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Reduction of (+)- and (−)-camphorquinones by cyanobacteria

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Abstract

Reduction of (+)- and (−)-camphorquinones (**1a**, **1b**) by cyanobacteria (*Synechococcus elongatus* PCC 7942 and *Synechosystis* sp. PCC 6803) under illumination gave α-hydroxycamphor selectively. It was found that (+)-camphorquinone was reduced to give (−)-3*S*-*exo*-hydroxycamphor as major product in high stereoselectivity and the stereoselectivity decreased in the dark.

Keywords: Cyanobacteria; Biocatalytic reduction; (+)-Camphorquinone; (−)-Camphorquinone; Stereoselectivity

1. Introduction

Chiral α-hydroxyketones are useful and important intermediates for the synthesis of optically active natural products having stereodirecting groups. Although many biocatalytic reductions of ketone have been reported [1], only a few reductions of camphorquinone using biocatalyts were reported such as baker's yeast [2–4]; the biotransformation of (+)-camphorquinone (**1a**) by *Absidia orchidis* to give (−)-3*S*-*exo*-hydroxycamphor (**1a-3**) as major products [5]; the biotransformation of (+)-camphorquinones (**1a**) by *Glomerella cingulata* to give (−)-3*S*-*exo*-hydroxycamphor (**1a-3**, 70%), and the biotransformation of (−)-camphorquinone (**1b**) by *Aspergillus niger* to give (+)-2*R*-*endo*-hydroxyepicamphor (**1b-2**, **80%**) [6].

Recently, we have been reported that the biotransformation of the synthetic substance into more useful substance by plant cultured-cells is an important reaction in synthetic chemistry [7–10]. It is known that the plant cultured-cells have abilities of regio- and stereoslective hydroxylation, oxidation-reduction, hydrogenation, glycosylation, and hydrolysis for various organic compounds [11]. There is little information on the biotransformation of diketones by cultured-cells. During the course of studies, we have investigated the biotransformation of 3,6-dialkylcyclohexane-1,2-dions by plant cultured-cells of *Marchantia polymorpha* [7] and *Caragana chamlagu* [9].

Moreover, we reported the biotransformation of (+)- and (−)-camphorquinones (**1a**, **1b**) by *N. tabacum* and *C. roseus*, and it was found that (+)-camphorquinone (**1a**) was reduced in moderato stereoselectivity by *C. roseus* to provide (−)-3*S*-*exo*-hydroxycamphor (**1a-3**, 66%) [10].

Here, we report that the biotransformation of (+)- and (−)-camphorquinones (**1a**, **1b**) by cyanobacteria such as *Synechococcus elongatus* PCC 7942 and *Synechosystis* sp. PCC 6803 yields (−)-3*S*-*exo*-hydroxycamphor with high selctivities.

2. Experimental

2.1. Analytical and substrates

2.1.1. Gas chromatography–mass spectrometry (GC–MS)

Shimadzu GCMS-QP5050 (EI-MS 70 eV) using DB1 (0.25 mm × 30 m, 0.25 μm) capillary column GC; GC: GC-

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17A at a column temperature of 80–200 °C at 10 °C/min. The injector and detector temperatures were 200–230 °C.

2.1.2. Infrared (IR) spectroscopy

The spectra were measured on a Jasco FT-IR 230. KBr disk was used.

2.1.3. Nuclear magnetic resonance (NMR) spectroscopy

The NMR spectra were measured on a JEOL GSX 400 spectrometer. $CDCl_3$ with tetramethysilane as the internal standard was used. The mixtures of three or four isomeric α-keto alcohols were identified from the peak areas of ^{1}H NMR spectral data: δ 3.55 (*s*, 1H for **1-1**), 3.75 (*s*, 1H for **1-3**), 3.85 (*s*, 1H for **1-2**), and 4.22 (*d*, 1H for **1-4**) [12].

