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The biocatalytic oxidation of thujopsene by plant cultured-cells

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Abstract

The biotransformation of thujopsene (**1**) using plant suspension cultured-cells was investigated. It was found that the cultured-cells oxidate thujopsene to 3β-hydroxy-4-thujopsene (**2**), mayurone (**3**), and 3β-epoxythujopsan-5β-ol (**4**). And, it was shown that the biotransformation activity of cultured-cells of *Hibiscus cannabinus* is significantly higher than that of cultured-cells of *Nicotiana tabacum* and *Catharanthus roseus*.

Keywords: Plant cultured-cells; Biocatalytic oxidation; Thujopsene; Mayurone; *Hibiscus cannabinus*

1. Introduction

Biotransformation of exogenous substrates has been studied extensively in plant cultured-cells [1]. During the course of our studies, we have reported: the oxidative cleavage of 3,6-dialkylcyclohexane-1,2-diones by cultured-cells of *Marchantia polymorpha* [2], and *Caragana chamlagu* [3]; the biocatalytic oxidation of thujopsene using cultured-cells of *C. chamlagu* [4].

The introduction of a functional group into terpenoids is important reaction in synthetic chemistry. Many studies have been reported on specific oxidation and reduction of alkenes and alicyclic compounds are known [5–7]. Recently, we succeeded to induce callus tissues from stems of *Hibiscus cannabinus* (Kenaf). In order to investigate the ability of cultured-cells of *H. cannabinus* as a new biocatalyst, we studied the biotransformation of thujopsene. However, chemical oxidation of the tricyclic sesquiterpene thujopsene (**1**), which was originally isolated from the wood neutral oil of the Japanese Hiba tree, generally research is complex mixtures. Some papers have been reported mayurone as an oxidation product of thujopsene [8–11]. Further, we wanted to compare the biotransformation activity of cultured-cells of *H. cannabinus* with that of cultured-cells of *Nicotiana tabacum* and *Catharanthus roseus*.

2. Experimental

The cultured-cells of *Nicotiana tabacum* and *Catharanthus roseus* were provided by Prof. H. Hamada, Department of Applied Science, Faculty of science, Okayama University of Science.

2.1. Analytical and substrates

Melting points were determined on a Yanaco micro melting point apparatus. IR spectra were recorded on a Jasco FT-IR 230 spectrometer. Optical rotations were determined using a Horiba SEPA-200 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded in a JEOL GSX 400 spectrometer. Samples were carried out $CDCl_3$ with tetramethylsilane as internal standard. GC-MS (EI) analyses were performed on a Shimadzu GC-MS QP5050 with an ionizing energy of 70 eV. HRMS (EI) analyses were performed on a JMS-GC mate II/HP-6890 with an ionizing energy of 70 eV. HREIMS analyses were performed on a Hitachi M-80B spectrometer.

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

2.2. Cultivation of suspension cells

The callus tissues of *N. tabacum* were transferred to freshly prepared MS medium [12] containing 1 ppm of 2,4-dichlorophenoxyacetic acid and 3% sucrose, and then were grown under continuous shaking (110 rpm) at 25 °C in the light (2000 lx).

