[Chem. Pharm. Bull.]
32(2) 504-509 (1984)

Stereochemistry of Microbial Hydrogenation of (–)- α -Santonin to (+)-1,2-Dihydro- α -santonin by *Streptomyces cinereocrocatus* NRRL 3443

YOSHIHIRO SATO,^{*,a} TAIKO ODA,^a JUNKO INOUE,^a
MASAYUKI KUNUGI,^b and KAZUO T. SUZUKI^b

*Kyoritsu College of Pharmacy,^a Shibakoen 1-chome, Minato-ku, Tokyo 105, Japan
and National Institute for Environmental Studies,^b
Yatabe, Tsukuba, Ibaraki 305, Japan*

(Received June 11, 1983)

The stereochemistry of microbial transformation of (–)- α -santonin (**1**) by *Streptomyces cinereocrocatus* is described. Fermentation of (–)- α -santonin (**1**) with *S. cinereocrocatus* led to the formation of (+)-1,2-dihydro- α -santonin (**2**). To elucidate the stereochemistry of the microbial hydrogenation of (–)- α -santonin, (–)-[1,2-²H]- α -santonin (**1a**) was synthesized from **1**, and subjected to the microbial transformation. Analysis of the 400 MHz proton nuclear magnetic resonance spectrum of the deuterated product clearly revealed that the microbial hydrogenation of **1a** proceeds stereo-specifically with *trans*-addition of hydrogens *via si* face attacks at the 1 and 2 positions.

Keywords—microbial transformation; *Streptomyces cinereocrocatus*; (–)- α -santonin; deuterated (–)- α -santonin; (+)-1,2-dihydro- α -santonin; deuterated (+)-1,2-dihydro- α -santonin; stereochemistry; *si* face attack

In the previous papers,¹⁾ we reported the microbial transformation of (–)- and (+)-dehydrogriseofulvin and their analogs by *Streptomyces cinereocrocatus* NRRL 3443 and it was demonstrated that hydrogenations of the dienones proceed in *trans*-diaxial manner to give the corresponding dihydro derivatives.

In order to elucidate the microbial transformation activity of *S. cinereocrocatus*, (–)- α -santonin (**1**) was selected as a cross-conjugated dienone compound. Microbial transformation of (–)- α -santonin (**1**) by *Cunninghamella blakesleeana* and *Streptomyces aureofaciens* has already been reported by Hikino *et al.*,²⁾ providing (+)-1,2-dihydro- α -santonin as a product. The microbial hydrogenation of the carbon-carbon double bond of α,β -unsaturated carbonyl compounds has been reviewed for many substrates.³⁾ The microbial hydrogenation of steroids and alkaloids has also been described.⁴⁻⁶⁾ Recently, Veschambre and his coworkers⁷⁾ have described the general properties of microbial reduction of α,β -unsaturated carbonyl compounds.

This paper describes the microbial transformation of (–)- α -santonin (**1**) by *S. cinereocrocatus*. The product was (+)-1,2-dihydro- α -santonin (**2**), and therefore its stereochemistry of hydrogenation was investigated by using the deuterated substrate, (–)-[1,2-²H]- α -santonin (**1a**), whose microbial transformation led to the formation of (+)-[1 β ,2 α -²H]-1,2-dihydro- α -santonin (**2c**). The structures of the substrates and the products were elucidated mainly from the 400 MHz proton nuclear magnetic resonance (¹H-NMR) spectra.

