THE STEREOCHEMISTRY OF HYDROGEN ADDITION BY A Δ^1-STEROID REDUCTASE IN BAKER'S YEAST

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SUMMARY

The reduction of the Δ^1 double bond in 5α-androstan-1-en-3-one was investigated utilizing a reductase present in fermenting baker's yeast. The stereochemistry of the hydrogen addition was found to be trans diaxial, as determined by suitable deuterium labeling and lanthanide induced shift nmr spectroscopy.

INTRODUCTION

Both microorganisms and mammalian tissues possess Δ^1 steroid reductases which are active in the in vivo metabolism of Δ^1 steroids (1,2). Yet, despite the importance of this functional group in many steroid drugs, the mechanistic anatomy of this reduction has not been closely examined. In the present communication we wish to report on the stereochemistry of hydrogen addition during such a reduction by Saccharomyces cerevisiae, a reaction first observed by Butenandt and coworkers in 1940 (3). Our approach consisted of carrying out the reduction on steroidal substrates labeled with deuterium at positions 1 and 2, followed by an elucidation of the stereochemistry of the added hydrogen atoms by nmr spectroscopy, using the lanthanide induced shift technique (4).

MATERIALS AND METHODS

The substrates used in the present reductase experiments were 5α-androst-1-en-3-one-2,4,4-d_3 (5) and 5α-androst-1-en-3-one-1-d, the latter being preparted by a multistep synthesis which will be described elsewhere. The deuterium content of the products was monitored by mass spectrometry, using a Hitachi Perkin-Elmer RMU6E spectrometer; melting points are uncorrected and were determined on a Thomas-Hoover capillary apparatus.

Reduction of 5α-androst-1-en-3-one-2,4,4-d_3 — A solution of 400 mg of 5α-androst-

1-en-3-one-2,4,4-d_3 (94% D_3, 4% D_2, 2% D_1; M^+- ketene peak, 97% D_1, 3% D_0) in 12 ml of ethanol was added to a stirred mixture of 80.0 g sucrose, 15.0 g glucose, 45.0 g yeast (Fleischmann's Active Dry), and 1200 ml of water. Stirring was continued at room temperature for 72 hours, 400 ml of ether added and gentle stirring continued for 2 hours. The ether layer was decanted, 400 ml of fresh ether added and the ether extraction process repeated for an additional 9 times. The combined organic layers were dried ($MgSO_4$), concentrated under reduced pressure, filtered through a cotton plug, and the remaining solvent evaporated. The brown residue was chromatographed on Silica Gel (E. Merck, 100 g) using an ethyl acetate/benzene eluent, which yielded the following products in their respective order of elution: 5α-androstan-3-one, 233 mg (58%); mp 98-99° (ether/benzene); 22% D_3, 50% D_2, 23% D_1, 5% D_0; 5α-androst-1-ene-3-one, 9 mg (2%); 64% D_3, 14% D_2, 10% D_1, 12 % D_0; M^+- ketene peak 82% D_1, 17% D_0; 5α-androstan-3β-ol, 20 mg (5%), mp 125-135° (ether/benzene); 65% D_3, 25% D_2, 9% D_1, 1% D_0. In addition, small amounts of sterols and what appeared to be fatty acids were also obtained, but which were not further identified.

5α-Androstan-3β-yl acetate — The 5α-androstan-3β-ol obtained above (20 mg) was acetylated by stirring with a 1:1 mixture of pyridine/acetic anhydride (2.0 ml) for 10 hours at room temperature. The mixture was diluted with water, let stand one-half hour, and extracted with ether. The ether layer was washed, dried ($MgSO_4$), and the organic solvent evaporated. The residue (28 mg) was purified by two successive preparative thin layer chromatograms on silica gel using 4% ethyl acetate/benzene for development, yielding 11 mg of 5α-androstan-3β-yl acetate which showed no molecular ion peak in the mass spectrum; M^+- acetic acid peak, 12% D_3, 66% D_2, 19% D_1, 3% D_0.

Reduction of 5α-androst-1-en-3-one-1-d — The reduction of this substrate (100% D_1) was carried out in the same manner as that described above for the trideuteriated analog. The products showed no deuterium exchange.

Nmr analyses of the products (4) — The spectra of the products were obtained on a Varian HA 100 (100 MHz) spectrometer under the following conditions: deuteriated and unlabeled 5α-androstan-3-ones, ρ = 0.53, $CDCl_3$; 5α-androstan-3β-yl acetate-2α,4,4-d_3 and unlabeled acetate, ρ = 0.27, CCl_4.

RESULTS AND DISCUSSION

Upon nmr analysis, the 5α-androstan-3-one obtained from the reduction of 5α-androst-1-en-3-one-1-d showed the exclusive addition of an α (axial) hydrogen

atom at the C-1 position, in that the multiplet and splitting patterns indicative of the 1β proton were absent from the spectrum. The results observed with the 5α-androst-1-en-3-one-2,4,4-d_3 reduction products were less clear-cut due to some not unexpected deuterium exchange at the C-2 position. A measure of the extent of this exchange at C-2 can be ascertained from the recovered starting material (2%). On the basis of the mass spectrum, only 64% of this material retained three deuterium atoms, while the M^+- ketene peak (6) showed a C-2 deuterium content of 83%, compared to 97% in the starting material used initially.

Yet, that the hydrogen addition at C-2 was also predominantly (and probably exclusively) axial could be readily determined from the nmr spectra. Thus, the spectrum for the major reaction product, 5α-androstan-3-one-2α,4,4-d_3 (58%), exhibited only a low intensity multiplet corresponding to the 2α proton, while the splitting patterns observed for the 1α, 1β and 2β protons were those expected for the absence of the 2α proton.

The second reduction product, 5α-androstan-3β-ol-2α,4,4-d_3, was isolated in 5% yield, which contrasts with an approximately 25% yield of 3,17-diol and no ketonic product obtained from the reduction of the 17-oxo analog (3). This product exhibited less deuterium exchange (65% D_3, 25% D_2) than did the ketone, and because of the poorer results observed with 3β-hydroxy steroids in the nmr lanthanide induced shift technique than with their acetates (4), the product was converted to the corresponding acetyl derivative. The loss of signal observed in the nmr spectrum of the labeled *vs.* unlabeled acetate again corresponded to the addition of a β (axial) hydrogen atom at C-2.

Thus, the preferred mode of addition of hydrogen to a steroidal Δ^1 double bond is *trans* diaxial in this case and parallels stereochemically the *mammalian* enzyme catalyzed reductions of a Δ^4-3-oxo (to a 3-oxo-5β) steroid (7), a Δ^{16}-20-oxo steroid (8), and the Δ^7 and Δ^{14} double bond reductions in the conversion of lanosterol to cholesterol (9, 10). On the other hand, the reduction of the Δ^6 double bond of 3α,12α-dihydroxychol-6-enoic acid appears to proceed in a *trans* diequatorial manner (11), while the conversion of a Δ^4-3-oxo steroid to a 3-oxo-5α product has been reported to occur by at least 50% *cis* addition of hydrogen (12).

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