2.2. Cyanobacteria

S. elongatus PCC 7942 and *Synechosystis* sp. PCC 6803 were obtained from the Institut Pasteur.

2.3. Cultivation

S. elongatus PCC 7942 and *Synechosystis* sp. PCC 6803 were grown in BG-11 medium [13] (pH 8.0) under continuous illumination provided by fluorescent lamps (2000 lx) with air-bubbling (50 ml/min) at 25 °C.

*2.4. Biotransformation of (+)- and (−)-camphorquinones (**1a**, **1b**)*

(+)- and (−)-camphorquinones (**1a**, **1b**, 20 mg) were added to suspended culture of *Synechococcus elogatus* PCC 7942 or *Synechosystis* sp. PCC 6803 (1 g/L as dry weight) in BG-11 medium (50 ml). The mixture was treated with air-bubbling (50 ml/min) at 25 °C in the light or darkness and the resulting mixture was extracted with EtOAc–Et_2O (1:1). The chemical and stereoselective purities were determined by IR, GC–MS and ^{1}H NMR analyses.

*2.5. (−)-3S-exo-Hydroxycamphor (**1a-3**)*

After incubation, the medium was filtered. The supernatant was then saturated with NaCl and extracted with EtOAc–EtO (1:1). The resulting residue was applied to a silica gel column and eluted with *n*-hexane–Et_2O (3:1) to give: (−)-3*S*-*exo*-hydroxycamphor (**1a-3**), IR (KBr): ν 3448, 1751 and 1123 cm^{-1}; EI-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.

2.6. Chemical compounds

The substrates used were from Aldrich Chemical Co. (+)-Camphorquinone: MW 166.22, mp 200–202 °C, $[\alpha]_D^{25}$ +100 (c = 1.9, CH_3OH). (−)-Camphorquinone: MW 166.22, mp 200–203 °C, $[\alpha]_D^{25}$ −101 (c = 2.0, $C_6H_5CH_3$).

3. Results and discussion

*3.1. Biotransformation of (+)- and (−)-camphorquinones (**1a**, **1b**) in the light condition*

(+)- and (−)-Camphorquinones (**1a**, **1b**) were reduced by *S. elongatus* PCC 7942 and *Synechosystis* sp. PCC 6803 (Figs. 1 and 2). (+)-Camphorquinone (**1a**) afforded a mixture of diastereoisomers of three α-keto alcohols: (+)-2*R*-*exo*-hydroxyepicamphor (**1a-1**), (−)-3*S*-*exo*-hydroxycamphor (**1a-3**) and (+)-3*R*-*endo*-hydroxycamphor (**1a-4**). However, (−)-2*S*-*endo*-hydroxyepicamphor (**1a-2**) could not be obtained from (+)-camphorquinone (**1a**) used in this work (Scheme 1).

(−)-Camphorquinone (**1b**) was transformed to give the corresponding four isomers: (−)-2*S*-*exo*-hydroxyepicamphor (**1b-1**), (+)-2*R*-*endo*-hydroxyepicamphor (**1b-2**),

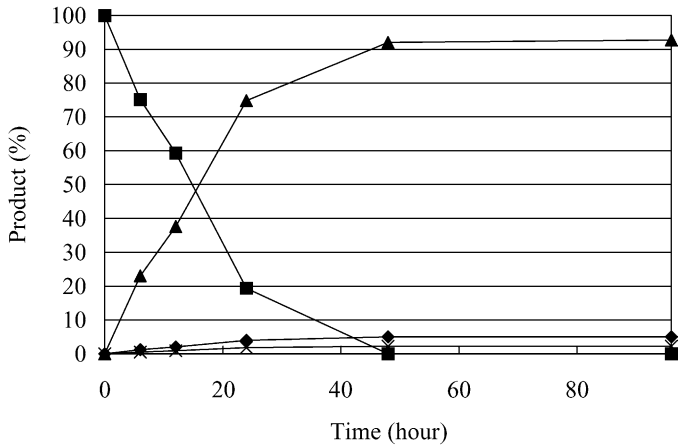


Fig. 1. Reduction of (1*S*)-(+)-camphorquinone (**1a**) by *S. elongatus* PCC 7942 in the light: (■), (+)-camphorquinone (**1a**); (×), 2-*exo*-hydroxycamphor (**1-1**); (▲), 3-*exo*-hydroxycamphor (**1-3**); (♦), 3-*endo*-hydroxycamphor (**1-4**).

