Table I. Bioassays of Vomiting Factor and Trichothecene

Fraction	Yield (g)	Ducklings		Reticulocytes[a] % of the control	TLC
		Dose (mg/kg, s.c.)	Vomition		
IV-1	0.770	500	−	30	
2	0.750	500	+	69	
3	0.259	300	++	16	deoxynivalenol
4	0.239	500	+	58	
5	0.898	500	+	100	
6	3.0	500	−	84	

a) inhibition of protein synthesis at concentration of 100 μg/ml

As presented in Table I, another fraction, IV-5, exhibited the vomition to ducklings without an inhibitory effect to the reticulocyte bioassay. This finding may indicate a possible contamination of non-trichothecene compound(s) which is able to induce the vomition to ducklings.

Acknowledgment The authors are greatly indebted to Dr. C. W. Hesseltine, the Northeren Regional Research Laboratory, for supplying the corn sample. We also wish to thank Dr. N. Morooka, Kagawa University, for the authentic sample of deoxynivalenol. This work was supported in part by grants from the Ministry of Welfare and the Ministry of Agriculture and Forestry.

[Chem. Pharm. Bull. 23(9)2164—2166(1975)]

UDC 547.92.02 : 542.98

Stereochemistry of $\Delta^{1,4}$ Unsaturation in Microbial Transformation of Cholesterol[1]

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(Received January 31, 1975)

The stereochemistry of hydrogen loss from C-2 and C-4 in cholesterol during microbial degradation into androsta-1,4-diene-3,17-dione has been studied. Each of the substrates, epimeric 2- and 4-deuteriocholesterols (I—IV), was incubated with respiring cultures of *Arthrobacter simplex*. Determination of the labeled isotope retained in the biotransformation products revealed that metabolic transformation of cholesterol into $\Delta^{1,4}$-3-ketosteroid proceeds by a stereospecific removal of 4β- and 2β-hydrogens.

In recent years considerable attentions have been focused on an interesting finding that some microorganisms are capable of transforming cholesterol into androsta-1,4-diene-3,17-dione (ADD) with elimination of the side chain.[3] Although the metabolic route has been

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3) M. Nagasawa, M. Bae, G. Tamura, and K. Arima, *Agr. Biol. Chem.* (Tokyo), **33**, 1644 (1969).

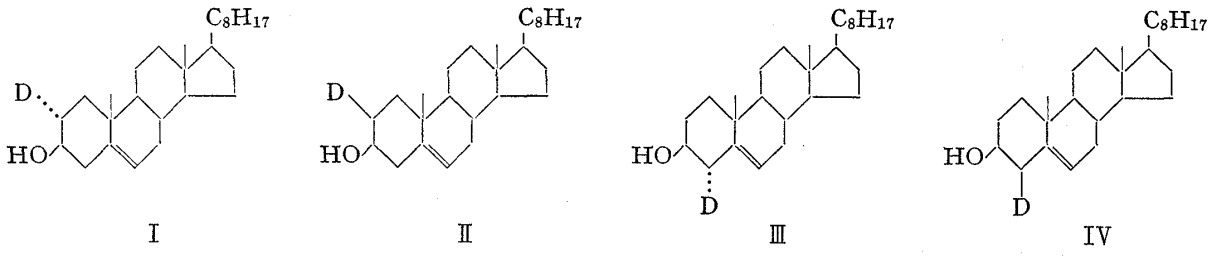

Chart 1

disclosed to a certain extent,[4] the steric mechanism of unsaturation in ring A still remains unclear. As a series of our studies on the biotransformation mechanism of steroids, elucidation of the stereochemistry of hydrogen loss from C-2 and C-4 during microbial dehydrogenation has been undertaken.

The required substrates for this purpose, epimeric 2- and 4-deuteriocholesterols (I—IV), were prepared by the route previously established in these laboratories.[5] Inspection of the molecular ion peak in the mass spectra revealed that the deuterium content of the stereospecifically labeled compounds was 93 to 98%. Each of these substrates was incubated with respiring cultures of *Arthrobacter simplex* (IAM 1660) in the presence of α,α′-dipyridyl. The incubation mixture was extracted with ethyl acetate and the extract was then separated by preparative thin-layer chromatography (TLC) to provide ADD in a satisfactory yield. The deuterium content of the biotransformation products was determined by means of mass and nuclear magnetic resonance (NMR) spectrometry.

