PERGAMON

Phytochemistry 57 (2001) 669–673

PHYTOCHEMISTRY

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Biotransformation of (+)- and (−)-camphorquinones by plant cultured cells

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Received 15 August 2000; received in revised form 23 January 2001

Abstract

Biotransformation of (+)- and (−)-camphorquinones with suspension plant cultured cells of *Nicotiana tabacum* and *Catharanthus roseus* was investigated. It was found that the plant cultured cells of *N. tabacum* and *C. roseus* reduce stereoselectively the carbonyl group of (+)- and (−)-camphorquinones to the corresponding α-keto alcohols. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *Nicotiana tabacum*; Solanaceae; *Catharanthus roseus*; Apocynaceae reduction; Biotransformation; (+)-Camphorquinone; (−)-Camphorquinone; 2-*exo*-Hydroxyepicamphor; 2-*endo*-Hydroxyepicamphor; 3-*exo*-Hydroxycamphor; 3-*endo*-Hydroxycamphor

1. Introduction

Asymmetric reductions of ketones by biocatalysts are useful preparative methods to obtain chiral alcohols (Sih and Chen, 1984). Chiral α-keto alcohols are useful and important intermediates for the synthesis of optically active natural products having stereodirecting groups. In previous work, microbes such as baker's yeast have been used widely to reduce camphorquinone. (Pfrunder and Tamm, 1969; Chenevert and Thiboutot, 1988; Rebolledo et al., 1991; Miyazawa et al., 1995). In the report of Miyazawa, (+)-camphorquinone (**1a**) was stereoselectivity reduced by *Glemerella cingulata* to give (−)-3*S*-*exo*-hydroxycamphor (**4a**).

On the other hand, plant cultured cells have been studied for the ability to transform foreign substrates into useful substances (Suga and Hirata, 1990). However, hitherto known biochemical reduction of the carbonyl group by plant culture cells is only NADH-dependent reduction of the C–C double bond of α,β-unsaturated ketones (Hirata et al., 1982; Shimoda et al., 1998). There is very little information on the biotransformation of diketones by plant cultured cells. Here, we report that the biotransformation of (+)- and (−)-camphorquinones (**1a** and **1b**) by *N. tabacum* and *C. roseus* yields the corresponding α-keto alcohols.

2. Results and discussion

2.1. Biotransformation of (+)-camphorquinone (*1a*) and (−)-camphorquinone (*1b*) by N. tabacum

It was found that cultured cells of *N. tabacum* reduce substrates **1a** and **1b** into α-keto alcohols. (−)-Camphorquinone (**1b**) afforded a mixture of diastereomeric isomers of four α-keto alcohols: (−)-2*S*-*exo*-hydroxy epicamphor (**3b**); (+)-3*R*-*exo*-hydroxycamphor (**2b**); (−)-3*S*-*endo*-hydroxycamphor (**4b**) and (+)-2*R*-*endo*-hydroxyepicamphor (**5b**). (+)-Camphorquinone (**1a**) was bioselectively transformed to give three corresponding enantiomers: (+)-2*R*-*exo*-hydroxyepicamphor (**3a**); (−)-3*S*-*exo*-hydroxycamphor (**4a**) and (+)-3*R*-*endo*-hydroxycamphor (**5a**). However, (−)-2*S*-*endo*-hydroxyepicamphor (**2a**) could not be formed from **1a** in this work (Schemes 1 and 2). Table 1 shows results of time-course experiments. (+)-Camphorquinone (**1a**) was easily reduced to obtain (−)-3*S*-*exo*-hydroxycamphor (**4a**, 57%) and (+)-2*R*-*exo*-hydroxyepicamphor (**3a**, 37%) as the major products after 10 h incubation. Substrate **1b** was transformed to (−)-2*S*-*exo*-hydroxy

