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Stereoselective reduction of ketones by various vegetables

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Abstract

Reduction of (+)-and (−)-camphorquinones (**1a**, **1b**) by various vegetables (carrot, potato, sweet potato, apple, Japanese radish, cucumber, burdock and onion) gave α-hydroxycamphor selectively. Using burdock, (+)-camphorquinone was reduced to give (−)-3*S*-*exo*-hydroxycamphor (**4a**) as major product in high stereoselectivity with high yield. Moreover, 1,2-cyclohexanedione (**1c**) and 2-methylcyclohexanone (**1d**) with various vegetables afford enantiomerically pure *trans*- and/or *cis*-alcohol, respectively. Various vegetable reduction gave a new idea of a biotechnological process.
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1. Introduction

Chiral α-hydroxyketones are useful and important intermediates for the synthesis of optically active natural products. It is known that the synthesis of chiral alcohols can be achieved from the corresponding prochiral ketones by asymmetric reduction. Although many biocatalytic reductions of ketone have been reported [1–7], only a few reductions of camphorquinone using biocatalysts were reported, such as baker's yeast [8–10]; the biotransformation of (+)-camphorquinone (**1a**) by *Absidia orchidis* to give (−)-3*S*-*exo*-hydroxycamphor (**4a**) as major products [11]; the biotransformation of (+)-camphorquinones (**1a**) by *Glomerella cingulata* to give (−)-3*S*-*exo*-hydroxycamphor (**4a**, 70%), and the biotransformation of (−)-camphorquinone (**1b**) by *Aspergillus niger* to give (+)-2*R*-*endo*-hydroxyepicamphor (**3b**, 80%) [12].

Recently, we have reported that the biotransformation of a synthetic substance into a more useful substance by plant cultured-cells is an important reaction in synthetic chemistry [13–16]. It is known that the cultured suspension cells *Eucalyptus perriniana* are capable of regioselective hydroxylation and glycosylation of bisphenol A [17]. Moreover, plant cultured-cells have the ability of regio- and stereoselective hydroxylation, oxidation, reduction, hydrogenation, glycosylation, and hydrolysis for various organic compounds [18]. 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-diones by plant cultured-cells of *Marchantia polymorpha* [13] and *Caragana chamlagu* [15].

Recently, 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 moderate stereoselectivity by *C. roseus* to provide (−)-3*S*-*exo*-hydroxycamphor (**4a**, 66%) [16]. Moreover, we reported that (+)-camphorquinone (**1a**) was reduced to high stereoselectivity by cyanobacteria (*Synechococcus elogatus* PCC 7942 and *Synechosystis* sp. PCC 6803) to provide (−)-3*S*-*exo*-hydroxycamphor (**4a**, 94%) [19].

We report on a convenient and simple procedure for the preparation of chiral alcohols from various ketones with various vegetables as the reduction process, under mild conditions [20–24].

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2. Experimental

2.1. Analytical and substrates

Shimadzu GCMS-QP5050 (EI-MS 70 eV) using DB1 (0.25 mm × 30 m, 0.25 μm) capillary column GC; GC: GC-17A at a column temp. of 80–200 °C at 10 °C/min. The injector and detector temperatures were 200–230 °C. The IR spectra were measured on a Jasco FT-IR 230.

2.1.1. Enatiomer separation

For reduction of 1,2-cyclohexanedione (**1c**), enantiomer separation is achieved on a Chirasil-Dex CB capillary column (25 mm × 0.25 mm); carrier gas: helium 70 kPa; column temperature: 120 °C. The injector temperature was 150 °C. The peaks in chromatogram were identified by *cis*- and *trans*-1,2-cyclohexanediol (**3c**) (Aldrich). The absolute configuration of the preferentially formed enantiomer was determined by comparison with (*R*, *R*)-**3c** and (*S*, *S*)-**3c** from Aldrich. Diastereomeric excess is expressed as diastereomeric excess (%) = 100 × (|*trans* − *cis*|)/(|*trans* + *cis*|).

