# Biotransformation of Thujopsene by *Caragana chamlagu*

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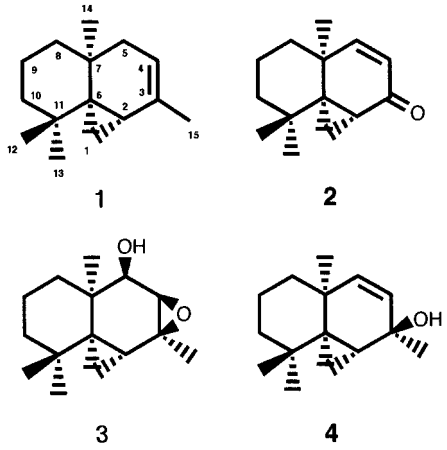
Biotransformation of thujopsene (**1**) using a cell suspension culture of *Caragana chamlagu* for 14 days gave mayurone (**2**, 52%) and two new compounds, 3$\beta$-hydroxy-4-thujopsene (**4**, 16%) and 3$\beta$-epoxythujopsa-5$\beta$-ol (**3**, 22%).

Biotransformation of foreign substrate into more useful substances by cultured plant cells is one of the important reactions in synthetic chemistry.[1,2] Suga and Hirata reported on recent progress in the biotransformation of exogenous substrate by plant cell cultures.[3] Kitanaka et al. reported that *Caragana chamlagu* (Leguminosae) produced the antiinflamatory principle (+)-viniferine, and callus cell cultures have been prepared to study biosynthesis of this compound.[4] However, no biotransformation of terpenes using cultured cells of *C. chamlagu* has been reported. It was of interest to investigate the behavior of the cultured cells toward terpenes having molecular distortion due to a cyclopropane ring. Here we report the biotransformation of thujopsene (**1**) by a cell suspension culture of *C. chamlagu*.

Biotransformation results of thujopsene (**1**) are summarized in Table 1. Incubation of **1** with a cell suspension of *C. chamlagu* for 14 days gave mayurone (**2**) as the major product. Its melting point and spectral data were in agreement with an authentic sample of **2** obtained by Collins oxidation of **1**.[5] Compound **2** is known to occur in certain species of the Coniferales.[6,7] Product (**3**, mp 90−92 °C, $[\alpha]_D$ +4.7°) was isolated in 22% yield. Compound **3** had a band at 3492 cm$^{-1}$ (OH) in its IR spectrum, and HREIMS of **3** gave a molecular ion peak at $M^+$ *m/z* 236.1753 ($C_{15}H_{24}O_2$). The $^1$H NMR spectrum showed methine proton signals at $\delta$ 2.78 (1H, d, 4.2 Hz CH−OH) and $\delta$ 3.00 (1H, q, 2 Hz CH−O), respectively. The $^{13}$C NMR spectrum showed four tertiary methyls, four quaternary carbons, four methylenes, and three methine carbon signals. The HMBC spectrum of compound **3** showed a C−H correlation between the signal of a methyl proton at C-14 and those of C-5, C-6, C-7, and C-8 and between the signal of a methyl proton at C-15 and those of C-2, C-3, and C-4. The relative stereochemistry of **3** was assigned from an NOE experiment. Enhancements were observed between 14α-H and 5α-H, between 5α-H and 4α-H, and between 4α-H and 15α-H. As a result, it was determined that the hydroxyl group at C-5 and an epoxy group at C3−C4 have a $\beta$-configuration. On the basis of these spectral data, compound **3** was determined to be 3$\beta$-epoxythujopsa-5$\beta$-ol.