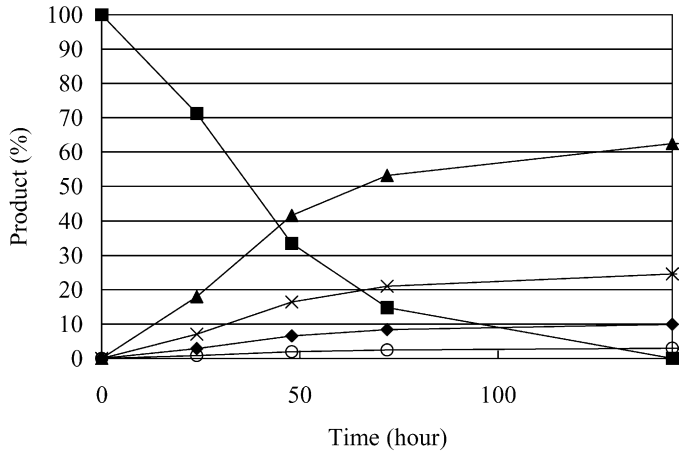
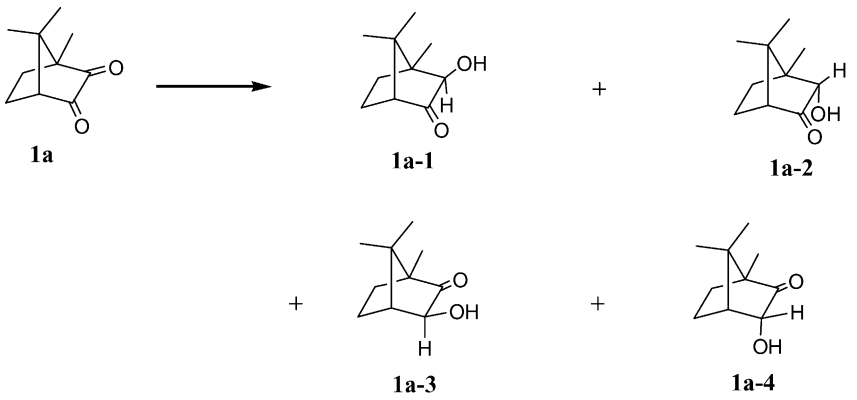


Fig. 2. Reduction of (1*R*)-(−)-camphorquinone (**1b**) by *S. elongatus* PCC 7942 in the light: (■), (−)-camphorquinone (**1a**); (×), 2-*exo*-hydroxycamphor (**1-1**); (◯), 2-*endo*-hydroxycamphor (**1-2**); (▲), 3-*exo*-hydroxycamphor (**1-3**); (♦), 3-*endo*-hydroxycamphor (**1-4**).

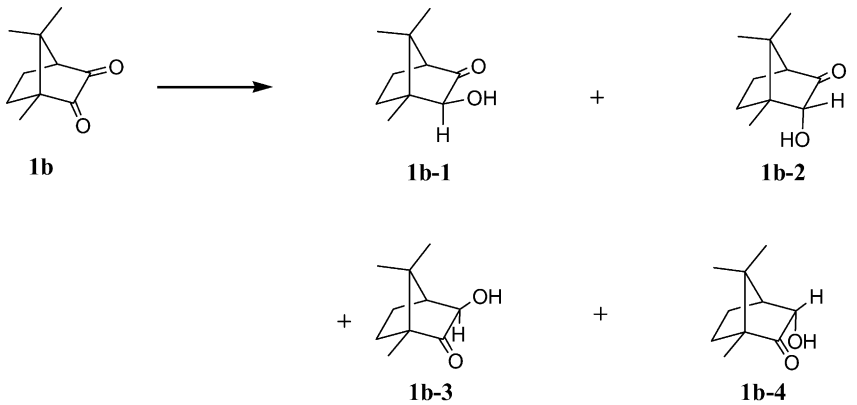


Scheme 1. Reduction of (+)-camphorquinone (**1a**) by biocatalyst.

