation of cell free bacterial dehydrogenases.

9 See ref. 3 for general procedure.

70 per cent formation of III which had also lost 93 per cent deuterium. In the latter case the recovered I exhibited a 75 per cent loss of deuterium establishing, incidentally, the reversibility of dehydrogenation in the whole cell.

Finally, dehydrogenation of I with dichlorodicyanoquinone in boiling dioxane interrupted short of completion (19 hr reflux) gave III with an identical loss of 93 per cent of the C-1 deuterium atom while the recovered I showed no deuterium loss.

Since both chemical and bacterial dehydrogenation of I led to the loss of 93 per cent deuterium from C-1, only two possibilities exist: (1) both processes are 93 per cent specific for loss of the 1α-hydrogen (deuterium) atom; (2) both processes are 100 per cent specific for 1α, but the original deuteration was not completely specific. It would be a highly unlikely coincidence for both the chemical and enzymatic dehydrogenation processes to exhibit precisely 93 per cent specificity, therefore it is most probable that the original reduction of Δ^1-androstene-3,17-dione gave 93 per cent 1α-deutero compound, 7 per cent 1β-deutero compound and that dehydrogenation proceeds stereospecifically by 1α-proton or -deuterium loss.