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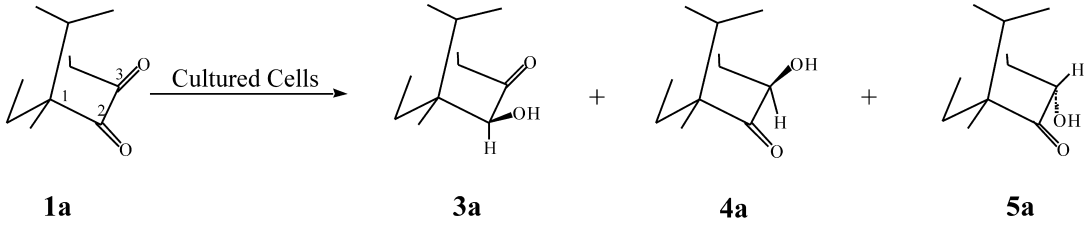
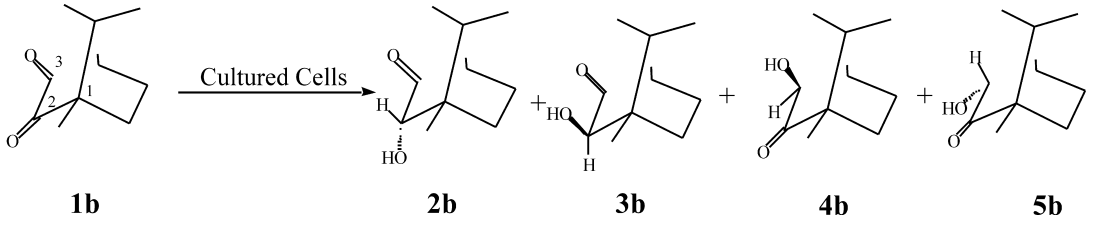
0031-9422/01/$ - see front matter
PII: S0031-9422(01)00133-9



Scheme 1. Biotransformation of (+)-camphorquinone (**1a**) by plant cultured cells.



Scheme 2. Biotransformation of (−)-camphorquinone (**1b**) by plant cultured cells.

Table 1
Biotransformation of camphorquinone by plant cultured cells

Substrate	Plant cultured cells	T/h[a]	Total yield/%[b]	Product ratio/%			
(+)-Camphorquinone (**1a**)				**2a**	**3a**	**4a**	**5a**
	C. roseus	3	96	/	11	66	23
	N. tabacum	10	82	/	37	57	6
(−)-Camphorquinone (**1b**)				**2b**	**3b**	**4b**	**5b**
	C. roseus	10	93	25	5	29	41
	N. tabacum	18	98	8	49	37	6

[a] T is reaction time.
[b] Total yield is the yield of product isolated.

epicamphor (**3b**, 49%) and (−)-3*S*-*endo*-hydroxycamphor (**4b**, 37%) after 18 h. (Figs. 1 and 2).

2.2. Biotransformation of (+)-camphorquinone (*1a*) and (−)-camphorquinone (*1b*) by C. roseus

Incubation of substrates **1a** and **1b** with cultured cells of *C. roseus*, also gave the corresponding α-keto alcohols. Compound **1a** rapidly disappeared and was undetectable after 3 h incubation. Compound **1a** was converted to (−)-3*S*-*exo*-hydroxy-camphor (**4a**, 66%) as the major product. Compound **1b** was transformated to (+)-2*R*-*endo*-hydroxyepicamphor (**5b**, 41%) as the major product at the end of 10 h.

2.3. Discussion

After incubation, the column mixtures of α-keto alcohols were applied to a silica gel. Elution with *n*-hexane–Et_2O (3:1) gave two products: 2β-hydroxycamphor (**3**) and 3β-hydroxycamphor (**4**) were identified by their IR, GC–MS and NMR spectral data. In addition, the ^1H NMR spectral data of the proton at C_2 or C_3 position of products **3** and **4** agreed with those of the authentic samples (Thoren, 1970). From the ^1H NMR spectral data of the reaction mixtures, the presence of 2α-hydroxycamphor (**2**) and 3α-hydroxycamphor (**5**) has been shown (Table 2). Then, the structures and relative yields of the products were determined on the basis of the ^1H NMR spectral peak areas of the proton at C_2 or C_3 position. The transformation products and their yields are shown in Table 1.