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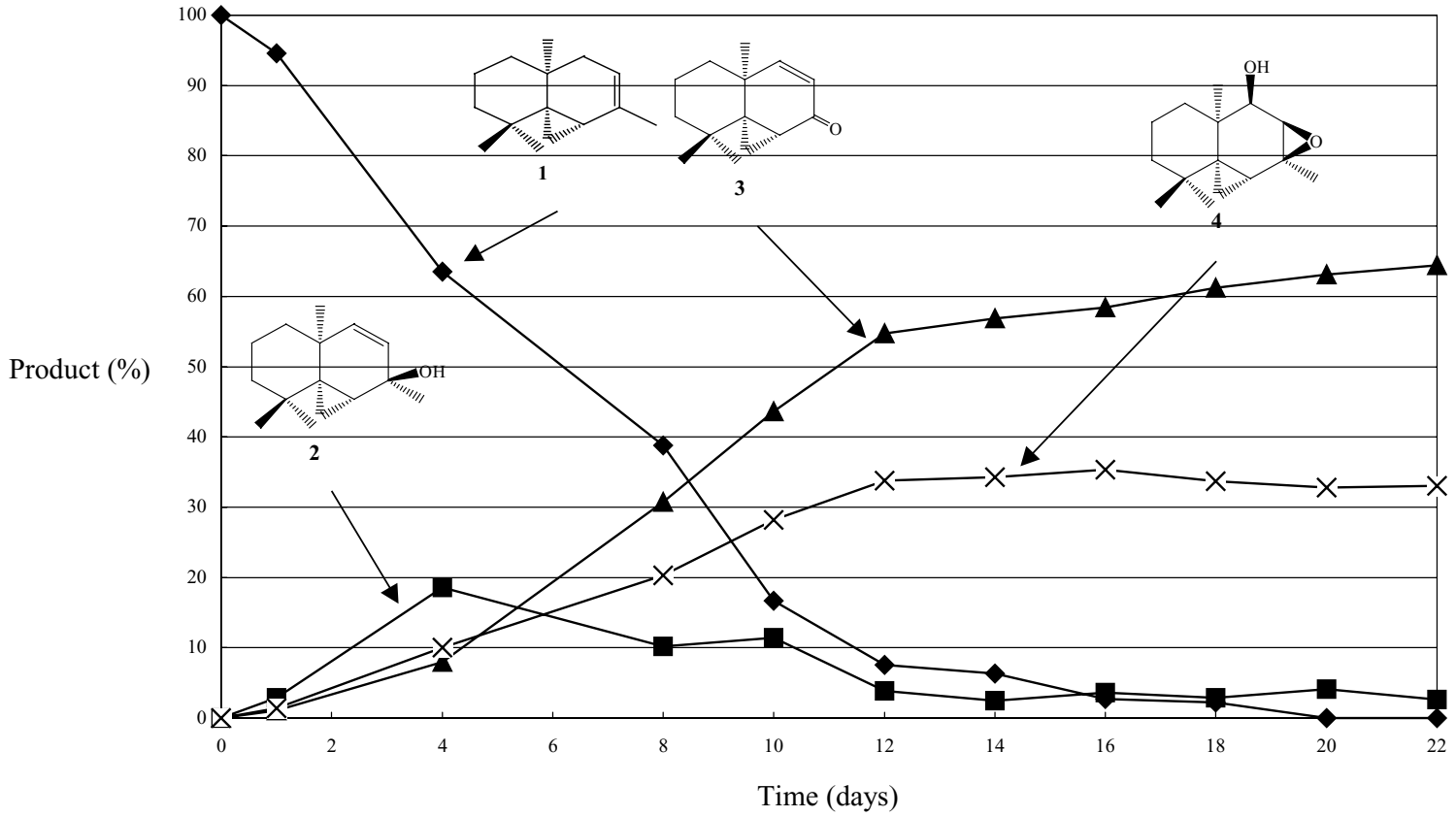


Fig. 1. Biotransformation of thujopsene (**1**) using *H. cannabinus*.