For reduction of 2-methylcyclohexanone (**1d**), enantiomer separation is achieved on a Chiraldex B-PM column (30 mm × 0.25 mm) containing Permethyl β-cyclodextrin; carrier gas: helium 70 kPa; column temperature: 70–200 °C (2.0 °C/min). The injector temperature was 200 °C. The *cis*-2-methylcyclohexanol acetate and *trans*-2-methylcyclohexanol acetate were prepared with acetic anhydride and pyridine. Retention time (min): 1*R*, 2*R*-**2d**, 13.0 (as acetyl derivative); 1*S*, 2*S*-**2d**, 13.3 (as acetyl derivative); 1*R*, 2*S*-**2d**, 13.6 (as acetyl derivative); 1*S*, 2*R*-**2d**, 14.2 (as acetyl derivative).

The absolute configurations of the compounds were determined by comparing the specific rotation with those of the literature [11,24,25]: for (−)-3*S*-*exo*-hydroxycamphor (**4a**) $[\alpha]_D^{27} = -78$ (ca. 1.5, $CHCl_3$); (+)-3*R*-*exo*-hydroxycamphor (**4b**) $[\alpha]_D^{27} = +99$ (ca. 0.42, $CHCl_3$); (1*S*, 2*S*)-*trans*-1,2-cyclohexanediol (**3c**) $[\alpha]_D^{27} = +36$ (ca. 0.66, C_2H_5OH); (1*S*, 2*S*)-*trans*-2-methylcyclohexanol (**2d**) $[\alpha]_D^{27} = +12$ (ca. 0.49, C_2H_5OH); (1*S*, 2*R*)-*cis*-2-methylcyclohexanol (**2d**) $[\alpha]_D^{27} = +10$ (ca. 0.27, C_2H_5OH).

2.1.2. Nuclear magnetic resonance (NMR) spectroscopy

The NMR spectra were measured on a JEOL GSX 400 spectrometer. $CDCl_3$ with tetramethylsilane as the internal standard was used. (+)- and (−)-Camphorquinones (**1a**, **1b**) were transformed to give the corresponding four isomers. The mixtures of four isomeric α-keto alcohols were identified from the peak areas of ^{1}H NMR spectral data: δ 3.55 (s, 1H for **2**), 3.75 (s, 1H for **4**), 3.85 (s, 1H for **3**), and 4.22 (d, 1H for **5**).

2.2. Vegetables

Fresh carrot (*Daucus carota*), potato (*Soanum tuberosum*), sweet potato (*Ipomoea batatas*), apple (*Malus pumila*), Japanese radish (*Raphanus sativus*), cucumber (*Cucumis sativus*), burdock (*Arctium lappa*) and onion (*Allium cepa*) were obtained from a local market.

2.3. General procedure for the reduction of ketones with various vegetables

Substrate (20 mg) was added to fresh vegetable (10 g, approximately 1 cm long slice) in water (70 ml). The mixture was treated by shaking (120 rpm) at 25 °C, and the resulting mixture was extracted with EtOAc–Et_2O (1:1). The chemical and stereoselective purities were determined by GC–MS and ^{1}H NMR analyses.

2.4. (−)-3S-exo-Hydroxycamphor (*4a*)

After incubation, the medium was filtered. The supernatant was then saturated with NaClaq 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 (**4a**), IR (KBr): ν 3448, 1751, and 1123 cm^{-1}; EI-MS *m*/*z*: $[M+H]^+$ 168; ^{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.

