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Biotransformation of (±)-α-ionone and β-ionone by cultured cells of *Caragana chamlagu*

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Abstract

Suspension cultures of *Caragana chamlagu* (Leguminosae) convert (±)-α-ionone (**1**) into (±)-3-oxo-α-ionone (**3**) as the major product and β-ionone (**2**) into 5,6-epoxy-β-ionone (**6**) as the sole product. It is interesting to note that the cultured cells of *C. chamlagu* convert regioselectively the cycloolefinic part of **1** into the corresponding unsaturated carbonyl compound, allylic alcohol and epoxide as the oxidation products, whereas the suspension cultures of *Nicotiana tabacum* (Solanaceae) convert the unsaturated carbonyl of **1** into the corresponding saturated ketones and alcohols as reduction products.

Keywords: Biotransformation; Plant catalyst; *Caragana chamlagu*; (±)-α-Ionone; β-Ionone

1. Introduction

The introduction of a functional group into terpenoids and steroids is an important reaction in synthetic chemistry. Many studies have been reported on the specific oxidation and reduction of olefins and alicyclic hydrocarbons with chemical reagents [1–3]. However, environmentally sensitive, milder, energy-saving and stereospecific methods have been recently required for synthetic chemistry. Therefore, plant cell cultures are considered to be useful biocatalysts for reactions such as hydroxylation at allylic positions, oxidation–reduction between an alcohols and ketones, and the reduction of carbon–carbon double bonds [4,5].

Racemic (±)-α-ionone (**1**) and β-ionone (**2**) are important materials in the fragrance industry. In addition, the related materials of **1** include many compounds, which show bioactivity [6,7], and **2** are important as a starting material for the chemical synthesis of abscisic acid [8,9].

Tang and Suga [10] reported that the biotransformation of **1** and **2** by the immobilized cells of *Nicotiana tabacum* (Solanaceae) is efficient in transforming an unsaturated ketone into the corresponding saturated ketone and alcohol.

In a previous paper [11], we revealed that *Caragana chamlagu* (Leguminosae) cell cultures converted thujopsene, which has molecular distortion due to a cyclopropane ring, into mayurone as the major product.

In this study, the biotransformation of **1** and **2** was carried out to examine the ability of the cultured cells of *C. chamlagu*.

The results of biotransformation of **1** and **2** are shown in Scheme 1. The incubation of **1** with suspension cultures of *C. chamlagu* for 20 days gave racemic (±)-3-oxo-α-ionone (**3**) in 50% yield. The spectral data for compound **3** agreed with those of an authentic sample [12].

Product (**4**) isolated in 16% yield had a band at 3492 cm^{-1} (OH) in its IR spectrum, and the HREIMS gave M^+ 208.1467 ($C_{13}H_{20}O_2$). The ^{13}C NMR spectrum showed four tertiary methyl, three quaternary, one methylene, and five methine carbon signals. The ^1H NMR spectrum showed a methine proton signal at δ 4.25 (1H, br m, CH–OH). The relative stereochemistry of **4** was assumed from the NOESY spectrum, which showed an H–H correlation between the methine proton signal at C-3 and methyl proton signal at C-12. Furthermore, the stereochemistry of **4** was assumed