Results and Discussion

The microbial transformation of (–)- α -santonin (**1**) by *S. cinereocrocatus* was performed under the same conditions as described in the previous paper.^{1b)} A gas liquid chromatogram of the neutral extract which was obtained from the microbial treatment showed two peaks

corresponding to the recovered substrate (38%) and a product (50%). The extract was subjected to repeated column chromatographies on silica gel to yield a microbial transformation product along with the recovered substrate. The mass spectrum (MS) of the product showed the molecular ion at m/z 248, corresponding to a dihydro derivative of (–)- α -santonin. The ^1H -NMR spectrum exhibited the presence of a C-4 methyl group (2.01 ppm) on a vinyl carbon, as in the substrate (**1**). However, the signals originating from the C-1 and C-2 vinyl hydrogens, which appeared at 6.69 and 6.26 ppm in the spectrum of the substrate (**1**), were not observed (Fig. 1a). These data indicated that the product might be the 1,2-dihydro derivative (**2**) of (–)- α -santonin (Chart 1). Therefore, (–)- α -santonin was partially hydrogenated over 5% palladium–charcoal catalyst in ethyl acetate to give (+)-1,2-dihydro- α -santonin (**2**) (Chart 2). The ^1H -NMR, MS and circular dichroism (CD) data of the microbial transformation product were identical with those of **2**,⁸⁾ demonstrating that the microbial hydrogenation of **1** by *S. cinereocrocatus* occurs at the 1 and 2 positions. Since Inayama *et al.*⁸⁾ have shown that **2** is in a half-chair conformation with respect to the cyclohexenone ring on the basis of the CD spectrum, which exhibited a negative CD Cotton effect, 400 MHz ^1H -NMR spectroscopy with selective proton decoupling established the assignments of all of the proton chemical shifts of (+)-1,2-dihydro- α -santonin (**2**) (Fig. 2 and Experimental).

Chart 1

Chart 2

Fig. 1. The 400 MHz ^1H -NMR Spectra of the 1 and 2 Positions of (–)- α -Santonin (**1**) (a) and [1,2- ^2H](–)- α -Santonin (**1a**) (b)

Fig. 2. Parts of the 400 MHz ^1H -NMR Spectra of (+)-1,2-Dihydro- α -santonin (a and b) and (+)-[1 β ,2 α - ^2H]-1,2-Dihydro- α -santonin (a' and b'): the Spectral Regions of 1.4—2.1 ppm and of 2.3—2.7 ppm

* Indicates the signal of H_2O in CDCl_3 .

Consequently, to elucidate the stereochemistry of microbial hydrogenation of (–)- α -santonin (**1**) by *S. cinereocrocatus*, the deuterated substrate, (–)-[1,2- ^2H]- α -santonin (**1a**), was synthesized from **1**. First, **2** was treated with selenium dioxide in *tert*-butanol and formation of **1** was confirmed (Chart 2) by ^1H -NMR, MS, CD, and optical rotatory dispersion (ORD) analyses. Compound **2a, b** ($^2\text{H}_0$ 4%, $^2\text{H}_1$ 25%, $^2\text{H}_2$ 71%) was prepared from **1** as described above, except for the use of deuterium in the catalytic hydrogenation. The ^1H -NMR spectrum of **2a, b** was identical with that of **2** except for decrease in the signal intensities for 1 α -, 1 β -, 2 α - and 2 β -H to 43, 64, 46 and 71%, respectively, and some differences of the spectral patterns in the proton regions. This result suggests that the catalytic reduction using deuterium proceeds somewhat preferentially from the α -side rather than the β -side by *cis* attacks to afford [1 α ,2 α - ^2H]- and [1 β ,2 β - ^2H]-(+)-1,2-dihydro- α -santonin (**2a** and **2b**) as the major deuterated products. Compound **1a** ($^2\text{H}_0$ 12%, $^2\text{H}_1$ 58%, $^2\text{H}_2$ 30%) was prepared by dehydrogenation of **2a, b** with selenium dioxide in *tert*-butanol. The ^1H -NMR spectrum of **1a** showed

decreases in the original doublet peaks at δ 6.26 and 6.69 that correspond to the vinylic 1-H and 2-H, respectively, showing triplet-like peaks (Fig. 1b).