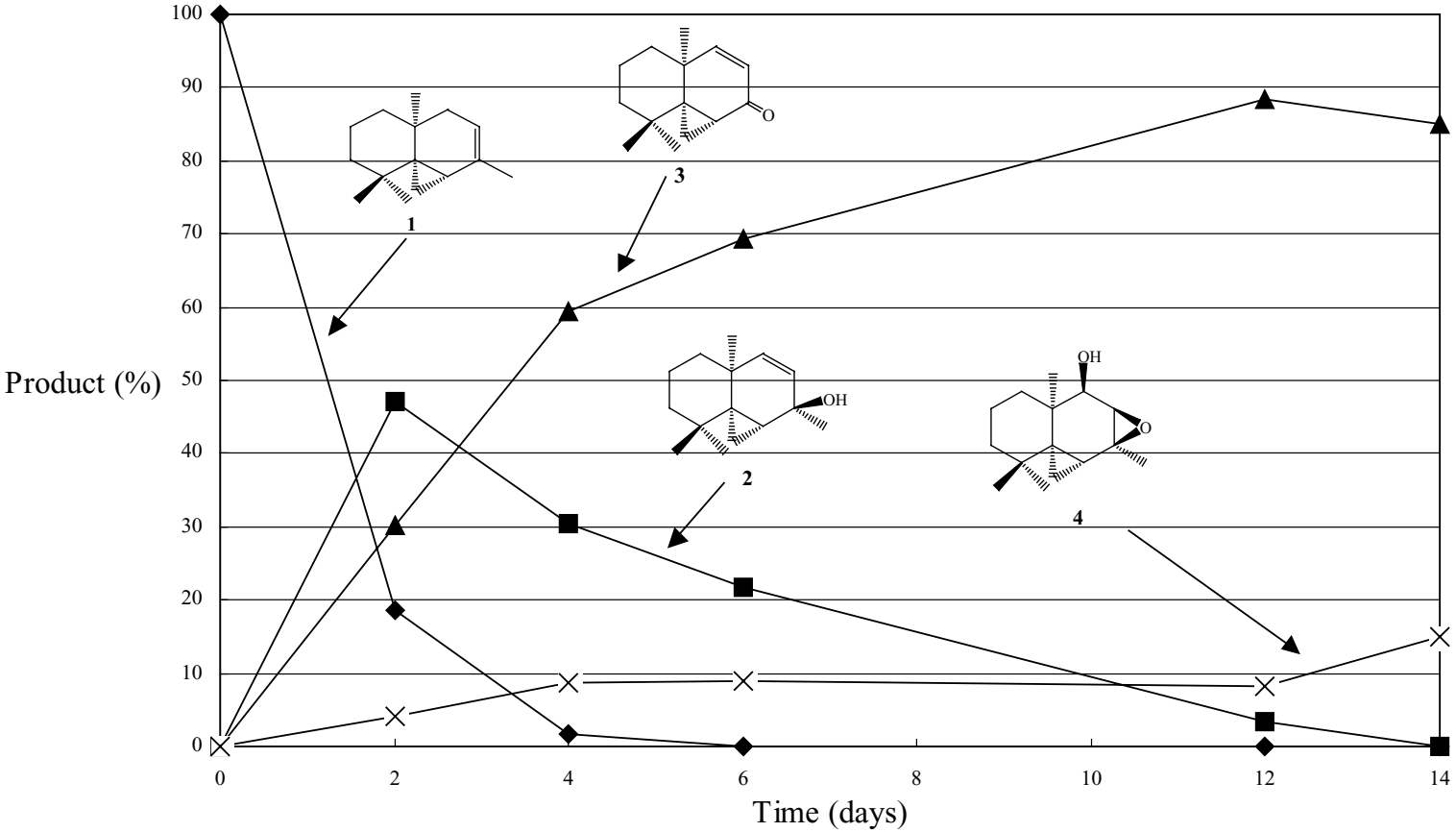


Fig. 2. Biotransformation of thujopsene (**1**) using *N. tabacum*.

The callus tissues of *C. roseus* were transferred to freshly prepared SH medium [13] containing 2 ppm of 2,4-dichlorophenoxyacetic acid and 3% sucrose, and then were grown under continuous shaking (110 rpm) at 25 °C in the light (2000 lx).