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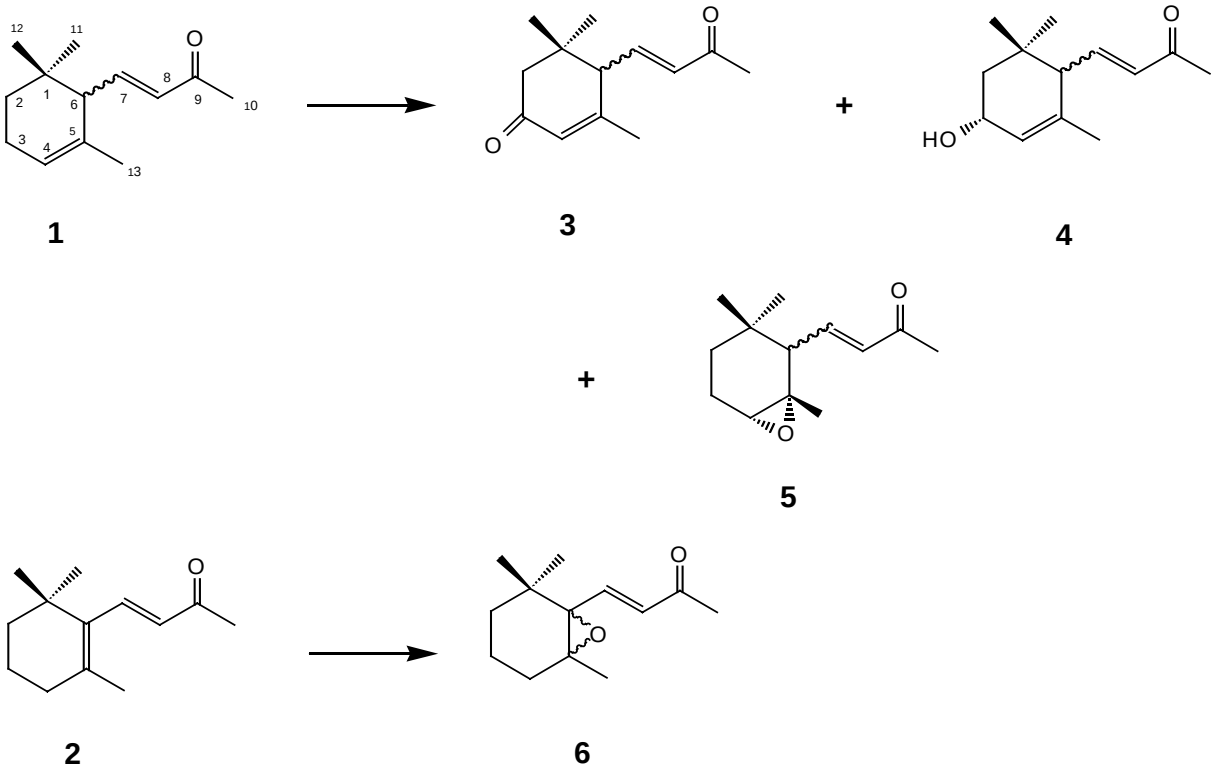

Scheme 1. Biotransformation of (**1**) and (**2**).

from the NOE experiment where enhancement was observed between 12β-H and 3β-H [13]. On the basis of these spectral data and its optical rotation, **4** was determined to be racemic (±)-3α-hydroxy-α-ionone.

Product (**5**) (20% yield) gave HREIMS M^+ 208.1496 ($C_{13}H_{20}O_2$) and the ^{13}C NMR spectrum revealed four tertiary methyl, three quaternary, two methylene, and four methine carbon signals. The ^1H NMR spectrum showed a methine proton signal at δ 3.11 (1H, t, $J = 2$ Hz CH–O). The relative stereochemistry of **5** was deduced from the NOE experiment, which showed enhancement between 4β-H and 13β-H, 13β-H and 6β-H, and 6β-H and 12β-H, and thus the epoxy group at C4–C5 has a α-configuration [14]. On the basis of these spectral data and its optical rotation, 5 is racemic (±)-4,5α-epoxy-α-ionone.

Compared with the optical rotation of reaction products, no transformation of **1** by *C. chamlagu* gave the optically substances.

On the other hand, the incubation of **2** with *C. chamlagu* cultured cells under the same conditions gave the epoxy ketone (**6**) in 87% yield. The HREIMS of **6** gave M^+ 208.1466 ($C_{13}H_{20}O_2$) and four tertiary methyl, four quaternary, two methine, and three methylene carbon signals in the ^{13}C NMR spectrum. The relative stereochemistry of **6** was assumed from its optical rotation [14] and determined to be a mixture of 5α- and 5β-epoxy-β-ionones (60:40).