Thus, the microbial transformation of (–)-[1,2- ^2H]- α -santonin (**1a**) by *S. cinereocrocatus* was performed under the same conditions as described above. The transformation product (**2c**) ($^2\text{H}_0$ 15%, $^2\text{H}_1$ 57%, $^2\text{H}_2$ 28%) was obtained in a yield of 36% and the substrate (**1a**) was recovered in a yield of 19%. The product (**2c**) was proved to be deuterated (+)-1,2-dihydro- α -santonin by MS and gas liquid chromatography comparisons with the standard compound (**2**). The 400 MHz ^1H -NMR spectrum of **2c** was almost identical with that of **2** except that decreases of the signal intensities at the 2α - and 1β -H regions and some differences in the 1α -, 1β -, 2α -, and 2β -H regions were observed as shown in Fig. 2. Further, a coupling pattern ($J_{2\beta-1\alpha} = 14.0$ Hz) was clearly observed at the 2β -H region, demonstrating the configurations of the deuterons of the microbial transformation product (**2c**) as 1β and 2α , respectively (Chart 3). Thus, the structure of **2c** was concluded to be (+)-[$1\beta, 2\alpha$ - ^2H]-1,2-dihydro- α -santonin.

Chart 3

The results mentioned above clearly indicate that the microbial hydrogenation of **1a** to **2c** proceeds with *trans*-addition of hydrogens *via* attack from the *si* face at both the 1 and 2 positions (Chart 3). Comparison of the above result with those for griseofulvin derivatives indicates that the microbial hydrogenations of dienone compounds by *S. cinereocrocatus* proceed with the same stereochemistry, *i.e.*, in a *trans* diaxial manner.

Experimental

All melting points were obtained on a micro-melting point apparatus, type MM2 (Shimadzu Seisakusho Ltd.), and are uncorrected. Gas liquid chromatography was carried out on a Shimadzu GC-6a gas liquid chromatograph by using a flame ionization detector with nitrogen as the carrier gas. A glass column (2 m $\times$ 3 mm i.d.) of 1.5% OV-17 on Chromosorb W was used. ^1H -NMR spectra were obtained on a JEOL JNM-GX 400 FT NMR spectrometer. All ^1H -NMR data were recorded in deuteriochloroform and reported as parts per million downfield from Me_4Si ($\delta = 0$). Abbreviations used: s=singlet, d=doublet, t=triplet, br=broad, m=multiplet, dd=double doublet, and ddd=double double doublet. Mass spectra were recorded on a JEOL D-100 spectrometer at 75 eV ionizing potential and are reported as m/z . Optical rotations were measured on a JASCO DIP-SL automatic polarimeter with a cell of 10-cm light path length, and circular dichroism spectra were taken in a 0.5-mm cell at room temperature (24–25 $^\circ\text{C}$) in chloroform on a JASCO J-20 recording spectropolarimeter. Column chromatography was performed with Kanto Kagaku silica gel (100 mesh).

(+)-1,2-Dihydro- α -santonin (2)—A suspension of 5% palladium–charcoal catalyst (100 mg) in an ethyl acetate solution (200 ml) of **1** (1 g) was shaken under a stream of hydrogen at atmospheric pressure and at room temperature (23 $^\circ\text{C}$). The hydrogenation was stopped after 1 h. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo*. The ratio of the product (**2**) to the unknown product (63:37) was determined by gas liquid chromatography. The crude product (1.1 g) in benzene was chromatographed on silica gel (70 g). Elution with benzene–methylene chloride (60:40) and recrystallization of the product from AcOEt gave (+)-1,2-dihydro- α -santonin (**2**) as colorless plates, mp 101 $^\circ\text{C}$. *Anal.* Calcd for $\text{C}_{15}\text{H}_{20}\text{O}_3$: C, 72.55; H, 8.12. Found: C, 72.01; H, 8.02. $[\alpha]_D^{23} + 80^\circ$ ($c = 0.10$, CHCl_3). MS m/z : 248 (M^+), 192 (base peak), 136, 91, 79, 55. ^1H -NMR δ (ppm): 1.27 (3H, d, $J = 6.9$ Hz, 11- CH_3), 1.34 (3H, s, 10- CH_3), 1.48 (1H, ddd, $J_{9\alpha-9\beta} = 14.0$, $J_{9\alpha-8\beta} = 12.9$, and $J_{9\alpha-8\beta} = 4.0$ Hz, 9 α -H), 1.68