For induce the callus tissues of *H. cannabinus*, the tissues were obtained by surface sterilization of stem for 30 s in 70% EtOH. The tissues of stem were washed twice with sterile distilled water and were then transferred to agar MS medium. The callus tissues of *H. cannabinus* were transferred to freshly prepared MS medium containing 1 ppm of 2,4-dichlorophenoxyacetic acid and 3% sucrose, and were grown under continuous shaking (110 rpm) at 25 °C in the dark.

2.3. Incubation of thujopsene (*1*)

In the case of *N. tabacum* (or *C. roseus*), the callus tissues (30 g) were transferred to MS medium (100 ml) and the suspension cell was incubated under shaking (110 rpm) at 25 °C in the light for 8 days. Thujopsene (**1**, 40 mg) was added to suspension cells and then suspension cells were incubated.

In the case of *H. cannabinus*, the callus tissues (2 g) were incubated under shaking (110 rpm) at 25 °C in the dark for 5 days. Thujopsene (**1**, 60 mg) was added to suspension cells and then suspension cells were incubated.

After incubation, plant cultured-cells were removed by filtration, and then filtrate was extracted with EtOAc-Et_2O (1:1). Evaporation of the solvent gave a yellow oil. Then, the residue was chromatographed on silica gel. Elution with *n*-hexane-EtOAc (3:1) gave 3β-hydroxy-4-thujopsene (**2**), mayurone (**3**), and 3β-epoxythujopsan-5β-ol (**4**) as judged by GC-MS and NMR [4].

3. Results and discussion

3.1. Biotransformation of thujopsene (*1*) using *H. cannabinus*

The biotransformation results are summarized in Fig. 1. The biotransformation of thujopsene (**1**, 60 mg) using cultured-cells of *H. cannabinus* gave a mixture (34 mg) for 20 days in aqueous solution at room temperature in the dark. Elution with *n*-hexane-EtOAc (3:1) gave three products: 3β-hydroxy-4-thujopsene (**2**, 4%), mayurone (**3**, 63%), and 3β-epoxythujopsan-5β-ol (**4**, 33%) were identified by their IR, HREIMS, GC-MS, and NMR spectroscopic analysis.

3.2. Biotransformation of thujopsene (*1*) using *N. tabacum* and *C. roseus*

The biotransformation results are summarized in Figs. 2 and 3. In the case of cultured-cells of *N. tabacum*, substrate **1** (40 mg) disappeared after 6 days. In a time-course experiment, it is shown that 3β-hydroxy-4-thujopsene (**2**) was the major product after 2 days rotating incubation. After 14 days, 30 mg reaction mixture was obtained. From

