The main biodegradation product of (±)-α-isomethylionone (2) with standard activated sludge was characterized as (±)-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)propan-2-one (1) by its analysis and synthesis. Both enantiomers (1a and 1b) of 1 were synthesized by starting from (R)- and (S)-2,4,4-trimethyl-2-cyclohexen-1-ol (3a and 3b), respectively.

Key words: 1-(2,6,6-trimethyl-2-cyclohexen-1-yl)propan-2-one; α-isomethylionone; biotransformation; standard activated sludge

Ionones and their derivatives, which are widely distributed in nature and presumably generated by the metabolism or degradation of carotenoids, are important constituents of many essential oils.1) Many reports have so far been published on the biotransformation of ionone analogues.2–8) For example, Mikami et al. have reported the microbial transformation of β-ionone,5,6) (±)-α-ionone and unnatural (±)-α-isomethylionone7) by Aspergillus niger into the corresponding oxidative hydroxylation products without any degradation. The fermentation of β-ionone with Lasiodiplodia theobromae has also been described by Krasnobajew et al.5) to give β-cyclohomogeraniol as the main degradation product. In the course of our studies on the fermentation of unnatural (±)-α-isomethylionone (2), we have found that standard activated sludge mainly gave (±)-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)propan-2-one (1) with a pleasant odor as the degradation product. We report here the biotransformation of 2 to 1 and an asymmetric synthesis of both enantiomers (1a and 1b) of 1 for an evaluation of their odors. The fermentation of 2 was conducted in a basic culture medium consisting of standard activated sludge at 25 °C for 28 days under continuous shaking. After extracting the metabolites from the culture broth, the residue by SiO₂ chromatography gave an inseparable mixture of A and unreacted 2 in a ca. 1 to 9 ratio. We were able to isolate analytically pure A with a molecular weight of 180 (M⁺) by preparative GLC. The IR absorption band at 1730 cm⁻¹ indicated the presence of a carbonyl group. The ¹H-NMR spectrum showed the presence of a geminal dimethyl at 0.76 and 0.91 ppm, a methyl on a double bond at 1.62 ppm, a methyl of methyl ketone at 2.18 ppm, and a proton on a double bond at 5.33 ppm. The ¹³C-NMR spectrum showed the presence of a geminal dimethyl at 0.76 and 0.91 ppm, a methyl on a double bond at 1.62 ppm, a methyl of methyl ketone at 2.18 ppm, and a proton on a double bond at 5.33 ppm. The ¹³C-NMR spectrum indicated the presence of 12 carbons. An analysis of these spectral data indicated A to be 1, which was then synthesized from (±)-2,4,4-trimethyl-2-cyclohexen-1-ol (3)⁹) with isopropenyl methyl ether in the presence of pivalic acid at 180 °C for 5 hr by a modification of the reported method.¹⁰) The spectral data for synthetic 1 were identical with those of A. Therefore, the main biotransformation product of 2 was characterized as (±)-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)propan-2-one (1) with an interesting floral-fruity odor. The formation of 1 suggested that a Baeyer–Villiger type of reaction had occurred in the first step of

Scheme 1. Synthesis of the Main Biotransformation Product of α-Isomethylionone.
Reagents: (a) standard activated sludge; (b) isopropenyl methyl ether and pivalic acid.

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biotransformation, as the same pathway has been reported by Krasnobajew et al.8)

Next, in order to evaluate the odors of both enantiomers (1a and 1b), we decided to synthesize these enantiomers. In the same manner as that just described, Claisen rearrangement of (R)- and (S)-2,4,4-trimethyl-2-cyclohexen-1-ol (3a and 3b)9) gave 1a and 1b in 50% and 61% yields, respectively. The optical purity of the starting materials 3a and 3b and resulting 1a and 1b were respectively found to be 97.8% e.e. 96.2% e.e. and 97.8% e.e. by GLC analyses with a chiral GLC column.11) (S)-1a was found to have a strongly diffusive floral-fruity odor with an iris-like note, while (R)-1b contained a weaker woody-aminaldic odor than that of (S)-1a. In summary, characterization of the biodegradation product of 2 and an odor evaluation of 1a and 1b were achieved.

Experimental. (S)- and (R)-2,4,4-Trimethyl-2-cyclohexen-1-ol (3a and 3b) were prepared by Mori’s method.9) Standard activated sludge, which was an aggregate of such microbes as sarcodia, ciliophora and rotifer, was purchased from Chemical Evaluation and Research Institute. All other chemicals were of analytical grade and commercially available. All boiling point (bp) data are uncorrected. GC/FTIR spectra were measured with a Jasco IR-500 spectrometer.1H-NMR spectra were recorded at 400 MHz by a Jeol JNM-LA400 spectrometer, the peak for TMS (at 0.00) being used as the internal standard.13) C-NMR spectra were recorded at 100 MHz by a Jeol JNM-LA400 spectrometer, the peak for CDCl3 (at 77.0) being used as the internal standard. MS spectra were obtained with a Hitachi M-80B spectrometer at 70 eV, and optical rotation values were measured with a Horiba SEPA-2000 polarimeter. GLC analyses were performed by a Hewlett-Packard 5890 gas chromatograph with a 0.25 mm i.d. × 50 m capillary column coated with a mixture of heptakis-(2,6-di-O-methyl-3-O-penty1)-β-CD and OV-1701 (20:80). The column temperature was programmed at 0.7°C/min from 70°C to 160°C; The carrier gas was He at 0.9 ml/min. Column chromatography was carried out on Merck Kieselgel 60 Art 7734. The odor evaluation was carried out with smelling strips by a panel of experts on the basis of 10% strength ethanolic solutions of 1-(2,6,6-trimethyl-2-cyclohexen-1-yl)propan-2-one.