(1H, dddd, $J_{8\beta-8\alpha}=14.0$, $J_{8\beta-9\alpha}=12.9$, $J_{8\beta-7}=12.1$ and $J_{8\beta-9\beta}=3.7$ Hz, 8 β -H), 1.75 (1H, ddd, $J_{9\beta-9\alpha}=14.0$, $J_{9\beta-8\beta}=3.7$ and $J_{9\beta-8\alpha}=2.7$ Hz, 9 β -H), 1.81 (1H, ddd, $J_{1\beta-1\alpha}=13.4$, $J_{1\beta-2\beta}=5.1$ and $J_{1\beta-2\alpha}=4.0$ Hz, 1 β -H), 1.92 (2H, dddd and ddd, $J_{7-8\beta}=12.1$, $J_{7-6}=11.0$, $J_{7-11}=11.0$ and $J_{7-8\alpha}=3.4$ Hz, and $J_{1\alpha-2\beta}=13.8$, $J_{1\alpha-1\beta}=13.4$ and $J_{1\alpha-2\alpha}=5.0$ Hz, 7-H and 1 α -H), 1.99, (1H, m, 8 α -H), 2.01 (3H, d, $J=1.6$ Hz, 4-CH₃), 2.35 (1H, dd, $J_{11-7}=12.1$ and $J_{11-13}=6.9$ Hz, 11-H), 2.45 (1H, ddd, $J_{2\alpha-2\beta}=16.0$, $J_{2\alpha-1\alpha}=5.0$ and $J_{2\alpha-1\beta}=4.0$ Hz, 2 α -H), 2.54 (1H, ddd, $J_{2\beta-2\alpha}=16.0$, $J_{2\beta-1\alpha}=13.8$ and $J_{2\beta-1\beta}=5.1$ Hz, 2 β -H), 4.69 (1H, dd, $J_{6-7}=11.5$ and $J_{6-4}=1.6$ Hz, 6-H). CD ($c=0.10$, CHCl₃) $[\theta]^{24}$ (nm): 0 (380), -2430 (320) (negative maximum), 0 (280), +29260 (242) (positive maximum). ORD ($c=0.10$, CHCl₃) $[\alpha]^{24}$ (nm): +740° (390), 0° (350), +4460° (300), +6940° (280), +17610° (258), 0° (241).