The time courses for biotransformations of **1** and **2** by *C. chamlagu* cultured cells are shown (Figs. 1 and 2, respectively). Biotransformation of **1** gave **3** as a major product, which steadily increased throughout the growth cycles whereas biotransformation of **2** afforded **6** as the sole product.

In order to prove the enzymes involved in the bioconversion of **1** by the *C. chamlagu*, cultures were grown in the presence of 1-aminobenzotriazole, cytochrom P450 inhibitor. As a result, **3**, **4** and **5** were obtained in 33%, 25% and 15% yields, respectively. Furthermore, biotransformation of **1** by peroxidase-H_2O_2 did not proceed. However, the enzyme system in biotransformation of **1** by *C. chamlagu* is investigating at the present time.

In conclusion, we have found that the *C. chamlagu* cultures convert regioselectively the cycloolefinic part structure in substrate into the corresponding unsaturated ketone, allyl alcohol and epoxide. Bioconversion of **1** having cyclic trisubstituted double bond produces regiospecificaly an unsaturated carbonyl compound as the major product, while **2** having cyclic tetra substituted double bond afford an epoxide as the sole product. It is interesting to note that the cell culture of *C. chamlagu* (Leguminosae) convert stereoselectively the cycloolefinic part structure of **1** into the corresponding unsaturated carbonyl compound, allylic alcohol and epoxide as the oxidation products, whereas the cell culture of *N. tabacum* (Solanaceae) convert an unsaturated carbonyl group of **1** into the corresponding saturated ketones and alcohols as reduction products [10].

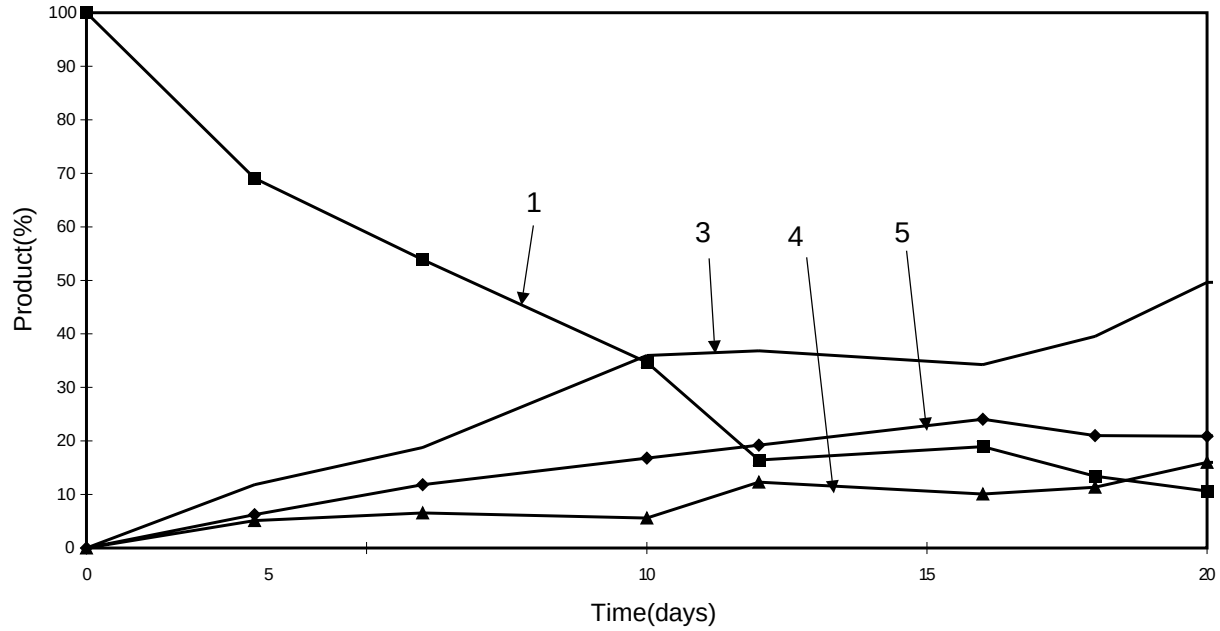

Fig. 1. Time course in the biotransformation of (±)-α-ionone(**1**).