In this paper, we have used plant suspension cells to transform camphorquinone. (+)-Camphorquinone (**1a**) was stereoselectively reduced by *C. roseus* and *N. tabacum* to give (−)-3*S*-*exo*-hydroxycamphor (**4a**) over a short time period (3–10 h). It was found that it is more rapid than the reduction of **1a** by *Glemerella cingulata* and *Mucor mucedo* (Miyazawa et al., 1995) with the same stereoselectivity (9–24 h).

3. Experimental

3.1. Analytical and substrates

IR: Jasco FT–IR 230; GC–MS: Shimadzu GCMS-QP5050 (EI–MS 70 eV) using DB1 (0.25 mm × 30 m,

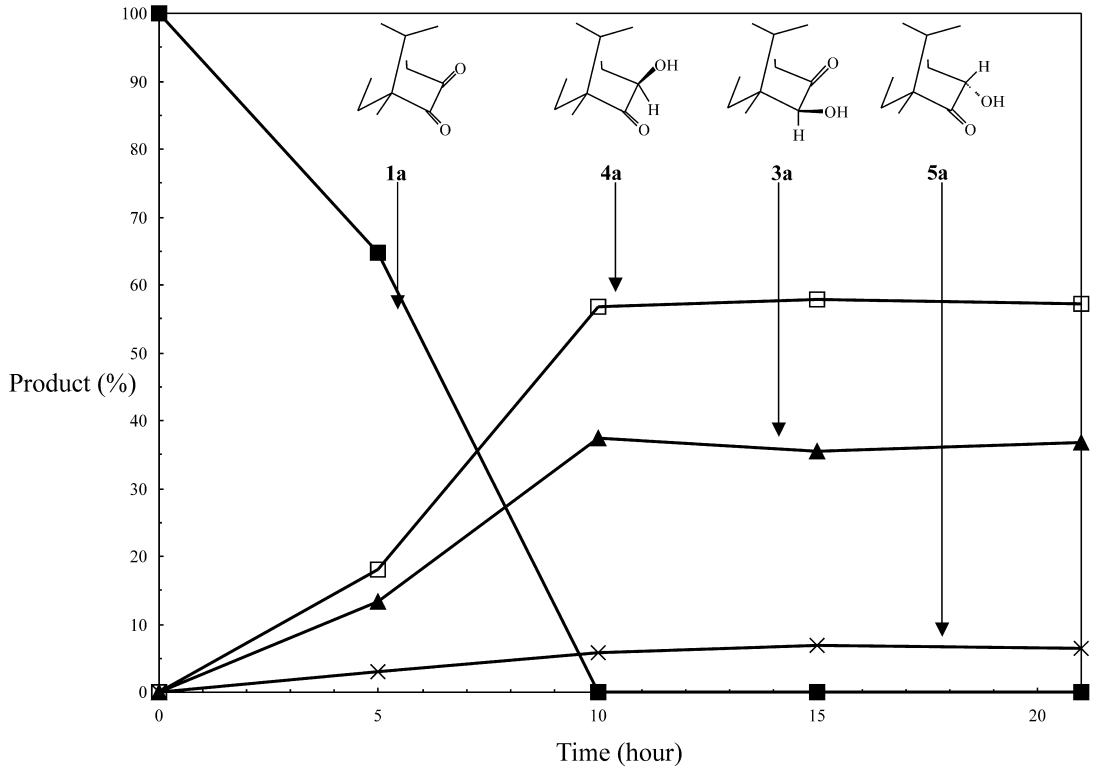


Fig. 1. Biotransformation of (1*S*)-(+)-camphorquinone (**1a**) using *Nicotiana tabacum*.

