Note

Microbial Conversion of 4-Oxoisophorone by *Aspergillus niger*Yoshinari YAMAZAKI, Yumiko HAYASHI,
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Many studies have been reported on the preparation of chiral compounds from achiral ones with enzymes or microorganisms. We have studied the conversion of terpenes by *Aspergillus niger* and reported that the main reaction was regio- and stereoselective hydroxylation.^{1,2)}

It has been reported that 2,6,6-trimethyl-2-cyclohexene-1,4-dione (4-oxoisophorone) is asymmetrically reduced to (6*R*)-2,2,6-trimethylcyclohexane-1,4-dione by yeasts³⁾ and actinomycetes.⁴⁾ We have reported that the conversion of isophorone and ionones by *Aspergillus niger* afforded chiral oxidation products.⁵⁾ Therefore, it is interesting as to whether the main reaction is reduction or oxidation on conversion of 4-oxoisophorone by the fungus.

This paper deals with identification of the conversion products of 4-oxoisophorone with *A. niger*, the conversion pathway and stereochemical aspects of the conversion.

Spores of *Aspergillus niger* JTS 191 (4×10^7) were inoculated into a 3-l Erlenmeyer flask containing one liter of medium consisting of 3% sucrose, 0.2% NaNO₃, 0.1% K₂HPO₄, 0.05% KCl, 0.05% MgSO₄·7H₂O, 0.1% yeast extract and distilled water (pH 7.2). Cultivation was carried out at 28°C for 48 hr under gyratory shaking at 200 rpm. To the resulting culture broth, one ml of 4-oxoisophorone (1) was added as the substrate. The broth was incubated at 28°C under continuous shaking. Ten ml aliquots of the cultured broth were extracted with ethyl acetate every 24 hr. The extract was analyzed by gas chromatography.²⁾ The substrate peak disappeared after 48 hr and seven peaks appeared concomitantly with the decrease in the substrate (Fig. 1).

After 33 and 120 hr, the conversion products were extracted with ethyl acetate. The organic solution was washed with a 5% NaHCO₃ aq. solution and with water, and then evaporated to dryness to yield the neutral products. Silicic acid column chromatography (hexane:ethyl acetate, 80:20~65:35) and preparative high performance liquid chromatography of the extract gave seven pure products: (6*R*)-2,2,6-trimethylcyclo-

hexane-1,4-dione (2), (4*S*,6*R*)-4-hydroxy-2,2,6-trimethylcyclohexanone (3), *cis*-4-hydroxy-3,3,5-trimethylcyclohexanone (4), (4*S*)-4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one (5), (4*R*)-4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one (6), *trans*-4-hydroxy-2,2,6-trimethylcyclohexanone (7) and (2*S*)-2-hydroxy-2,6,6-trimethylcyclohexane-1,4-dione (8).

Most of the products were known compounds and the spectral data obtained for them were in good agreement with the previous data. The specific rotations of these products and the references are given in Table I. The optical purity of each product varied with the incubation

TABLE I. THE SPECIFIC ROTATIONS OF THE CONVERSION PRODUCTS

Compound ^{a)}	$[\alpha]_D^{23}$	$c^{b)}$	Solution	O.p. ^{c)}	Ref. ^{d)}
2	-243	0.96	MeOH	90	3~5)
3	-86	4.1	MeOH	82	3)
4	+6.2	1.8	MeOH	—	6)
5	-8.9	1.9	MeOH	18	7)
6	+42.6	1.6	MeOH	81	5)
7	+1.9	0.55	MeOH	—	3)
8	+13.6	0.28	CHCl ₃	102	8)

^a Incubation time: 2, 33 hr; 3~8, 120 hr.^b Concentration of solution (g/100 ml).^c Optical purity (%).^d Reference. FIG. 1. Gas Chromatograms for the Time Course of the Conversion of 4-Oxoisophorone by *Aspergillus niger* JTS 191.

1, 4-oxoisophorone; 2, (6*R*)-2,2,6-trimethylcyclohexane-1,4-dione; 3, (4*S*,6*R*)-4-hydroxy-2,2,6-trimethylcyclohexanone; 4, *cis*-4-hydroxy-3,3,5-trimethylcyclohexanone; 5, (4*S*)-4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one; 6, (4*R*)-4-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one; 7, *trans*-4-hydroxy-2,2,6-trimethylcyclohexanone; 8, (2*S*)-2-hydroxy-2,6,6-trimethylcyclohexane-1,4-dione.

FIG. 2. Proposed Pathway for the Conversion of 4-Oxoisophorone by *Aspergillus niger* JTS 191.

time, because all the products tended to undergo racemization with the lapse of incubation time. The optical purities of the diastereomer, **3** and **7** were 82% and racemic, respectively. The rate of racemization of these products might be different.

Among these compounds, **2** was the main product, corresponding to 55% of the added substrate after 30 hr and decreasing gradually thereafter. Then, **4** increased markedly to reach 40% by 144 hr, as shown in Fig. 1.

In order to clarify the conversion pathway, **2** was converted with the fungus. Compounds **3**, **4**, **7** and **8** were obtained as the conversion products. The rate of formation of **4** was very high. Therefore, as shown in Fig. 2, it is considered that the main pathways are from **1** to **3**, **4**, **7** and **8** via **2**, while there are other pathways, *i.e.*, from **1** to **5** and **6**.