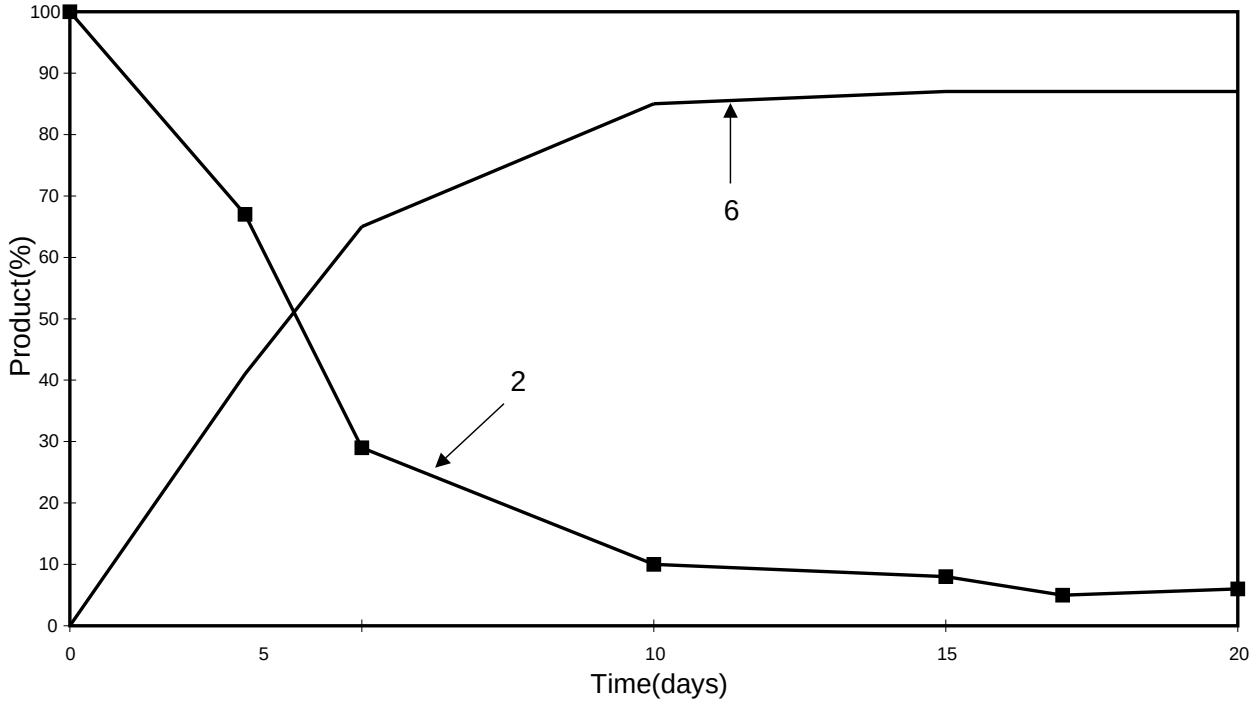

Fig. 2. Time course in the biotransformation of β-ionone(**2**).

2. General experimental procedures

IR spectra were recorded using a Hitachi 270-50 and a Jasco FT-IR 230 spectrophotometer. Optical rotation was determined using a Horiba SEPA-200 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz using a JEOL GX-400 spectrometer with $SiMe_4$ as the internal reference. High resolution mass spectra (HREIMS) were obtained on a Hitachi M-80B spectrometer. GC-MS were recorded a Shimadzu GC–MS QP5050 (EI-MS) 70 eV using DVI (0.25 mm × 30 m, 0.25 μm) capillary column. GLC was performed on Shimadzu GC-9A and 14A model equipped with 2% OV-17 column (5 mm × 3 m) at 170 °C.