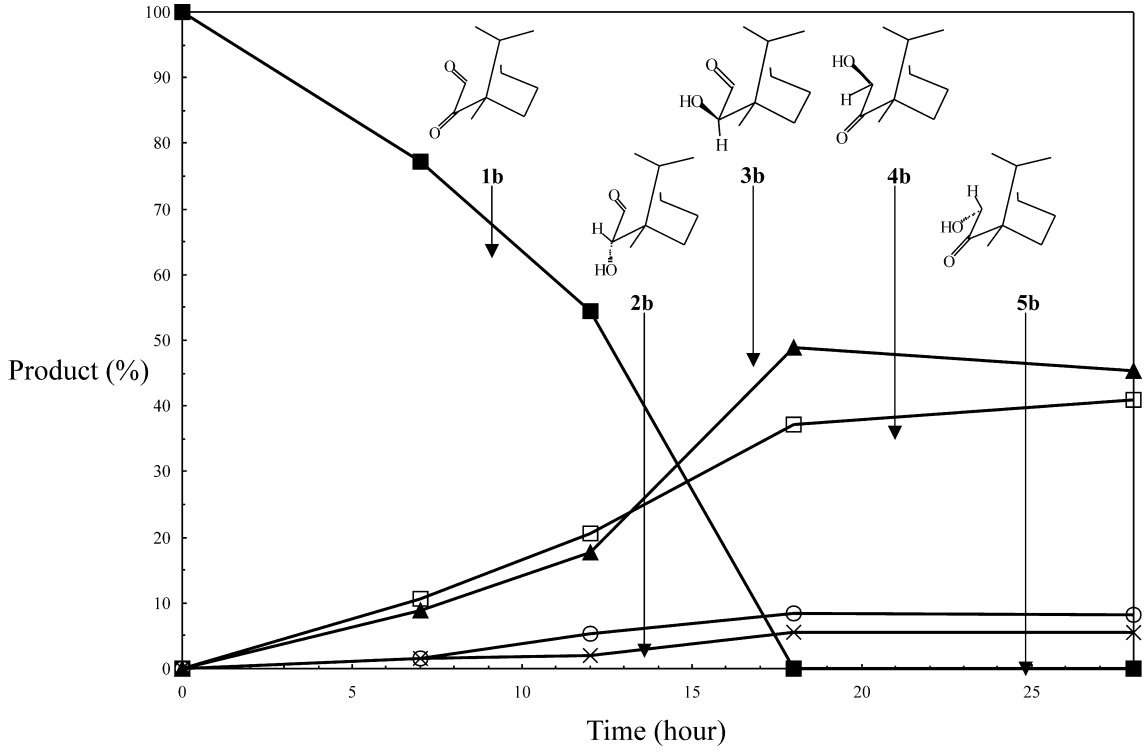


Fig. 2. Biotransformation of (1*R*)-(−)-camphorquinone (**1b**) using *Nicotiana tabacum*.

Table 2
^{1}H NMR spectra[a] of the α-ketols

Compound	$CDCl_3$: δ C_2 or C_3[b]	Compound	$CDCl_3$: δ C_2 or C_3
2α-Hydroxycamphor	3.87 (*s*, *b*, 1H)	**2**	3.86 (*s*, *b*, 1H)
2β-Hydroxycamphor	3.58 (*s*, 1H)	**3** (isolated)	3.54 (*s*, 1H)
3β-Hydroxycamphor	3.72 (*s*, 1H)	**4** (isolated)	3.75 (*s*, 1H)
3α-Hydroxycamphor	4.28 (*d*, *J* = 5 Hz, 1H)	**5**	4.21 (*d*, *J* = 5 Hz, 1H)

[a] Shifts are given in ppm downfield from the TMS signal; *s* = singlet; *d* = doublet; *b* = broadened.
[b] Thoren (1970).

0.25 μm) capillary column GC; GC: GC-17A at a column temp. of 80–200°C at 10°C/min; ^{1}H and ^{13}C NMR: Jeol GSX 400 spectrometer.