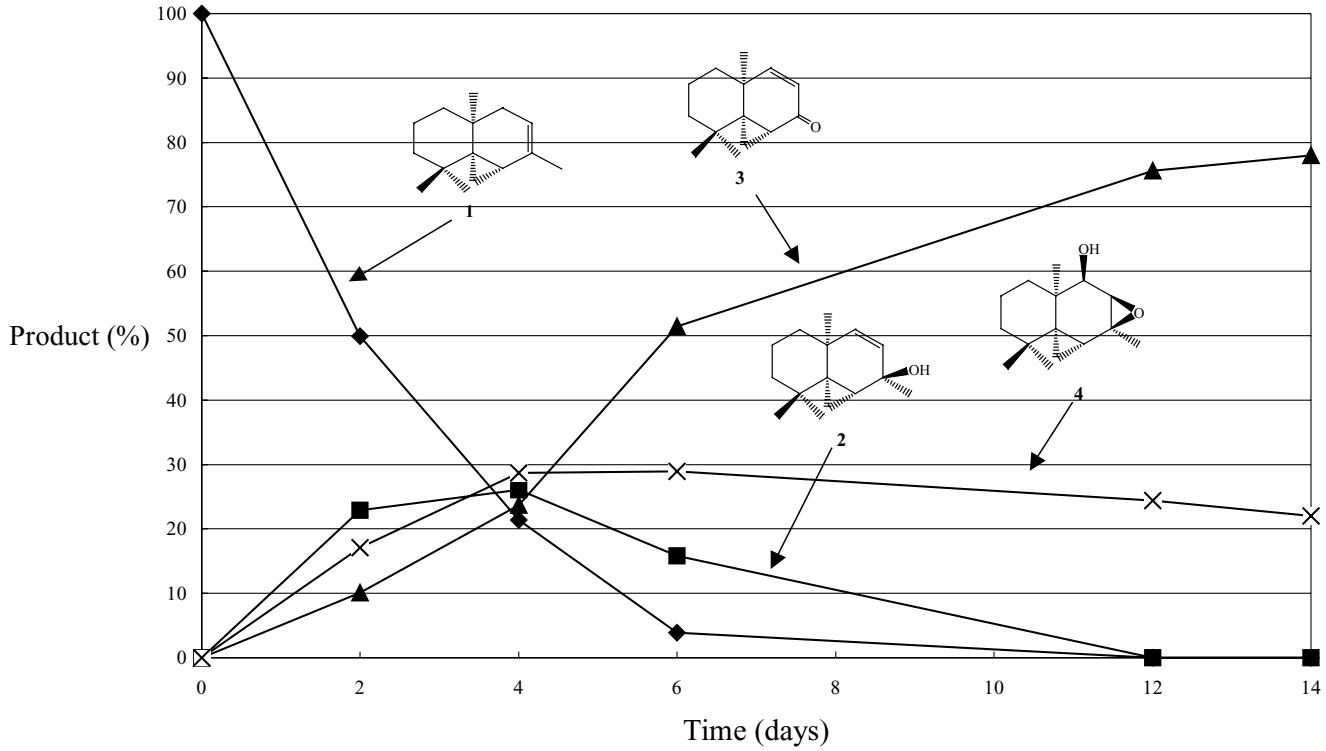
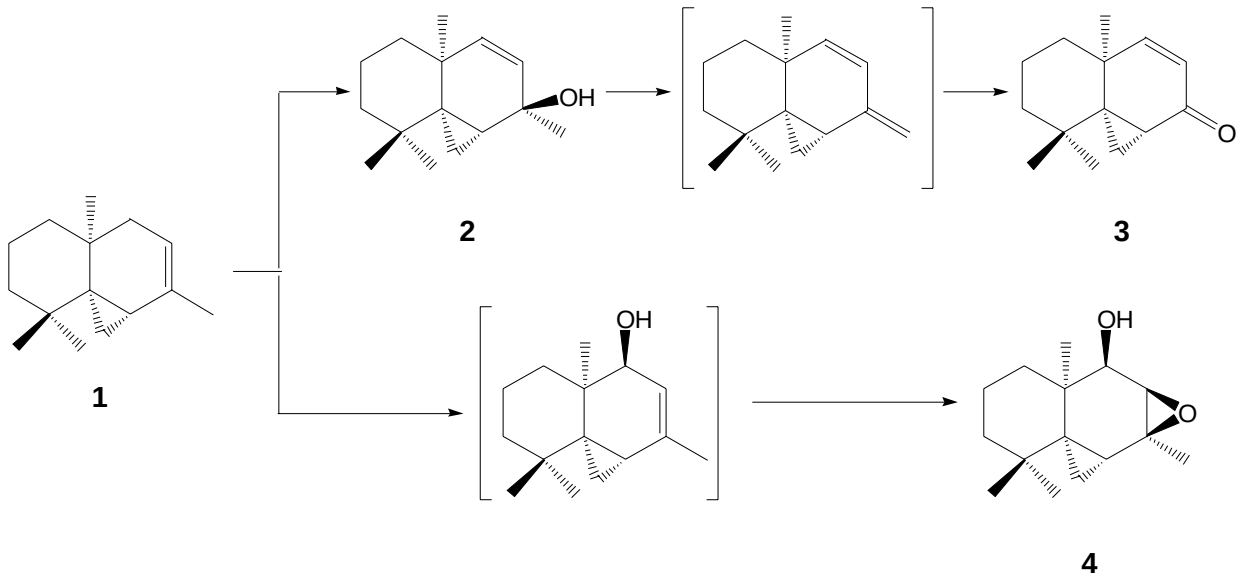


Fig. 3. Biotransformation of thujopsene (**1**) using *C. roseus*.