2.1. Substrates

(±)-α-Ionone (**1**) and β-ionone (**2**) were purchased from Aldrich Chem. Co. The purity of each was >98% by GLC

analysis. Peroxidase and 1-aminobenzotriazole were purchased from Wako Chem. Ind. Ltd.

2.2. Cultures

Callus tissues from leaves of *C. chamlagu* (Leguminosae) have been maintained our laboratory for approximately 8 years.

Unless otherwise stated all cultures were grown in MS medium plus 3% sucrose and 2,4-dichlorophenoxyacetic acid at 25 °C for 20 days in the dark on an orbital shaker (120 rpm).

2.3. Biotransformation of (1)

The callus tissues (2 g) were transferred to MS–medium (100 ml) containing 2 ppm of 2,4-D and 3% sucrose. (±)-α-Ionone (**1**, 60 mg) was added to the suspension in several 300 ml flask containing 100 ml MS-medium. The filtered culture medium was extracted with EtOAc, the solvent dried over Na_2SO_4, and removed in vacuo. The residue was chromatographed on SiO_2, using benzene–EtOAc (10:1) to give (±)-4,5-epoxy-α-ionone (**5**, 20% by GLC). Elution with benzene–EtOAc (5:1) afforded (±)-3-oxo-α-ionone (**3**, 50% by GLC). Elution with benzene–EtOAc (5:1) gave (±)-3α-hydroxy-α-ionone (**4**, 16% by GLC).

2.4. Biotransformation of (1) containing 1-aminobenzotriazole by C. chamlagu

(±)-α-Ionone (**1**, 60 mg) was added to a suspension culture (2 g callus) in 300 ml flask containing 100 ml MS-medium and 1-aminobenzotriazole (52 mg). Culture medium was extracted as above, the products were separated and assay by GLC giving **3**, **4** and **5** in 33, 25 and 15% yields, respectively.

2.5. Biotransformation of (2) by C. chamlagu

β-Ionone (**2**, 60 mg) was added to a suspension culture (from 2 g callus) in 300 ml flask containing 100 ml MS-medium. Metabolites were extracted as above and chromatographed on SiO_2, using benzene–EtOAc (10:1) as eluent, to give (±)-5,6-epoxy-β-ionone (**6**, 86% by GLC).

2.6. Time course experiment

A callus of *C. chamlagu* (2 g) was transferred to 50 ml MS-culture medium in a 100 ml Erlenmeyer flask grown with continuous shaking for 3 days at 25 °C in the dark. The substrate (60 mg) was added to the suspension culture and incubated at 25 °C in a rotary shaker (120 rpm) in the dark. At regular intervals, several of the flasks were taken out and the incubation mixture was filtered and extracted with EtOAc. The yield of the products were determined on the basis of the peak area from GLC traces.

(±)-3-Oxo-α-ionone (**3**): oil; $[\alpha]_D$: −4°(c 0.45, $CHCl_3$); IR ($CHCl_3$): ν 1660, 1625 cm^{-1}, ^1H NMR ($CDCl_3$): δ 1.01 (3H, s, H-11), 1.08 (3H, s, H-12), 1.89 (3H, s, H-13), 2.28 (3H, s, H-10), 2.71 (1H, d, J = 10 Hz, H-6), 5.98 (1H, br s, 4-H), 6.18 (1H, br s, J = 15 Hz, H-8), 6.60 (1H, q, J = 6, 10 Hz, H-7); ^{13}C NMR ($CDCl_3$): δ 198.3 (s, C-3), 197.5 (s, C-9), 159.1 (s, C-5), 143.6 (d, C-7), 133.8 (d, C-8), 127.0 (d, C-4), 55.5 (d, C-6), 47.4 (t, C-2), 36.7 (s, C-1), 27.9 (q, C-10), 27.6 (q, C-12), 27.3 (q, C-11), 23.5 (q, C-13); HREIMS *m/z*: 206.1306 (calcd for $C_{13}H_{28}O_2$, 206.1307).