2.5. Chemical compounds

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

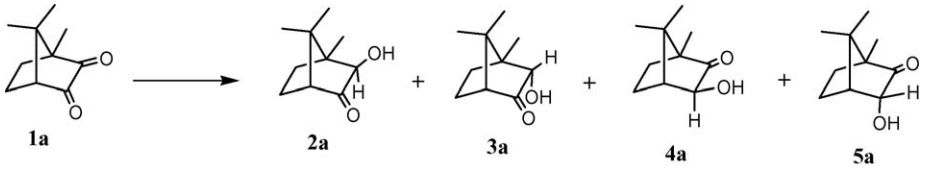
3. Results and discussion

3.1. Reduction of (+)- and (−)-camphorquinones (*1a*, *1b*) by the carrot (*D. carota*, root)

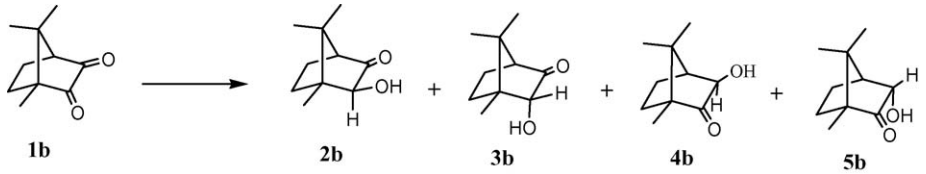
It is known that various enzyme is included in vegetables. For example, polyphenol oxidase (tyrosinase) is isolated from a mushroom and peroxidase is obtained from the potato [26]. It has been reported that the reduction of various prochiral ketones such as acetophenones, α-azido aryl ketones, β-ketoesters, aliphatic acyclic and cyclic ketones to the corresponding optically active secondary alcohols with moderate to excellent chemical yield was achieved using the carrot (*D. carota*, root) [20]. This method is an eco-friendly environmental reduction system employing the carrot as a biocatalyst. The advantages of this reduction are the easy availability and inexpensive of the carrot. Therefore, we tried the biotransformation of (+)- and (−)-camphorquinones (**1a**, **1b**) using the carrot.

(+)-Camphorquinone (**1a**) afforded a mixture of diastereoisomers of three α-keto alcohols: (+)-2*R*-*exo*-hydroxyepicamphor (**2a**), (−)-3*S*-*exo*-hydroxycamphor (**4a**) and (+)-3*R*-*endo*-hydroxycamphor (**5a**). However, (−)-2*S*-*endo*-hydroxyepicamphor (**3a**) could not be obtained from (+)-camphorquinone (**1a**) using the carrot (Scheme 1).

(−)-Camphorquinone (**1b**) was transformed to give the corresponding four isomers: (−)-2*S*-*exo*-hydroxyepicamphor (**2b**), (+)-2*R*-*endo*-hydroxyepicamphor (**3b**), (+)-3*R*-*exo*-



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



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

Table 1
Reduction of camphorquinone (**1a**, **1b**) by carrot[a]

Run	Substrate	Vegetable	Time (h)	Yield (%)[b]	Product ratio (%)[c]			
1	**1a**	Carrot (*Ducus carota*)	4	88	**2a** (4)	**3a** (−)	**4a** (88)	**5a** (8)
2	**1b**	Carrot (*D. carota*)	14	87	**2b** (52)	**3b** (15)	**4b** (28)	**5b** (5)

[a] Reaction conditions: substrate (20 mg), water (70 ml) and carrot (10 g) were cultivated with shaking at 25 °C.
[b] Isolated yields.
[c] Relative intensities by ^{1}H NMR peak area.

hydroxycamphor (**4b**) and (−)-3*S*-*endo*-hydroxycamphor (**5b**) (Scheme 2).

From these results, it was found that only hydroxy ketones were obtained and diols were not afforded. (+)-Camphorquinone (**1a**) reduced stereoselectivity by the carrot to give (−)-(3*S*)-*exo*-hydroxycamphor (**4a**) in 88% selectivity for short reaction times of 4 h. These results indicate that the reduction activities are higher than those of plant cultured-cells (*N. tabacum* and *C. roseus*) [16].

In the case of (−)-camphorquinone (**1b**), the reduction requires a long times and (−)-2*S*-*exo*-hydroxyepicamphor (**2b**, 52%) was obtained as a major product (Table 1). The products formed by each of the hydroxycamphor are showed in Figs. 1 and 2.

Furthermore, for consideration of the influence of a microbe, we tried under the sterilization condition (a carrot is sterilized with 70% ethanol and an ultraviolet lamp for 6 h). Consequently, yield and stereoselectivity did not change.

3.2. Reduction of (+)-and (−)-camphorquinones (*1a, 1b*) by various vegetables

It is known that the carrot can be reduced by acetophenones and other ketones, with excellent selectivity. However, there are few reports that reduce the ketone with other vegetables. In addition to carrot (*D. carota*), potato (*S. tuberosum*), sweet potato (*I. batatas*), apple (*M. pumila*), Japanese radish (*R. sativus*), cucumber (*C. sativus*), burdock (*A. lappa*) and onion (*A. cepa*) were selected for the biotransformation. These vegetables can be obtained in all seasons. Furthermore, these vegetables have the advantage of low cost.