Scheme 1. Metabolic pathway of thujopsene (**1**) by plant cultured-cells.

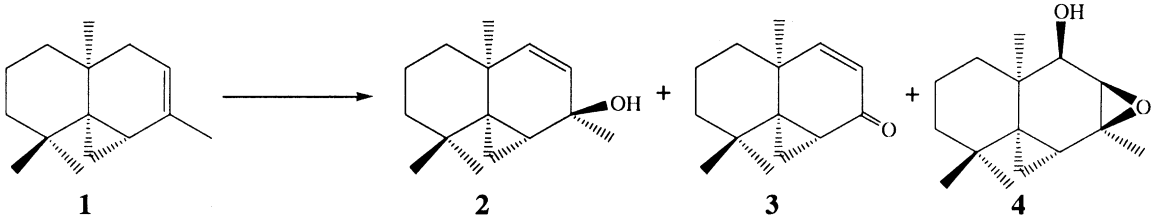
GC-MS data, it was found that 3β-hydroxy-4-thujopsene (**2**) disappeared and mayurone (**3**, 85%) was obtained as the major product. The biotransformation of thujopsene (**1**, 40 mg) using cultured-cells of *C. roseus* gave a mixture (32 mg) for 14 days. The substrate was oxidized to mayurone (**3**, 78%) and 3β-epoxythujopsan-5β-ol (**4**, 22%).

3.3. Discussion

From the results of the time-course experiments, the metabolic pathway of thujopsene (**1**) is the same as in Scheme 1. They support that mayurone is formed via 3β-hydroxy-4-thujopsene as an intermediate. From our previous work, it was known that the alcohol was easily transformed to the alkene by dehydration [14]. It was assumed that 3β-hydroxy-4-thujopsene was dehydrated to a dialkene, and then the dialkene was oxidized to mayurone. 3β-Epoxythujopsan-5β-ol (**4**) was directly obtained from thujopsene (**1**) by epoxidation.

And these results show that thujopsene (**1**) is biotransformed to mayurone (**3**) as the major product in high yield (63–85%) (Table 1). As a synthetic method of mayurone, the present biotransformation is more convenient and cleaner than Collins oxidation [5]. Moreover, these experiments indicate that only 2 g of plant cultured-cells of *H. cannabinus* are required to transform 60 mg of substrate **1**. In the case of *N. tabacum* and *C. roseus*, 30 g plant cultured-cells are required to transform 40 mg of substrate **1**. Thus the reactivity of *H. cannabinus* is about 22 times that of *N. tabacum* and *C. roseus*. It was found that as a new biocatalyst, the mayurone's yield using *H. cannabinus* was raised 10% or

Table 1
Biotransformation of thujopsene (**1**) by plant cultured-cells



Run	Cultured-cells	Time (day)	Yield (%)[a]
1[b]	*H. cannabinus*	20	**2**(4), **3**(63), **4**(33)
2[c]	*N. tabacum*	14	**3**(85), **4**(15)
3[c]	*C. roseus*	14	**3**(78), **4**(22)
4 [4]	*C. chamlagu*	14	**2**(16), **3**(52), **4**(22)

[a] GLC yield.
[b] Reaction conditions: substrate (60 mg), callus tissues (2 g) and culture medium (100 ml) were employed in the dark at 25 °C.
[c] Reaction conditions: substrate (40 mg), callus tissues (30 g) and culture medium (100 ml) were employed in the light (2000 lx) at 25 °C.

more rather than that of the cultured-cells of *C. chamlagu* [4].

Acknowledgements

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