(±)-3α-Hydroxy-α-ionone (**4**): oil; $[\alpha]_D$: −1.2°(c 0.45, $CHCl_3$); IR ($CHCl_3$): ν 3492, 1658, 1615 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.89 (3H, s, H-11), 0.98 (3H, s, H-12), 1.63 (3H, s, H-13), 2.26 (3H, s, H-10), 2.28 (1H, d, J = 2 Hz, H-6), 4.25 (1H, br m, H-3), 5.59 (1H, br s, H-4), 6.08 (1H, d, J = 16 Hz, H-8), 6.63 (1H, dd, J = 10,16 Hz, H-7); ^{13}C NMR ($CDCl_3$): δ 198.4 (s, C-9), 147.7 (d, C-7), 135.5 (s, C-5), 132.7 (d, C-8), 126.4 (d, C-4), 66.5 (d, C-3), 54.3 (d, C-6), 40.7 (t, C-2), 35.0 (s, C-1), 29.1 (q, C-11), 27.0 (q, C-12), 26.9 (q, C-10), 22.4 (q, C-13). HREIMS *m/z*: 208.1467 (calcd for $C_{13}H_{20}O_2$, 208.1463).

(±)-4,5-Epoxy-α-ionone (**5**): oil; $[\alpha]_D$: −1.3°(c 0.67, $CHCl_3$); IR ($CHCl_3$): ν 1666, 1625 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.75 (3H, s, H-12), 0.93 (3H, s, H-11), 1.26 (3H, s, H-13), 2.09 (1H, d, J = 10 Hz, H-6), 2.31 (3H, s, H-10), 3.11 (1H, t, J = 2 Hz, H-4), 6.10 (1H, d, J = 6 Hz, H-8), 6.73 (1H, dd, J = 10,16 Hz, H-7); ^{13}C NMR ($CDCl_3$): δ 198.8 (s, C-9), 146.4 (d, C-7), 134.4 (d, C-8), 59.5 (d, C-4), 58.8 (s, C-5), 52.5 (d, C-6), 31.2 (s, C-1), 28.5 (t, C-3), 27.9 (q, C-11), 27.5 (q, C-12), 26.4 (q, C-13), 24.0 (q, C-10), 21.7 (t, C-2); HREIMS *m/z*: 208.1496 (calcd for $C_{13}H_{20}O_2$, 208.1463). (±)-5,6-Epoxy-β-ionone (**6**): oil; $[\alpha]_D$: −7.6°(c 0.67, $CHCl_3$); IR ($CHCl_3$): ν 1700, 1680, 1630 cm^{-1}, ^1H NMR ($CDCl_3$): δ 0.94 (3H, s, H-11), 1.15 (6H, s, H-12,13), 2.28 (3H, s, H-13), 6.30 (1H, d, J = 15 Hz, H-8), 7.02 (1H, d, J = 16 Hz, H-7); ^{13}C NMR ($CDCl_3$): δ 197.6 (s, C-9), 142.7 (d, C-7), 132.5(d, C-8), 70.6 (d, C-6), 65.9 (s, C-5), 35.5 (t, C-2), 33.6 (s, C-1), 29.8 (t, C-4), 28.3 (q, C-10), 25.9 (q, C-12), 25.8 (q, C-11), 20.8 (q, C-13), 16.9 (t, C-3); HREIMS *m/z*: 208.1466 (calcd for $C_{13}H_{20}O_2$, 208.1463).

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