Reduction of (+)-camphorquinone (**1a**) with burdock provided (−)-3*S*-*exo*-hydroxycamphor (**4a**) in 100% selectivity. The result indicates that reduction of (+)-camphorquinone (**1a**) yielded (−)-3*S*-*exo*-hydroxycamphor (**4a**) with high stereoselectivity. Reduction of (+)-camphorquinone (**1a**) from the apple, Japanese radish, cucumber and onion were transformed to (−)-

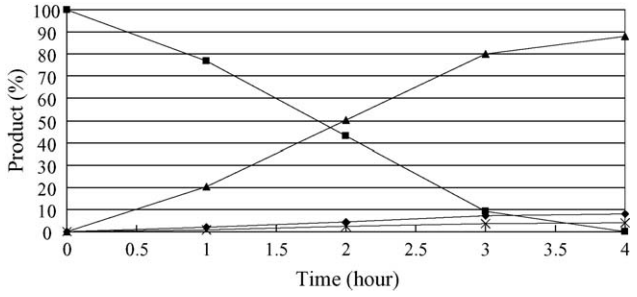


Fig. 1. Reduction of (1*S*)-(+)-camphorquinone (**la**) by carrot.

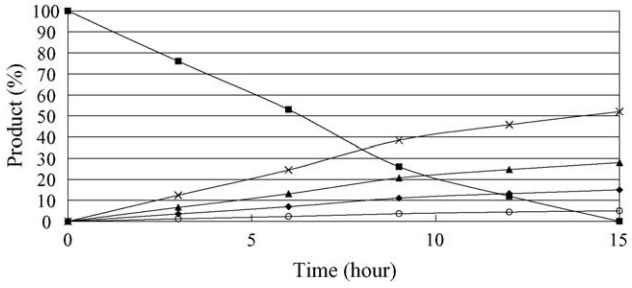


Fig. 2. Reduction of (1*R*)-(−)-camphorquinone (**lb**) by carrot.

Table 2
Reduction of (+)-camphorquinone (**1a**) by various vegetables[a]

Run	Substrate	Vegetable	Yield (%)[b]	Product ratio (%)[c]			
1	**1a**	Carrot (*Ducus carota*)	88	**2a** (4)	**3a** (−)	**4a** (88)	**5a** (8)
2	**1a**	Potato (*Soanum tuberosum*)	89	**2a** (36)	**3a** (−)	**4a** (27)	**5a** (37)
3	**1a**	Sweet potato (*Ipomoea batatas*)	86	**2a** (79)	**3a** (−)	**4a** (14)	**5a** (7)
4	**1a**	Apple (*Malus pumila*)	84	**2a** (5)	**3a** (−)	**4a** (59)	**5a** (36)
5	**1a**	Radish (*Raphanus sativus*)	82	2a (20)	**3a** (−)	**4a** (63)	**5a** (17)
6	**1a**	Cucumber (*Cucumis sativus*)	85	**2a** (19)	**3a** (−)	**4a** (49)	**5a** (32)
7	**1a**	Burdock (*Arctium lappa*)	81	**2a** (−)	**3a** (−)	**4a** (100)	**5a** (−)
8	**1a**	Onion (*Allium cepa*)	84	**2a** (20)	**3a** (−)	**4a** (63)	**5a** (17)

[a] Reaction conditions: substrate (20 mg), water (70 ml) and vegetables (10 g) were cultivated with shaking at 25 °C for 48 h.
[b] Isolated yields.
[c] Relative intensities by ^{1}H NMR peak area.

Table 3
Reduction of (−)-camphorquinone (**lb**) by various vegetables[a]

Run	Substrate	Vegetable	Yield (%)[b]	Product ratio (%)[c]			
1	**1b**	Carrot (*Daucus carota*)	87	**2b** (52)	**3b** (15)	**4b** (28)	**5b** (5)
2	**1b**	Potato (*Soanum tuberosum*)	82	**2b** (32)	**3b** (36)	**4b** (26)	**5b** (6)
3	**1b**	Sweet potato (*Ipomoea batatas*)	86	**2b** (43)	**3b** (20)	**4b** (30)	**5b** (7)
4	**1b**	Apple (*Malus pumila*)	84	**2b** (17)	**3b** (19)	**4b** (49)	**5b** (15)
5	**1b**	Radish (*Raphanus sativus*)	84	**2b** (47)	**3b** (10)	**4b** (35)	**5b** (8)
6	**1b**	Cucumber (*Cucumis sativus*)	82	**2b** (35)	**3b** (8)	**4b** (45)	**5b** (12)
7	**1b**	Burdock (*Arctium lappa*)	81	**2b** (36)	**3b** (36)	**4b** (28)	**5b** (−)
8	**1b**	Onion (*Allium cepa*)	85	**2b** (−)	**3b** (9)	**4b** (85)	**5b** (6)