Ketols **3** and **4** were produced through asymmetric reduction of a carbonyl group. The mode of reduction of **1** by the fungus was similar to in the cases of yeast and actinomyces, although in the conversion of isophorone by this fungus, the oxidation was the major reaction and the reduction of the double bond was minor reaction.⁵⁾ The fungus introduced a hydroxyl group at the C-6 position of **2**. This mode of hydroxylation is the same as that of menthone isomers by the fungus.¹⁰⁾

The fungus rapidly incorporated **1** into mycelia, reduced the substrate immediately to **2** and released the latter into the culture broth. *A. niger* JTS 191 could convert up to 0.5% of **1**, but not above 0.7%, owing to the antimicrobial activity of the substrate.

The spectrometric data for these products are summarized below. **2**: MS m/z (%): 154 (M^+ , 6), 139 ($M^+ - 15$, 11), 70 (38), 69 (45), 56 (100). NMR δ_C ($CDCl_3$): 213.7 (C-1, s), 207.5 (C-4, s), 52.7 (C-3, t), 44.9 (C-5, t), 44.2 (C-2, s), 39.8 (C-6, d), 26.5 (C2-Me, q), 25.6 (C2-Me, q), 14.6 (C6-Me, q). NMR δ_H ($CDCl_3$): 3.02 (1H, m, Me-CH<), 1.21 (3H, s, C2-CH₃), 1.91 (3H, d, $J=5.9$ Hz, C6-CH₃), 1.12 (3H, s, C2-CH₃). **3**: MS m/z (%): 156 (M^+ , 7), 141 ($M^+ - 15$, 6), 138 (4), 83 (61), 69 (56), 57 (96), 41 (100). NMR δ_C ($CDCl_3$): 215.3 (C-1, s), 65.6 (C-4, d), 49.4 (C-3, t), 44.3 (C-5, t), 44.3 (C-2, s), 37.9 (C-6, d), 26.2 (C2-Me,

q), 25.7 (C2-Me, q), 14.8 (C6-Me, q). NMR δ_H ($CDCl_3$): 4.32 (1H, m, HO-CH<), 2.74 (1H, m, CH₃CH<), 1.20 (3H, s, C2-CH₃), 1.07 (3H, s, C2-CH₃), 1.00 (3H, d, $J=6.4$ Hz, C6-CH₃). IR ν_{max} cm^{-1} : 3380 (-OH), 1700. **4**: MS m/z (%): 156 (M^+ , 7), 141 (8), 83 (36), 69 (49), 58 (81), 43 (100), 41 (86). NMR δ_C ($CDCl_3$): 212.0 (C-1, s), 77.0 (C-4, d), 48.5 (C-2, t), 43.1 (C-6, t), 39.7 (C-3, s), 33.1 (C-5, d), 27.1 (C3-Me, q), 25.6 (C3-Me, q), 18.2 (C5-Me, q). NMR δ_H ($CDCl_3$): 3.33 (1H, m, broad, HO-CH<), 2.60 (1H, d, $J=13.8$ Hz, CO-CH₂-C (Me)₂, eq), 1.89 (1H, d, $J=13.8$ Hz, CO-CH₂-C (Me)₂, ax), 1.11 (3H, s, C3-CH₃), 1.08 (3H, d, $J=5.3$ Hz, C5-CH₃), 0.89 (3H, s, C3-CH₃). IR ν_{max} cm^{-1} : 3450 (-OH), 1700. **5**: MS m/z (%): 154 (M^+ , 5), 126 (4), 111 (14), 98 (93), 70 (100), 41 (99). NMR δ_H ($CDCl_3$): 6.62 (1H, m, CH=C (Me)-), 4.56 (1H, m, -CH(OH)-), 2.24 (1H, dd, $J=1.8, 5.5$ Hz, CH₂-CH (OH), eq), 2.10 (1H, dd, $J=1.9, 5.4$ Hz, CH₂-CH (OH), ax), 1.79 (3H, s, C2-CH₃), 1.15 (3H, s, C6-CH₃), 1.12 (3H, s, C6-CH₃). **6**: MS m/z (%): 154 (M^+ , 1), 112 (55), 98 (73), 70 (82), 42 (100). NMR δ_H ($CDCl_3$): 5.79 (1H, broad, CO-CH=C-), 4.37 (1H, broad, CH(OH)-), 3.97 (1H, broad, CH(OH)-), 2.26 (2H, q, $J=1.6$ Hz, CH₂-CO), 2.03 (3H, s, C2-CH₃), 1.07 (3H, s, C6-CH₃), 1.01 (3H, s, C6-CH₃). **7**: MS m/z (%): 156 (M^+ , 4), 138 (4), 83 (65), 57 (90), 41 (100). NMR δ_H ($CDCl_3$): 4.19 (1H, m, HO-CH<), 3.14 (1H, m, CH₃-CH<), 1.33 (3H, s, C2-CH₃), 1.05 (3H, s, C2-CH₃), 1.04 (3H, d, $J=6.6$ Hz, CO-CH (CH₃)-). **8**: MS m/z (%): 170 (M^+ , 1), 142 (5), 127 (3), 69 (21), 58 (100), 43 (89). NMR δ_C ($CDCl_3$): 214.2 (C-1, s), 205.5 (C-4, s), 73.8 (C-2, s), 51.6 (C-5, t), 50.1 (C-3, t), 42.3 (C-6, s), 27.5 (C2-Me, q), 26.7 (C6-Me, q), 26.3 (C6-Me, q). NMR δ_H ($CDCl_3$): 3.37 (1H, broad, C (Me) OH-), 2.82 (2H, s, CO-CH₂+C (Me)), 2.74 (1H, s, CO-CH₂-C (OH) Me-, eq), 2.72 (1H, s, CO-CH₂-C (OH) Me-, ax), 1.44 (3H, s, C2-CH₃), 1.25 (3H, s, C6-CH₃), 1.25 (3H, s, C6-CH₃).

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