C-200 column to give 5.9 g (42%) of pure VII. Crystallization from methanol gave prisms of mp 127°, $[\alpha]_D^{23}$ −66.5° (c=1.00, chloroform). *Anal.* Calcd. for $C_{24}H_{38}O_{14}S$: C, 49.48; H, 6.57; S, 5.51. Found: C, 49.39; H, 6.74; S, 5.45.

2,3;5,6-Di-O-isopropylidene-D-mannofuranosyl Chloride (IX)——To 12.5 ml of an ethereal solution containing 1.3 g (5×10^{-3} mole) of 2,3;5,6-di-O-isopropylidene-α-D-mannofuranose (VIII) and 0.40 g (5×10^{-3} mole) of anhydrous pyridine was added dropwise during 30 min, with vigorous stirring and keeping the temperature below 0°, 5 ml of an ethereal solution containing 0.30 g (2.5×10^{-3} mole) of thionyl chloride. The reaction mixture was allowed to stand below 0° for an additional hour, and the precipitate was filtered off. The filtrate was evaporated to dryness, absorbed on 2 g of Wakogel C-200, placed on a Wakogel C-200 column (1.5×45 cm), and eluted with the solvent system, hexane–ether (7:3). From the fractions 11—15 (each fraction was 10 ml) 58 mg (4.2%) of a syrup was obtained, which gave a single spot on TLC. $[\alpha]_D^{25}$ +17° (c=0.89, chloroform) (lit.[2]: $[\alpha]_D^{21}$ of the α-anomer, +85.7°). *Anal.* Calcd. for $C_{12}H_{19}O_5Cl$: C, 51.73; H, 6.87; Cl, 12.70. Found: C, 51.73; H, 7.28; Cl, 12.67.

3-Deoxy-1,2;5,6-di-O-isopropylidene-α-D-*erythro*-hex-3-enofuranose (IV)——The mixture of an *o*-xylene solution containing 346 mg of (III) and 5 ml of 1N methanolic sodium methoxide was heated at 100° for 10 min to evaporate methanol. Then the remaining solution was refluxed for 3 hr. The reaction mixture was evaporated to dryness and the residue was extracted twice with 10 ml of ether. After evaporation of the solvent 156 mg of a dark brown syrup was obtained. Thin-layer chromatography (TLC) of the syrup showed a fast moving spot (major) along with two slower moving spots (minor). The major component was isolated by Wakogel C-200 column (0.5×30 cm) chromatography to give 78 mg (54%) of a syrup, which was crystallized from ether to give needles of mp 50.5° (lit.,[3] 51°), $[\alpha]_D^{21}$ +19.7° (c=0.61, chloroform) (lit.[3]: $[\alpha]_D^{20}$ +19.8°, c=3.0, ethanol). *Anal.* Calcd. for $C_{12}H_{18}O_5$: C, 59.45; H, 7.50. Found: C, 59.51; H, 7.42.

2) K. Freudenberg and A. Wolf, *Ber.*, **60**, 232 (1927).
3) F. Weygand and H. Wolz, *Chem. Ber.*, **85**, 256 (1952).

[Chem. Pharm. Bull. 18(10)2127—2128(1970)]

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Biochemical Syntheses. VIII.[1] Microbial Transformation of α-Santonin to 1,2-Dihydro-α-santonin[2]

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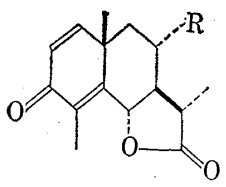
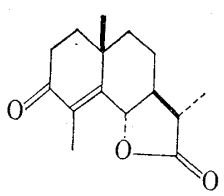
In the recent few years we have performed microbial transformation of a number of sesquiterpenoids obtained from plants and found that some biological procedures (*i.e.* selective hydroxylation) which actually take place in the plants can be reproduced by enzymes induced by microorganisms.[4–6]

α-Santonin (I) and artemisin (II) are the sesquiterpenic constituents of *Artemisia* spp. (Compositae) and the latter must be biosynthesized by enzymatic hydroxylation of the former in the plants.