[a] Reaction conditions: substrate (20 mg), water (70 ml) and vegetable (10 g) were cultivated with shaking at 25 °C, 48 h.
[b] Isolated yields.
[c] Relative intensities by ^{1}H NMR peak area.

3*S*-*exo*-hydroxycamphor (**4a**) as the major products. In the case of reduction of (+)-camphorquinone (**1a**) by the potato, there was no selectivity. On the contrary, the sweet potato gave (+)-2*R*-*exo*-hydroxyepicamphor (**2a**) in 79% selectivity.

(−)-Camphorquinone (**1b**) with various vegetables was transformed to the corresponding four isomers. Reduction of (−)-camphorquinone (**1a**) with onion provided (+)-3*R*-*exo*-hydroxycamphor (**4b**) in 85% selectivity. However, apple and cucumber were transformed to (+)-3*R*-*exo*-hydroxycamphor (**4b**), whereas sweet potato gave (−)-2*S*-*exo*-hydroxyepicamphor (**2b**) low selectivity as a major product. The yields and products formed by each of the vegetables are summarized in Tables 2 and 3.

In addition, it was found that the stereoselectivity and yield do not depend on places of production, lot and season of the vegetables (for example, it is Saitama, Chiba, Hokkaido, etc.).

It is known that the plant cultured-cells have the ability to various organic compounds. However, the problem using plant cultured-cells as biocatalysts is that they usually grow very slowly and require a large amount of the catalyst. On the other hand, the experiment of the enzymatic system using various vegetables can be operated easily.

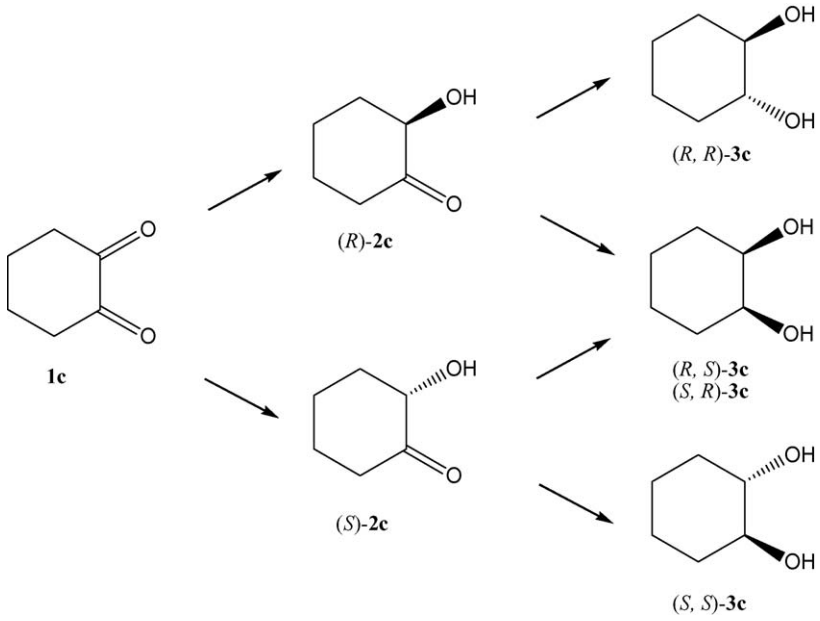
*3.3. Reduction of (+)- and (−)-camphorquinones (**1a, 1b**) in buffer*

The stereoselectivity of reduction was influenced by the reaction conditions. For example, the reduction of carbonyl compound was carried out according to the experimental proce-

Table 4
Reduction of camphorquinone (**1a**, **1b**) by carrot[a]