Dehydrogenation of (+)-1,2-Dihydro- α -santonin(2)—A solution of **2** (190 mg) and selenium dioxide (190 mg) in *tert*-butanol (15 ml) was refluxed for 20 h. The ratio of the product to the starting material was determined to be 79:21 by gas liquid chromatography. After removal of the solvent under reduced pressure, the residue was taken up in benzene and the solution was directly subjected to column chromatography over silica gel (50 g). Elution with benzene-methylene chloride (60:40) and recrystallization of the product from methanol gave (-)- α -santonin (**1**) as colorless needles, mp 178–179°C, $[\alpha]_D^{23} -173.3^\circ$ ($c=0.09$, CHCl₃). Anal. Calcd for C₁₅H₁₈O₃: C, 73.15; H, 7.37. Found: C, 72.64; H, 7.46. ¹H-NMR δ (ppm): 1.28 (3H, d, $J=7.0$ Hz, 11-CH₃), 1.33 (3H, d, $J=0.5$ Hz, 10-CH₃), 1.52 (1H, ddd, $J_{9\alpha-9\beta}=13.2$ Hz, $J_{9\alpha-8\beta}=12.2$ and $J_{9\alpha-8\alpha}=4.0$ Hz, 9 α -H), 1.69 (1H, dddd, $J_{8\beta-8\alpha}=12.8$, $J_{8\beta-7}=12.2$, $J_{8\beta-9\alpha}=12.2$ and $J_{8\beta-9\beta}=3.8$ Hz, 8 β -H), 1.82 (1H, dddd, $J_{7-8\beta}=12.2$, J_{7-6} and $J_{7-11}=11.7$, and $J_{7-8\alpha}=3.3$ Hz, 7-H), 1.90 (1H, ddd, $J_{9\beta-9\alpha}=13.2$, $J_{9\beta-8\beta}=3.8$ and $J_{9\beta-8\alpha}=2.3$ Hz, 9 β -H), 2.03 (1H, dddd, $J_{8\alpha-8\beta}=12.8$, $J_{8\alpha-9\alpha}=4.0$, $J_{8\alpha-7}=3.3$ and $J_{8\alpha-9\beta}=2.3$ Hz, 8 α -H), 2.14 (3H, d, $J=1.5$ Hz, 4-CH₃), 2.42 (1H, dd, $J_{11-7}=12.1$ and J_{11-13} methyl = 7.0 Hz, 11-H), 4.79 (1H, dd, $J_{6-7}=11.3$ and J_{6-4} methyl = 1.5 Hz, 6-H), 6.26 (1H, d, $J=9.9$ Hz, 2-H), 6.69 (1H, d, $J=9.9$ Hz, 1-H). MS m/z : 246 (M⁺), 231, 173 (base peak), 172, 135, 121, 91. CD ($c=0.11$, CHCl₃) $[\theta]^{24}$ (nm): 0 (294), -20670 (256) (negative maximum), -1370 (226). ORD ($c=0.11$, CHCl₃) $[\alpha]^{24}$ (nm): 0° (589), -1640° (336), -11780° (274) (trough), 0° (257), +13400° (249) (peak), +11210° (236).

(-)-[1,2-²H]- α -Santonin (1a)—Deuterated (+)-1,2-dihydro- α -santonins (**2a** and **2b**) were obtained from **1** (2 g) by the same procedures as described for the preparation of **2** from **1**, except for the use of deuterium. mp 98–99°C. MS: ²H₀ 4%, ²H₁ 25%, ²H₂ 71%. The deuteration ratios: 57% (1 α), 36% (1 β), 54% (2 α), and 29% (2 β). **1a** was obtained from the deuterated dihydrosantonins (**2a** and **2b**) by the same procedures as described for the preparation of **1** from **2**, mp 178–179°C. MS: ²H₀ 12%, ²H₁ 58%, ²H₂ 30%. ¹H-NMR δ (ppm): 1.28 (3H, d, $J=7.0$ Hz, 11-CH₃), 1.33 (3H, d, $J=0.5$ Hz, 10-CH₃), 1.52 (1H, ddd, $J_{9\alpha-9\beta}=13.2$ Hz, $J_{9\alpha-8\beta}=12.2$ and $J_{9\alpha-8\alpha}=4.0$ Hz, 9 α -H), 1.69 (1H, dddd, $J_{8\beta-8\alpha}=12.8$, $J_{8\beta-7}=12.2$, $J_{8\beta-9\alpha}=12.2$ and $J_{8\beta-9\beta}=3.8$ Hz, 8 β -H), 1.82 (1H, dddd, $J_{7-8\beta}=12.2$, J_{7-6} and $J_{7-11}=11.7$ and $J_{7-8\alpha}=3.3$ Hz, 7-H), 1.90 (1H, ddd, $J_{9\beta-9\alpha}=13.2$, $J_{9\beta-8\beta}=3.8$ and $J_{9\beta-8\alpha}=2.3$ Hz, 9 β -H), 2.03 (1H, dddd, $J_{8\alpha-8\beta}=12.8$, $J_{8\alpha-9\alpha}=4.0$, $J_{8\alpha-7}=3.3$ and $J_{8\alpha-9\beta}=2.3$ Hz, 8 α -H), 2.14 (3H, d, $J=1.5$ Hz, 4-CH₃), 2.42 (1H, dd, $J_{11-7}=12.1$ and J_{11-13} methyl = 7.0 Hz, 11-H), 4.79 (1H, dd, $J_{6-7}=11.3$ and J_{6-4} methyl = 1.5 Hz, 6-H), 6.26 (0.29H, d, $J=9.9$ Hz and s, 2-H), 6.69 (0.5H, d, $J=9.8$ Hz and s, 1-H). The CD and ORD spectra were identical with those of **1**.