Biotransformation of (±)-α-isomethylionone (2). The standard biotransformation medium consisted of the following components (per liter of distilled water): 0.063 g of K2HPO4, 0.026 g of KH2PO4, 0.134 g of Na2HPO4·7H2O, 0.005 g of NH4Cl, 0.068 g of MgSO4, 0.083 g of CaCl2, and 0.0008 g of FeCl3·6H2O. To the resulting standard medium (900 ml), 108 mg of α-isomethylionone (2) and 120 mg of standard activated sludge were added. The mixture was incubated at 25°C for 28 days under continuous shaking.

Isolation of (±)-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)propan-2-one (1). The above metabolites were extracted with ether, and the organic layer was washed with brine, dried over MgSO4, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with n-hexane–ethyl acetate (10:1) to give a mixture of 1 and 2 (100 mg, 1:2 = 1:9 by GLC analysis). An analytical sample was further purified by preparative GLC to afford 1 (10 mg, 99.5% purity). GC/FTIR \( \nu_{\text{max}} \text{cm}^{-1} \): 1730 (m, C=O). NMR \( \delta_H \) (400 MHz, CDCl3): 0.76 (3H, s, 6-CH3), 2.26 (1H, dd, \( J = \frac{1}{4} \text{Hz}, \) 4.4 Hz, COC6-H), 2.53 (1H, dd, \( J = 17.6, 6.4 \text{Hz}, \) COCH2), 2.33–2.35 (2H, m, 1-C6-H), 1.62 (3H, s, 2-CH3), 1.95–1.99 (2H, m, 4-C6-H), 1.15–1.38 (2H, m, 5-C6-H), 1.62 (3H, s, 2-CH3), 1.95–1.99 (2H, m, 4-C6-H), 2.18 (3H, s, COCH3), 2.26 (1H, dd, \( J = 17.6, 4.4 \text{Hz}, \) COCH2), 2.33–2.35 (2H, m, 1-CH), 2.53 (1H, dd, \( J = 17.6, 6.4 \text{Hz}, \) COCH2), 5.33 (1H, brs, 3-CH). NMR \( \delta_C \) (100 MHz, CDCl3): 22.75, 22.88, 26.50, 26.72, 30.11, 31.47, 32.00, 43.58, 45.38, 120.97, 135.58, 208.72. MS m/z: 180 (M+), 165, 137, 122, 107, 81, 43. The enantiomeric purity of resulting 1 was almost 0% e.e. by GLC analysis: \( \tau_R \) 73.40 min [50.2%], \( \tau_R \) 75.60 min [49.8%].

Synthesis of (±)-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)propan-2-one (1). A mixture of (±)-2,4,4-Trimethyl-2-cyclohexen-1-ol (3) (2.8 g, 0.02 mol), isopropenyl methyl ether (25.0 g, 0.35 mol), and pivalic acid (0.6 g, 5.9 mmol) was heated at 180°C for 5 hr in an autoclave under a nitrogen atmosphere. The reaction mixture was poured into Na2CO3 aq. and then extracted with ether. The organic layer was washed with brine, dried over MgSO4 and concentrated in vacuo. The residue by SiO2 chromatography, eluting with n-hexane–ethyl acetate (95:5), gave 1 (2.0 g, 56%), bp 73–74°C/1 mmHg. Its IR, MS and NMR spectra were identical with those of racemate product 1. HRMS m/z (M+): calcd. for C12H20O, 180.1514; found, 180.1522.

Synthesis of (S)-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)propan-2-one (1a). In the same manner as that described for the preparation of 1, 3a (2.8 g, 97.8% e.e.) gave 1a (1.8 g, 50%). [\( \alpha \)]D25 = −104.1 (c 1.10, CHCl3). Its IR, MS and NMR spectra were identical with those of racemate 1. HRMS m/z (M+): calcd. for C12H20O, 180.1514; found, 180.1538. The enantiomeric purity of resulting 1a was
found to be 96.2% e.e. by GLC analysis: $t_R$ 73.4 min [98.1%, 1a], $t_R$ 75.6 min [1.9%, 1b].

*Synthesis of (R)-1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-propan-2-one (1b).* In the same manner as that described for the preparation of 1, 3b (2.8 g, 98.1% e.e.) gave 1b (2.2 g, 61%). $[\alpha]_D^{20} +104.9$ (c 1.06, CHCl$_3$). Its IR, MS and NMR spectra were identical with those of racemate 1. HRMS $m/z$ (M$^+$): calcd. for C$_{12}$H$_{20}$O, 180.1514; found, 180.1532. The enantiomeric purity of resulting 1b was found to be 97.8% e.e. by GLC analysis: $t_R$ 73.4 min [1.1%, 1a], $t_R$ 75.6 min [98.9%, 1b].

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