**Table 1.** Biotransformation of Thujopene (**1**) by a Cell Suspension Culture of *C. chamlagu*

| culture time (days) | yield of products (%) | | | | |
|---|---|---|---|---|---|
| | recov. **1** | **2** | **3** | **4** | others |
| 7 | 89 | 5 | | 6 | |
| 12 | 12 | 23 | 15 | 46 | 4 |
| 14 | 5 | 52 | 22 | 16 | 5 |




Compound **4** ($[\alpha]_D$ −30.1°) was isolated in 16% yield. Compound **4** had a band at 3408 cm$^{-1}$ (OH) in its IR spectrum, and HREIMS of **4** gave a molecular ion peak at $M^+$ *m/z* 220.1819 ($C_{15}H_{24}O$). The $^1$H NMR spectrum showed methine proton signals at $\delta$ 5.14 (1H, dd, 12 Hz CH=CH) and $\delta$ 5.36 (1H, d, 10 Hz CH=CH), respectively. The $^{13}$C NMR spectrum showed four tertiary methyls, four quaternary carbons, four methylenes, and three methine carbon signals. The HMBC spectrum of **4** showed a C−H correlation between the signal of a methyl proton at C-14 and those of C-5, C-6, C-7, and C-8 and between the signal of a methyl proton at C-15 and those of C-2, C-3, and C-4. The relative stereochemistry of **4** was assigned from the NOESY experiment. NOESY enhancements were observed between 14α-H and 5-H, between 5-H and 4-H, and between 4-H and 15α-H. As a result, it was determined that the hydroxyl group at C-3 has a $\beta$-configuration. On

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the basis of these spectral data, compound **4** was determined to be 3$\beta$-hydroxy-4-thujopsene.

After 12 days, **4** was obtained as the major product (46% yield). After 14 days, **4** decreased and **2** was increased in 52% yield. Therefore, it is considered that the biotransformation of **1** by *C. chamlagu* proceeds through a rearrangement of double bonds and then demethylation of **4**. For the formation of **3**, it was assumed that hydroxylation of **1** occurs at the allylic position and then epoxidation of the double bond proceeds.

In conclusion, the biotransformation of **1**, having a molecular distortion due to the cyclopropane group, yields the $\alpha,\beta$-unsaturated ketone **2** as the major product after 14 days. Hydroxylation at the allylic position and regio- and stereoselective epoxidation yields alcohol **3**. This biotransformation afforded a simple new route to mayurone (**2**).

## Experimental Section

**General Experimental Procedures.** Melting points were measured using a Shimazu apparatus and are uncorrected; IR spectra were recorded using a Hitachi 270−50 spectrophotometer. Optical rotations were determined using a Horiba SEPA-200 polarimeter. $^1$H NMR and $^{13}$C NMR measurements ($\delta$ in ppm) were obtained using a JEOL JNM GX 400 NMR spectrophotometer with $SiMe_4$ as the internal reference. Mass spectra were recorded using a Hitachi M-80B spectrometer. HLPC was performed using a Gasukuro Kogyo Model 576 apparatus. GLC was performed on a Shimazu GC-9A gas chromatograph equipped with a 2% OV-17 column (5 mm × 3 m) at 170 °C. Si gel (Merck) was used for column chromatography.

**Substrate**. Thujopsene (**1**) was supplied by T. Hasegawa Co., Ltd. The purity of **1** was >98% by GLC analysis.

**Biotransformation Experiments.** Callus tissues from leaves of *Caragana chamlagu* (Leguminosae) have been maintained in our laboratory for approximately 6 years. The callus tissues (2 g) were transferred to MS-medium (100 mL) containing 2 ppm of 2,4-dichlorophenoxy acetic acid and 3% sucrose and then were grown with continuous shaking (125 rpm) for 5 days at 25 °C in the dark. Thujopsene (**1**, 60 mg) was added to the suspension cells in 300 mL flasks containing 100 mL of MS-medium, and the cultures were incubated at 25 °C in the dark for 14 days on a shaker. Culture medium was filtered from the callus and extracted with ether. The organic layer was dried over $Na_2SO_4$, and the solvent was removed in vacuo. The residue was chromatographed on Si gel. Elution with benzene−ethyl acetate (20:1) gave **4** (16% by GLC). The next fraction, eluted with benzene−ethyl acetate (15:1), gave mayurone **2** (52% by GLC). The third fraction, eluted with benzene−ethyl acetate (10:1), afforded **3** (22% by GLC).