Table I. Results of Deuterium Retention

Product	Substrate (%)			
	2α-D (I)	2β-D (II)	4α-D (III)	4β-D (IV)
Androsta-1,4-diene-3,17-dione	75	0	80	0

The results of the isotope retention are shown in Table I. The products formed from the 2β- and 4β-deuterated substrates (II, IV) showed the complete loss of the labeled isotope, while those derived from the 2α- and 4α-epimers (I, III) retained more than 75% of the label. It has been proved that microbial transformation of cholesterol into ADD proceeds by a stereospecific removal of 4β- and 2β-hydrogens.

The present result is fairly consistent with the recent reports on the stereoselective elimination of 2β-hydrogen in the degradation of cholesterol into ADD by *Mycobacterium phlei*[6] and the loss of 4β-hydrogen in androst-5-ene-3,17-dione on isomerization with mammalian enzyme preparations, *i.e.* beef adrenal microsomes and rat liver 100000×***g*** supernatant.[7] However, it has already been postulated that isomerization catalyzed by pure Δ^5-3-ketosteroid isomerase from *Pseudomonas testosteroni* occurs by direct transfer of 4β-hydrogen to C-6β.[8,9] In the present study it is shown that the isotope in ADD was not present at C-6β. Accordingly the enzymatic mechanism involved in the fate of 4β-hydrogen during Δ^5-Δ^4-isomerization by *Arthrobacter simplex* seems to be different from that during reaction with *Pseudomonas testo-*

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steroni, although the degradation process leading to ADD from cholesterol has not yet fully been elucidated.

Experimental

Material——Epimeric 2- and 4-deuteriocholesterols (I—IV) were prepared by the method previously developed in these laboratories.[5] Mass spectral analysis of these substrates indicated that the deuterium content was *ca.* 93 to 98%.

Mass and NMR Spectrometry——Mass spectral measurements were run on a Shimadzu Model LKB-9000S under the following conditions: ion source temperature 290°, ionization current 60 μA, ionization voltage 70 eV, and accelerating voltage 3.5 KV. NMR spectra were recorded with CCl_4 solution on a JEOL Model PS-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard. Abbreviation used s= singlet, d=doublet, and dd=doublet of doublets.

Microbial Transformation of Cholesterol into ADD——The fermentation medium consited of corn steep liquor 1.0%, meat extract 0.2%, and K_2HPO_4 0.05% to make pH 7.0. *Arthrobacter simplex* (IAM 1660) was inoculated to the sterile medium (100 ml) in a 500 ml Sakaguchi flask and was incubated at 30° for 2 days on a reciprocal shaker. To the fresh medium (10 ml) was added a seed culture (0.4 ml) of *Arthrobacter simplex* and was incubated at 30° for 74 hr. During the course of incubation deuterated cholesterol (5 ml) dissolved in dimethylformamide (0.1 ml) and α,α′-dipyridyl (1.6 mg) were added to the culture at 21 hr and 27 hr after the start of incubation, respectively.

Separation of ADD——The broth culture was extracted with AcOEt (20 ml×3) and the extract was combined, washed with H_2O, and dried over anhydrous Na_2SO_4. After evaporation of solvent an oily residue obtained was submitted to preparative TLC on a plate of Silica gel HF_{254} using benzene–ether (1 : 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.40) with AcOEt and evaporation of solvent gave ADD in *ca.* 27% yield. The deuterium content of ADD formed from each substrate was determined by inspection of the molecular ion peak in the mass spectra (Table I). The NMR spectral data on ADD derived from I, II, and IV were as follows:

Substrate	C_1-H	C_2-H	C_4-H
I	6.82 (broad s)	——	5.85 (s)
II	6.82 (d, J=10 Hz)	6.01 (dd, J=10, 1 Hz)	5.84 (s)
IV	6.82 (d, J=10 Hz)	6.01 (dd, J=10, 1 Hz)	5.84 (s)

Acknowledgement The authors express their thanks to all the staffs of central analytical laboratory of this Institute and Research Laboratory of Medicine, Nippon Kayaku, Co., Ltd. for spectral measurements.