(+)-3*R*-*exo*-hydroxycamphor (**1b-3**) and (−)-3*S*-*endo*-hydroxycamphor (**1b-4**) (Scheme 2).

From these results, it was found that only hydroxyketones were obtained and diols were not afforded. (+)-Camphorquinone (**1a**) was reduced stereoselectively by *S. elongatus* PCC 7942 and *Synechosystis* sp. PCC 6803 to give (−)-(3*S*)-*exo*-hydroxycamphor (**1a-3**) in 93% and 94% yield, respectively. These results indicate that the reduction activities of *S. elongatus* PCC 7942 and *Synechosystis* sp. PCC 6803 are higher than that of plant cultured cells (*N. tabacum* and *C. roseus*) [10].

In the case of (−)-camphorquinone (**1b**), biotransformation using *S. elongatus* PCC 7942 and *Synechosystis* sp. PCC 6803 gave (+)-3*R*-*exo*-hydroxycamphor (**1b-3**) in 62% and 57% yield, respectively (Table 1).



Scheme 2. Reduction of (−)-camphorquinone (**1b**) by biocatalyst.

Table 1
Reduction of camphorquinone (**1a**, **1b**) by *S. elongatus* PCC 7942 and *Synechococcus* sp. PCC 6803 in the light[a]

Run	Substrate	Cyanobacteria	Time (h)	Yield (%)[b]	Product ratio (%)[c]			
1	**1a**	*S. elongatus* PCC 7942	48	92	**1a-1**(2)	**1a-2**(–)	**1a-3**(93)	**1a-4**(5)
2	**1a**	*Synechococcus* sp. PCC 6803	48	92	**1a-1**(3)	**1a-2**(–)	**1a-3**(94)	**1a-4**(3)
3	**1b**	*S. elongatus* PCC 7942	144	91	**1b-1**(25)	**1b-2**(3)	**1b-3**(62)	**1b-4**(10)
4	**1b**	*Synechococcus* sp. PCC 6803	144	91	**1b-1**(21)	**1b-2**(4)	**1b-3**(58)	**1b-4**(17)

[a] Reaction conditions: substrate (20 mg), medium (50 ml), *S. elongatus* PCC 7942 and *Synechococcus* sp. PCC 6803 were cultivated with air-bubbling at 25 °C in the light.
[b] Isolated yields.
[c] Relative intensities by ^{1}H NMR signals.

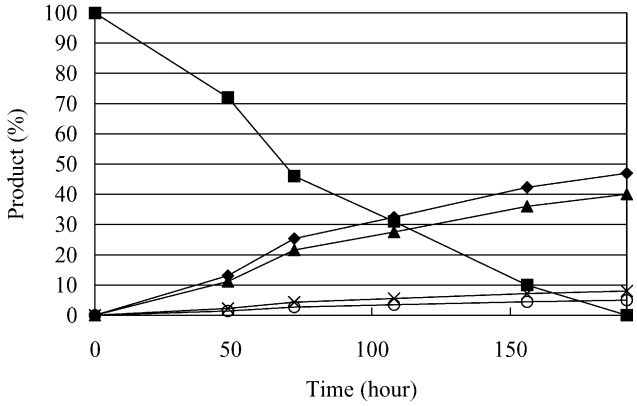


Fig. 3. Reduction of (1*S*)-(+)-camphorquinone (**1a**) by *S. elongatus* PCC 7942 in the dark: (■), (+)-camphorquinone (**1a**); (×), 2-*exo*-hydroxycamphor (**1-1**); (◯), 2-*endo*-hydroxycamphor (**1-2**); (▲), 3-*exo*-hydroxycamphor (**1-3**); (♦), 3-*endo*-hydroxycamphor (**1-4**).