**Collins Oxidation of 1.**[5] Thujopsene (**1**, 350 mg) in dry $CH_2Cl_2$ (20 mL) was added at 0 °C to the $CrO_3$−$(C_5H_5N)_2$ complex as a slurry in dry $CH_2Cl_2$ (20 mL). After 16 h of stirring at room temperature, $CH_2Cl_2$ solution was passed through a Florisil column and then washed with aqueous HCl. The organic layer was dried over $Na_2SO_4$, and the solvent was removed in vacuo. The crude product was chromatographed on Si gel, using benzene−ethyl acetate (20:1) as the eluent, to give mayurone (**2**) (87 mg, mp 66 °C, $[\alpha]_D$ +229 (*c* 0.46, $CHCl_3$)).[5]

**Mayurone (2):** white solid; mp 65−66 °C; $[\alpha]_D$ +229.1 (*c* 0.46, $CHCl_3$), IR ($CHCl_3$) $\nu_{max}$ 1658 (C=O), 1625 (C=C) cm$^{-1}$; $^1$H NMR (400 MHz $CDCl_3$) $\delta$ 0.65 (3H, s, 12-H), 1.14 (3H, s, 13-H), 1.35 (3H, s, 14-H), 5.60 (1H, dd, 4-H), 6.10 (1H, d, 5-H); $^{13}$C NMR (100 MHz $CDCl_3$) $\delta$ 199.4 (s, C-3), 158.8 (d, C-5), 121.7 (d, C-4), 28.6 (q, C-12), 27.3 (q, C-13), 25.9 (q, C-14); HREIMS *m*/*z* 204.1513 (calcd for $C_{14}H_{20}O$ 204.1514).

**3$\beta$-Epoxythujopsa-5$\beta$-ol (3):** white solid; mp 90−92 °C, $[\alpha]_D$ +4.7 (*c* 0.51, $CHCl_3$), IR ($CHCl_3$) $\nu_{max}$ 3492 cm$^{-1}$; $^1$HNMR (400 MHz $CDCl_3$) $\delta$ 0.54 (3H, s, 12-H), 1.01 (3H, s, 13-H), 1.35 (3H, s, 14-H), 1.43 (3H, s, 15-H), 2.78 (1H, d, 4.2 Hz, 5-H), 3.00 (1H, dd, 2 Hz, 4-H); $^{13}$C NMR (100 MHz $CDCl_3$) $\delta$ 67.9 (d, C-5), 67.9 (s, C-3) 60.5 (d, C-4), 28.4 (q, C-12), 40.4 (t, C-10), 36.7 (t, C-8), 34.6 (s, C-6), 33.7 (s, C-11), 31.7 (s, C-7), 30.3 (d, C-2), 27.8 (q, C-13), 27.4 (q, C-15), 24.7 (q, C-14), 17.9 (t, C-9), 9.3 (t, C-1); HREIMS *m*/*z* 237.1753 (calcd for $C_{15}H_{24}O_2$ 236.1776 ).

**3$\beta$-Hydroxy-4-thujopsene (4):** colorless oil; $[\alpha]_D$ −30.1° (*c* 0.95, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 3408 cm$^{-1}$; $^1$H NMR (400 MHz $CDCl_3$) $\delta$ 0.65 (3H, s, 12-H), 1.05 (3H, s, 13-H), 1.18 (3H, s, 14-H), 1.35 (3H, s, 15-H), 5.14 (1H, dd, 12 Hz, 4-H), 5.36 (1H, d, 10 Hz, 5-H); $^{13}$C NMR (100 MHz $CDCl_3$) $\delta$ 145.5 (d, C-5), 121.3 (d, C-4), 81.4 (s, C-3), 40.2 (t, C-10), 38.9 (t, C-8), 36.5 (s, C-7), 32.9 (s, C-11), 31.2 (s, C-6), 29.3 (q, C-12), 27.4 (q, C-14), 27.3 (q, C-13), 26.3 (d, C-2), 24.2 (q, C-15), 18.5 (t, C-9), 9.0 (t, C-1); HREIMS *m*/*z* 220.1819 (calcd for $C_{15}H_{24}O$ 220.1829).

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