(1*S*)-(+)-Camphorquinone was purchased from Aldrich Chem. Co. (mp 200–202°C, $[\alpha]_D^{25}$ +100° (C_6H_5 CH_3; c 1.9)). (1*R*)-(−)-Camphorquinone was purchased from Tokyo Kasei Kogyo Co., Ltd (mp 200°C).

3.2. Cultivation of suspension cells of *N. tabacum* and *C. roseus*

The callus tissues of *N. tabacum* were transferred to freshly prepared MS medium (Murashige and Skoog, 1962) containing 1 ppm of 2,4-dichlorophenoxyacetic acid as auxin and 3% sucrose, and then were grown with continuous shaking (110 rpm) for 8 days at 25°C under the light. The callus tissues of *C. roseus* were transferred to freshly prepared SH medium (Schenk and Hildebrandt, 1976) containing 2 ppm of 2,4-dichlorophenoxyacetic acid as auxin and 3% sucrose, and then were grown with continuous shaking (110 rpm) for 8 days at 25°C under the light.

3.3. Time-course experiment

The details are described below using **1a** as an example. A part of the callus tissues (15 g) was transferred to 50 ml culture medium in a 200 ml Erlenmeyer flask and grown with continuous shaking for 8 days at 25°C under light (about 2000 Lux). The substrate **1a** (20 mg) was administered to the suspension cells and the cultures were incubated at 25°C in a rotary shaker (110 rpm) under light. At regular intervals, one of the flasks was taken out and the incubation mixture was filtered and extracted with EtOAc–Et_2O (1:1). The extract so obtained was then subjected to ^{1}H NMR spectral analysis. The yields of the products were determined on the basis of the peak area from ^{1}H NMR spectra and expressed as a relative percentage to the total amount of the total reaction mixture extracted.

3.4. Isolation of the metabolic products

After incubation, the culture medium was filtered. The supernatent was then saturated with NaCl and extracted with EtOAc–Et_2O (1:1). The resulting residue was applied to a silica gel column, that was eluted with *n*-hexane–Et_2O (3:1) to give: 2β-hydroxycamphor (**3**), (IR (KBr): ν 3448, 1751 and 1098 cm^{-1}; CI–MS *m*/*z*: $[M+H]^+$ 169; ^{1}H NMR ($CDCl_3$): δ (ppm) 0.94 (*s*, 3H), 1.03 (*s*, 3H), 1.04 (*s*, 3H), 1.18–1.94 (*m*, 4H), 2.18 (*d*, *J* = 7 Hz, 1H), 2.65 (*s*, *b*, 1H) and 3.54 (*s*, 1H); ^{13}C NMR ($CDCl_3$): δ (ppm) 10.3, 18.9, 20.3, 21.2, 33.9, 46.6, 49.2, 58.6, 79.5 and 218.7) and 3β-hydroxycamphor (**4**), (IR (KBr): ν 3448, 1751 and 1123 cm^{-1}; CI–MS *m*/*z*: $[M+H]^+$ 169; ^{1}H NMR($CDCl_3$): δ (ppm) 0.94 (*s*, 3H), 0.95 (*s*, 3H), 1.00 (*s*, 3H), 1.20–2.00 (*m*, 4H), 2.10 (*d*, *J* = 5 Hz, 1H), 2.59 (*s*, *b*, 1H) and 3.75 (*s*, 1H); ^{13}C NMR($CDCl_3$): δ (ppm) 9.03, 20.1, 21.0, 25.2, 28.6, 48.2, 49.2, 57.0, 77.4 and 220.0) (Thoren, 1970)

The ratios of the isomers were calculated from the peak areas of ^{1}H NMR spectral data: δ 3.86 (*s*, *b*, 1H for **2**), 3.54 (*s*, 1H for **3**), 3.75 (*s*, 1H for **4**) and 4.21 (*d*, *J* = 5 Hz, 1H for **5**).

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