Previously, Nakamura et al. [14–16] reported that reduction of asymmetric ketone by cyanobacteria (*S. elongatus* PCC 7942) as biocatalyst under the light conditions gave the corresponding (*S*)-alcohols with excellent enantioselectivities. It is known that this method converts form CO_2 to O_2 by the direct use of light energy using photosynthetic microbe as biocatalysts.

The growth rate of cyanobacteria is faster than other typical microbe. The problem using plant cultured-cells as biocatalysts is usually grown very slowly and requires large amount of the catalyst.

Reduction of (+)- and (−)-camphorquinones (**1a**, **1b**) by *N. tabacum* and *C. roseus* 15 g plant cultured-cells are required to biotransform 20 mg of the substrates. However, only 50 mg (dry weight) of cyanobacteria could be biotransform stereoselectively.

*3.2. Biotransformation of (+)- and (−)-camphorquinones (**1a**, **1b**) in the dark condition*

S. elogatus PCC 7942 and *Synechosystis* sp. PCC 6803 are grown under illumination, and so we experimented under the

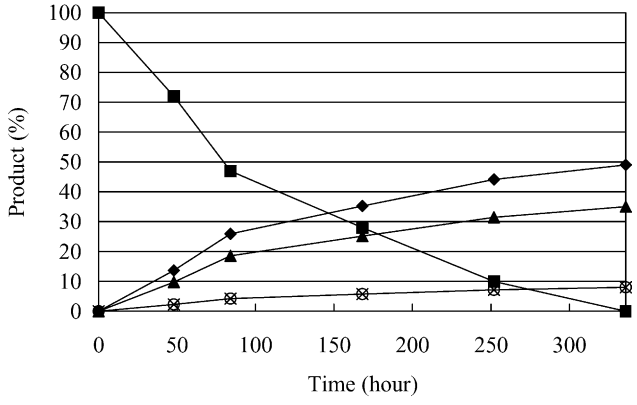


Fig. 4. Reduction of (1*R*)-(−)-camphorquinone (**1a**) by *S. elongatus* PCC 7942 in the dark: (■), (−)-camphorquinone (**1b**); (×), 2-*exo*-hydroxycamphor (**1-1**); (◯), 2-*endo*-hydroxycamphor (**1-2**); (▲), 3-*exo*-hydroxycamphor (**1-3**); (♦), 3-*endo*-hydroxycamphor (**1-4**).

Table 2
Reduction of camphorquinone (**1a**, **1b**) by *S.elongatus* PCC 7942 and *Synechococcus* sp. PCC 6803 in the dark[a]

Run	Substrate	Cyanobacteria	Time (h)	Yield (%)[b]	Product ratio (%)[c]			
1	**1a**	*S. elongatus* PCC 7942	192	89	**1a-1**(4)	**1a-2**(–)	**1a-3**(72)	**1a-4**(24)
2	**1a**	*Synechococcus* sp. PCC 6803	192	88	**1a-1**(6)	**1a-2**(–)	**1a-3**(68)	**1a-4**(26)
3	**1b**	*S. elongatus* PCC 7942	336	86	**1b-1**(8)	**1b-2**(5)	**1b-3**(40)	**1b-4**(47)
4	**1b**	*Synechococcus* sp. PCC 6803	336	85	**1b-1**(8)	**1b-2**(8)	**1b-3**(35)	**1b-4**(49)

[a] Reaction conditions: substrate (20 mg), medium (50 ml), *Synechococcus* sp. PCC 6803 and *S. elongatus* PCC 7942 were cultivated with air-bubbling at 25 °C in the dark condition.
[b] Isolated yields.
[c] Relative intensities by ^{1}H NMR signals.

