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Stereoselective Hydrogen Elimination at C-2 in the Transformation of 5β-Pregnane-3,11,20-trione by *Septomyxa affinis*[1]

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The stereochemistry of hydrogen loss at C-2 in the microbial transformation of 5β-pregnane-3,11,20-trione is described. Epimeric substrates stereospecifically labeled with deuterium at C-2 were incubated aerobically with *Septomyxa affinis*, and the biotransformation products, 5β-androst-1-ene-3,11,17-trione and 5β-androstane-3,11,17-trione, were separated. Inspection of their mass and nuclear magnetic resonance spectra showed that elimination of hydrogen at C-2 in Δ^1-dehydrogenation is stereoselectively β.

Keywords——microbial transformation; *Septomyxa affinis*; Δ^1-dehydrogenation; hydrogen elimination at C-2; steric mechanism; deuterium label; epimeric 2-d_1-5β-pregnane-3,11,20-triones; 5β-androst-1-ene-3,11,17-trione; 5β-androstane-3,11,17-trione

Various microorganisms are known to be capable of introducing a double bond into the 1,2- and 4,5-positions of 5α-,5β- and Δ^4-3-ketosteroids.[3] The stereochemistry of Δ^1-dehydrogenation has been extensively investigated by using labeled substrates, and the stereoselective elimination of axial hydrogen from the position adjacent to the carbonyl group at C-3 has been demonstrated.[4,5] However, an exception was reported in that Δ^1-dehydrogenation in the bioconversion of 5β-pregnane-3,11,20-trione by *Septomyxa affinis* (ATCC 6737) involves the loss of 2β-hydrogen.[6] This finding is not necessarily definitive because only 1α,2α-d_2-5β-pregnanetrione was used as a substrate for microbial transformation. To confirm the stereochemistry of hydrogen loss at C-2, the epimeric 2-d_1-5β-pregnane-3,11,20-triones (I, II) were used. The desired substrates were synthesized through the unequivocal route reported in the previous paper.[7] The isotopic purity of the deuterium-labeled substrates obtained was over 98%.

Chart 1

1) Part CLI of "Studies on Steroids" by T. Nambara; Part CL: K. Shimada, T. Tanaka, and T. Nambara, *J. Chromatogr.*, **178**, 350 (1979).
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6) Y.J. Abul-Hajj, *J. Biol. Chem.*, **247**, 686 (1972).
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Epimeric 2-d_1-5β-pregnane-3,11,20-triones were incubated with *Septomyxa affinis* under conditions similar to those described by Abul-Hajj.[6] The incubation mixture was extracted with ethyl acetate, and the extract was purified by column chromatography on silica gel. The transformation product was then subjected to high-performance liquid chromatography on a normal phase column, providing the desired 5β-androst-1-ene-3,11,17-trione together with 5β-androstane-3,11,17-trione.

Table I. Deuterium Retention in Biotransformation Products formed from 2-d_1-5β-Pregnane-3,11,20-triones by *Septomyxa affinis*

Product	Substrate (%)	
	2α-D (I)	2β-D (II)
5β-Androst-1-ene-3,11,17-trione	85	20
5β-Androstane-3,11,17-trione	65	86

The deuterium contents in these biotransformation products were determined by inspection of the molecular ion peaks in the mass spectra. As listed in Table I, the Δ^1-unsaturated compound formed from the 2β-deuterated substrate (II) showed loss of the isotope, while that derived from the 2α-epimer (I) retained the label intact. The loss of labeled deuterium in the dehydrogenation product from the 2β-deuterated substrate was confirmed by the nuclear magnetic resonance (NMR) spectral data. In addition, mass spectral analysis supported the substantial retention of the isotope label in 2-d_1-5β-androstane-3,11,17-triones produced from the two substrates.

It is evident from these data that during microbial transformation of 5β-pregnane-3,11,-20-trione into 5β-androst-1-ene-3,11,17-trione, the 2β-hydrogen is stereoselectively eliminated. The present results support the previous finding by Abul-Hajj.[6] To the best of our knowledge this is the first recorded case where 2β-hydrogen in 5β-3-ketosteroids is stereoselectively eliminated during microbial Δ^1-dehydrogenation. No plausible explanation is at present available for the unusual steric course of hydrogen loss at C-2. However, it can perhaps be explained if ring A exists in a boat or twist form. Precise knowledge of the spatial arrangement of C-2 hydrogens must await the conformational analysis of A/B-*cis* C_{21} steroid with an 11-keto group. It is hoped that further work in progress in this laboratory will provide the data necessary for conformational definition.

Experimental[8]

Materials——Epimeric 2-d_1-5β-pregnane-3,11,20-triones (I, II) were prepared by the method previously developed in this laboratory.[7] Mass spectral analysis of these substrates indicated that the deuterium content was over 98% at the indicated position.

High-Performance Liquid Chromatography——The apparatus used was a Waters ALC/GPC 202 R401 high-performance liquid chromatograph (Waters Assoc., Milford, Mass., U.S.A.) equipped with a UV detector monitoring the absorbance at 300 nm. A μ-Porasil (1 ft$\times$1/4 in I.D.) column was used under ambient conditions.

Microbial Transformation——The fermentation was carried out under conditions similar to those described by Abul-Hajj.[6] The deuterated substrates were dissolved in dimethylformamide (DMF) and added to a 48 hr culture medium in the following manner:

8) Melting points were taken on a micro hot-stage apparatus and are uncorrected. Mass spectra were obtained with a Hitachi M-529 spectrometer. NMR spectra were recorded on a JEOL PS-100 spectrometer at 100 MHz. Chemical shifts are quoted as ppm (δ) downfield from $Si(CH_3)_4$ as an internal standard. Abbreviations: s=singlet and d=doublet.

Substrate (mg)	DMF (ml)	Culture (ml)
2α-D (I) 103	0.8	400
2β-D (II) 150	1.0	500

The mixture was then incubated aerobically for 72 hr with continuous shaking at 28°.

Separation of Transformation Products——The incubation mixture was filtered to remove cell spores. The precipitate was washed with AcOEt, and the filtrate was extracted with AcOEt. The combined organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The oily product was subjected to column chromatography on silica gel. The eluate obtained with hexane–AcOEt (1: 1) was further purified by high-performance liquid chromatography on μ-Porasil using cyclohexane–AcOEt (1: 1) as an eluent. Recrystallization of the less polar eluate from hexane–ether gave 5β-androstane-3,11,17-trione as colorless plates. mp 130.5—131.5°. Yield 6%. Recrystallization of the more polar eluate from MeOH gave 5β-androst-1-ene-3,11,17-trione as colorless prisms. mp 174—175°. Yield 9%. Mixed melting points of these compounds on admixture with the corresponding authentic samples showed no depression. The deuterium contents of these products formed from the two substrates were determined by inspection of the molecular ion peaks in the mass spectra (Table I). The NMR spectra data of the Δ^1-unsaturated products in $CDCl_3$ were as follows:

Substrate	Chemical shift (δ) ppm			
	18-CH_3	19-CH_3	C_1–H	C_2–H
2α-D (I)	0.88 (s)	1.40 (s)	7.44 (s)	—
2β-D (II)	0.88 (s)	1.40 (s)	7.47 (d, J=11 Hz)	5.86 (d, J=11 Hz)

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