Microbial Transformation of (-)- α -Santonin by *S. cinereocrocatus*—All of the experiments were essentially the same as those described in the previous paper^{1b}) except that (-)- α -santonin (**1**) was used as the substrate. Column chromatography of the residue from the supernatant of a 5-d incubation mixture on silica gel and recrystallization of the product from AcOEt gave (+)-1,2-dihydro- α -santonin (**2**): mp 98°C. Anal. Calcd for C₁₅H₂₀O₃: C, 72.55; H, 8.12. Found: C, 72.53; H, 7.79. $[\alpha]_D^{23} +78^\circ$ ($c=0.12$, CHCl₃). ¹H-NMR δ (ppm): 1.27 (3H, d, $J=6.9$ Hz, 11-CH₃), 1.34 (3H, s, 10-CH₃), 1.48 (1H, ddd, $J_{9\alpha-9\beta}=14.0$, $J_{9\alpha-8\beta}=12.9$ and $J_{9\alpha-8\alpha}=4.0$ Hz, 9 α -H), 1.68 (1H, dddd, $J_{8\beta-8\alpha}=14.0$, $J_{8\beta-9\alpha}=12.9$, $J_{8\beta-7}=12.1$ and $J_{8\beta-9\beta}=3.7$ Hz, 8 β -H), 1.75 (1H, ddd, $J_{9\beta-9\alpha}=14.0$, $J_{9\beta-8\beta}=3.7$ and $J_{9\beta-8\alpha}=2.7$ Hz, 9 β -H), 1.81 (1H, ddd, $J_{1\beta-1\alpha}=13.4$, $J_{1\beta-2\beta}=5.1$ and $J_{1\beta-2\alpha}=4.0$ Hz, 1 β -H), 1.92 (2H, dddd and ddd, $J_{7-8\beta}=12.1$, $J_{7-6}=11.0$, $J_{7-11}=11.0$ and $J_{7-8\alpha}=3.4$ Hz, and $J_{1\alpha-2\beta}=13.8$, $J_{1\alpha-1\beta}=13.4$ and $J_{1\alpha-2\alpha}=5.0$ Hz, 7- and 1 α -H), 1.99 (1H, m, 8 α -H), 2.01 (3H, d, $J=1.6$ Hz, 4-CH₃), 2.35 (1H, dd, $J_{11-7}=12.1$ and $J_{11-13}=6.9$ Hz, 11-H), 2.45 (1H, ddd, $J_{2\alpha-2\beta}=16.0$, $J_{2\alpha-1\alpha}=5.0$ and $J_{2\alpha-1\beta}=4.0$ Hz, 2 α -H), 2.54 (1H, ddd, $J_{2\beta-2\alpha}=16.0$, $J_{2\beta-1\alpha}=13.8$ and $J_{2\beta-1\beta}=5.1$ Hz, 2 β -H), 4.69 (1H, dd, $J_{6-7}=11.5$ and $J_{6-4}=1.6$ Hz, 6-H). CD ($c=0.12$, CHCl₃) $[\theta]^{24}$ (nm): 0 (389), -2123 (320) (negative maximum), 0 (280), +27400 (242) (positive maximum). ORD ($c=0.12$, CHCl₃) $[\alpha]^{24}$ (nm): +770° (390), 0° (350), +4100° (300), +6800° (280), +17400° (241) (peak).