Table 3
Reduction of (+)-camphorquinone (**1a**) by various catalysts

Catalyst	Yield (%)[a]	Product ratio (%)[b]			
M. mucedo [6]	99	**1a-1**(14)	**1a-2**(–)	**1a-3**(71)	**1a-4**(14)
N. tabacum [10]	82	**1a-1**(37)	**1a-2**(–)	**1a-3**(57)	**1a-4**(6)
C. roseus [10]	96	**1a-1**(11)	**1a-2**(–)	**1a-3**(66)	**1a-4**(23)
Candida parapsilosis [18]	34	**1a-1**(–)	**1a-2**(–)	**1a-3**(–)	**1a-4**(>98)
Abaidia orchidis [5]	90	**1a-1**(–)	**1a-2**(–)	**1a-3**(100)	**la-4**(–)
Baker's yeast [3]	–	**1a-1**(3)	**1a-2**(–)	**1a-3**(61)	**1a-4**(36)
L-Selectride [19]	90	**1a-1**(17)	**1a-2**(–)	**1a-3**(83)	**la-4**(–)
S. elongatus PCC 7942	92	**1a-1**(2)	**1a-2**(–)	**1a-3**(93)	**1a-4**(5)
Synechococcus sp. PCC 6803	92	**1a-1**(3)	**1a-2**(–)	**1a-3**(94)	**1a-4**(3)

[a] Isolated yields.
[b] Relative intensities by ^{1}H NMR signals.

Table 4
Reduction of (−)-camphorquinone (**1b**) by various catalysts

Catalyst	Yield (%)[a]	Product ratio (%)[b]			
A. niger [6]	98	**1b-1**(3)	**1b-2**(80)	**1b-3**(12)	**1b-4**(3)
N. tabacum [10]	82	**1b-1**(49)	**1b-2**(8)	**1b-3**(37)	**1b-4**(6)
C. roseus [10]	96	**1b-1**(5)	**1b-2**(25)	**1b-3**(29)	**1b-4**(41)
Candida parapsilosis [18]	24	**1b-1**(–)	**1b-2**(–)	**1b-3**(–)	**1b-4**(>98)
Abaidia orchidis [5]	85	**1b-1**(–)	**1b-2**(30)	**1b-3**(30)	**1b-4**(40)
L-Selectride [19]	90	**1b-1**(17)	**1b-2**(–)	**1b-3**(83)	**1b-4**(–)
S. elongatus PCC 7942	91	**1b-1**(25)	**1b-2**(3)	**1b-3**(62)	**1b-4**(10)
Synechococcus sp. PCC 6803	91	**1b-1**(21)	**1b-2**(4)	**1b-3**(58)	**1b-4**(17)

[a] Isolated yields.
[b] Relative intensities by ^{1}H NMR signals.

light. It is considered that the associate enzymatic activities were influenced strongly by the amount of light in the environment. Therefore, the stereoselectivity changes because the reduction may depend on the amount of light. We tested that (+)- and (−)-camphorquinones (**1a**, **1b**) were added to suspension of *S. elongatus* PCC 7942 and *Synechosystis* sp. PCC 6803 with air-bubbling in the dark condition (Figs. 3 and 4).

As shown in Table 2, reaction of (+)-camphorquinone (**1a**) with *S. elongatus* PCC 7942 and *Synechosystis* sp. PCC 6803 in the dark proceeded slowly and provided (−)-3*S*-*exo*-hydroxycamphor (**1a-3**) in 72% and 68% yield, respectively. On other hand, 24–26% of (+)-3*R*-*endo*-isomer were obtained. In the case of (−)-camphorquinone (**1b**), the reduction requires long time and (+)-3*R*-*exo*-isomer (**1b-3**) and (−)-3*S*-*endo*-isomer (**1b-4**) were obtained as major products. Thus, dark conditions afford low selectivity of the reduction.

From these results, it is considered that *S. elongatus* PCC 7942 and *Synechosystis* sp. PCC 6803 perform photosynthesis during exposure to light, and all enzymatic activities change depending on whether the environment is dark or light [16,17].

Under illumination, cyanobacteria perform photosynthesis, absorb carbondioxide generate oxygen, and transform (+)- and (−)-camphorquinones (**1a**, **1b**) to the useful α-hydroxycamphor stereoselectively (Tables 3 and 4). Therefore, it was found that cyanobacteria are excellent biocatalysts for the reduction of (+)-camphorquinone (**1a**) (Table 3).

Acknowledgments

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