1) Part VII: H. Hikino, S. Nabetani, and T. Takemoto, *Yakugaku Zasshi*, **90**, 757 (1970).
2) This paper is Part XXXVI in the series on Sesquiterpenoids. Preceding paper, Part XXXV: H. Hikino, K. Agatsuma, C. Konno, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **18**, 752 (1970).
3) Location: *Aoba-yama, Sendai.*
4) H. Hikino, Y. Tokuoka, Y. Hikino, and T. Takemoto, *Tetrahedron*, **24**, 3147 (1968).
5) H. Hikino, K. Aota, Y. Tokuoka, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **16**, 1088 (1968).
6) H. Hikino, T. Kohama, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **17**, 1659 (1969).



I : R=H
II : R=OH



III

In the hope that the enzymatic hydroxylation of α-santonin to artemisin could be achieved by an enzyme produced by a microorganism, twelve species of microbes were subjected to screening test. As the result, it was found that fermentation with *Cunninghamella blakesleeana* and *Streptomyces aureofaciens* led to the transformation of α-santonin (I). Thus, incubation of α-santonin afforded, after ethyl acetate extraction, each fermentation product whose silica gel thin-layer chromatography (TLC) showed that it mainly consisted of the recovered starting material (I) but contained two minor metabolites, one being less polar and the other more polar than the substrate (I).

The isolation of the less polar product (III) was accomplished by silica gel chromatography. The retention of the γ-lactone (1785 and 1762 cm^{-1}) and the α,β-unsaturated carbonyl group (1678 cm^{-1}) in the product (III) was indicated by its infrared (IR) spectrum. While the nuclear magnetic resonance (NMR) spectrum exhibited the presence of the C-4 vinyl methyl (1.97 ppm), the C-10 tertiary methyl (1.32 ppm), the C-11 secondary methyl (1.23 ppm), and the C-6 hydrogen (4.67 ppm) as with the substrate (I). However, the signals originating from the C-1 and C-2 vinyl hydrogens which appeared at 6.71 and 6.21 ppm as an AB type quadruplet in the spectrum of the substrate (I) were not observed. These data demonstrated that the product (III) might be 1,2-dihydro derivative of α-santonin. Then α-santonin was partially hydrogenated over Adams' catalyst in methanol to give 1,2-dihydro-α-santonin which was identified as the above product (III).

Although the more polar product might possibly be identical with artemisin (II), it was found that on TLC the *Rf* value of the product was different from that of artemisin (II). Further investigation, however, was not carried out due to lack ofthe material.

It is of interest to note that while *Cunninghamella blakesleeana* is well known to conduct the oxidation of steroidal and terpenic substrates, the same mold is found also to catalyze the reduction of the terpenoid in the present work.

Experimental[7]

Fermentation of α-Santonin with *Cunninghamella blakesleeana* and *Streptomyces aureofaciens*—— *Cunninghamella blakesleeana* and *Streptomyces aureofaciens* were grown in a modified *Corticium* medium[4] (20 liter in twenty 500 ml flasks, respectively) at 27° on a reciprocal shaker. After 6 days of incubation, α-santonin (I) (0.4 g) dissolved in EtOH (20 ml) was distributed equally among the twenty flasks. The fermentation was continued at 27° for a period of 6 days. The filtrate of the culture broth was then extracted with AcOEt, and the extract was evaporated to give the fermentation product (0.51 g for *C. blakesleeana* and 0.45 g for *S. aureofaciens*). The residue was chromatographed over silica gel (20 g) and the column eluted with benzene. The crystalline fractions from the chromatogram were combined and crystallized from AcOEt to furnish 1,2-dihydro-α-santonin (III) as colorless plates (15 mg for *C. blakesleeana* and 21 mg for *S. aureofaciens*), mp 102—103°. IR ν_{max}^{KBr} cm^{-1}: 1785, 1762 (γ-lactone), 1678 (α,β-unsaturated carbonyl). NMR ($CHCl_3$): 3H doublet at 1.23 (J=8, $C_{(13)}\underline{H}_3$), 3H singlet at 1.32 ($C_{(15)}\underline{H}_3$), 3H doublet at 1.97 (J=1.5, $C_{(14)}\underline{H}_3$), 1H doublet at 4.67 (J=9, $C_{(6)}\underline{H}$). The identity with 1,2-dihydro-α-santonin, prepared from α-santonin by catalytic hydrogenation, was confirmed by the usual criteria (mp, mixed mp, TLC, IR, NMR).

Acknowledgement We are grateful to Analytical Laboratory, this Institute, for the NMR spectra.

7) NMR spectra were recorded at 60 MHz. Chemical shifts are given in ppm downfield from internal Me_4Si, and coupling constants (J) in Hz.