Microbial Transformation of (-)-[1,2-²H]- α -Santonin by *S. cinereocrocatus*—All of the experiments were the same as described above, except that (+)-[1,2-²H]- α -santonin (**1a**) was used as the substrate. Column chromatography of the residue from the supernatant of a 5-d incubation mixture on silica gel and recrystallization of the product from AcOEt gave (+)-[1 β ,2 α -²H]-dihydro- α -santonin (**2c**): mp 98–99°C, $[\alpha]_D^{23} +82^\circ$ ($c=0.10$, CHCl₃). MS: ²H₀ 15%, ²H₁ 57%, ²H₂ 28%. The ¹H-NMR spectrum (Fig. 2) was identical with that of **2**, except for decreases of the proton signals at 1 β and 2 α of **2** and the appearance of additional signals (ppm) at: 1.80 (0.6H, dd, $J_{1\beta-1\alpha}=13.4$ and $J_{1\beta-2\beta}=5.1$ Hz, 1 β -H), 1.89 (1H, d, $J_{1\alpha-2\beta}=14.0$, 1 α -H), 2.44 (0.4H, dd, $J_{2\alpha-2\beta}=16.0$, $J_{2\alpha-1\alpha}=4.8$ Hz, 2 α -H), 2.51 (1H, d, $J_{2\beta-1\alpha}=14.0$ Hz, 2 β -H). CD ($c=0.10$, CHCl₃) $[\theta]^{24}$ (nm): 0 (380), -2430 (320) (negative maximum), 0 (280), +28770 (242) (positive maximum). ORD ($c=0.10$, CHCl₃) $[\alpha]^{24}$ (nm): +790° (390), 0° (350), +4220° (300), +6700° (280), +18350° (258) (peak).

Acknowledgements Thanks are due to Dr. T. G. Pridham of ARS Culture Collection Research for providing the strain of *Streptomyces cinereocrocatu*s NRRL 3443, and to Dr. S. Iwasaki of the Institute of Applied Microbiology, University of Tokyo, for elemental analyses.

References

- 1) a) Y. Sato, T. Oda, and H. Saitô, *J. Chem. Soc., Chem. Commun.*, **1977**, 415; b) Y. Sato, T. Oda, and H. Saitô, *Chem. Pharm. Bull.*, **29**, 2313 (1981); c) Y. Sato and T. Oda, *Heterocycles*, **17**, 171 (1982); d) T. Oda and Y. Sato, *Chem. Pharm. Bull.*, **31**, 934 (1983); e) T. Oda and Y. Sato, *Chem. Pharm. Bull.*, **31**, 3446 (1983).
- 2) H. Hikino, Y. Tokuoka, and T. Takemoto, *Chem. Pharm. Bull.*, **18**, 2127 (1970).
- 3) K. Kieslich, "Microbial Transformations of Non-steroid Cyclic Compounds," G. Thieme Pub. Stuttgart, 1976, pp. 633—655.
- 4) H. Iizuka and A. Naito, "Microbial Transformation of Steroids and Alkaloids," University of Tokyo Press, Tokyo and University Park Press, State College, Pennsylvania, 1967, pp. 156—167.
- 5) W. Charny and H. L. Herzog, "Microbial Transformation of Steroids," Academic Press, New York, 1967, pp. 60—63.
- 6) H. Iizuka and A. Naito, "Microbial Conversion of Steroids and Alkaloids," University of Tokyo Press, Tokyo, 1981, pp. 202—204.
- 7) M. Desrut, A. Kergomard, M. F. Renard, and H. Veschambre, *Biochem. Biophys. Res. Commun.*, **110**, 908 (1983), and references cited therein.
- 8) S. Inayama, N. Shimizu, H. Hori, T. Ohsaka, T. Hirose, T. Shibata, and Y. Iitaka, *Chem. Pharm. Bull.*, **30**, 3856 (1982).