Run	Substrate	Vegetable	PH	Yield (%)[b]	Product ratio (%)[c]			
1	**1a**	Carrrot (*Daucus carota*)	4	85	**2a** (4)	**3a** (−)	**4a** (90)	**5a** (6)
2	**1a**	Carrot (*D. carota*)	7	85	**2a** (2)	**3a** (−)	**4a** (89)	**5a** (9)
3	**1a**	Carrot (*D. carota*)	10	85	**2a** (3)	**3a** (−)	**4a** (90)	**5a** (7)
4	**1b**	Carrot (*D. carota*)	4	84	**2b** (54)	**3b** (17)	**4b** (25)	**5b** (4)
5	**1b**	Carrot (*D. carota*)	7	86	**2b** (53)	**3b** (15)	**4b** (26)	**5b** (6)
6	**1b**	Carrot (*D. carota*)	10	86	**2b** (53)	**3b** (17)	**4b** (26)	**5b** (4)

[a] Reaction conditions: substrate (20 mg), water (70 ml) and carrot (10 g) were cultivated with shaking at 25 °C for 24 h.
[b] Isolated yields.
[c] Relative intensities by ^{1}H NMR peak area.



Scheme 3. Reduction of 1,2-cyclohexanedione (**1c**) by various vegetables.

Table 5
Reduction of 1,2-cyclohexanedione (**1c**) by various vegetables[a]

Run	Substrate	Vegetable	Product	Yield (%)[b]	d.e. (%)[c]	e.e. (%)[c]
1	**1c**	Carrot (*Ducus carota*)	**3c**	30	72	87(1*S*, 2*S*)
2	**1c**	Potato (*Soanum tuberosum*)	**3c**	22	28	2 (1*S*, 2*S*)
3	**1c**	Sweet potato (*Ipomoea batatas*)	**3c**	26	44	58 (1*R*, 2*R*)
4	**1c**	Apple (*Malus pumila*)	**3c**	46	56	90 (1*S*, 2*S*)
5	**1c**	Radish (*Raphanus sativus*)	**3c**	43	80	19 (1*R*, 2*R*)
6	**1c**	Cucumber (*Cucumis sativus*)	**3c**	40	56	62 (1*S*, 2*S*)
7	**1c**	Burdock (*Arctium lappa*)	**3c**	34	62	91 (1*S*, 2*S*)
8	**1c**	Onion (*Allium cepa*)	**3c**	43	60	85 (1*S*, 2*S*)

[a] Reaction conditions: substrate (20 mg), water (70 ml) and vegetables (10 g) were cultivated with shaking at 25 °C for 48 h.
[b] GLC yields.
[c] GLC peak area.

dures reported: one employs cut carrot in water [20], while the other minced carrot in 0.1 M phosphate buffer at pH 6.5. It was reported that reduction of ketones under the buffer conditions decreased the enatioselectivity [21]. Here, we report on the biotransformation of (+)- and (−)-camphorquinones (**1a**, **1b**) under buffer conditions.

Freshly cut carrot was suspended in 70 ml of 0.1 M phosphate buffer [pH 4, pH 7 and pH 10]. These results are shown in Table 4. The stereoselectivity of reduction was not influenced by pH conditions. (+)-Camphorquinone (**1a**) provided (−)-3*S*-*exo*-hydroxycamphor (**4a**) with high stereoselectivity, while (−)-camphorquinone (**1b**) transformed to give (+)-3*R*-*exo*-hydroxycamphor (**4b**) with moderate stereoselectivity.

*3.4. Reduction of 1,2-cyclohexanedione (**1c**) by various vegetables*

Several applications of the diastereoselectivity reduction product for 1,2-cyclohexanediol have been reported. (1*R*, 2*R*)-*trans*-1,2-cyclohexanediol (**3c**) is used in organic synthesis as a chiral auxiliary. The conjugate addition proceeds by organocuprate reagents and Grignard reagents to α,β-unsatured ester of (1*R*, 2*R*)-*trans*-1,2-cyclohexanediol (**3c**) with high diastereoselectivity [27]. For instance, it is used as an auxiliary for the enatio- and diastereoselective synthesis of β-substituted five-, or six-membered cyclohexanecarboxylates [28]. Moreover, asymmetric hydrogenation of 1,2-cyclohexanedione (**1c**) over cinchonidine-modified platinum was investigated [25].

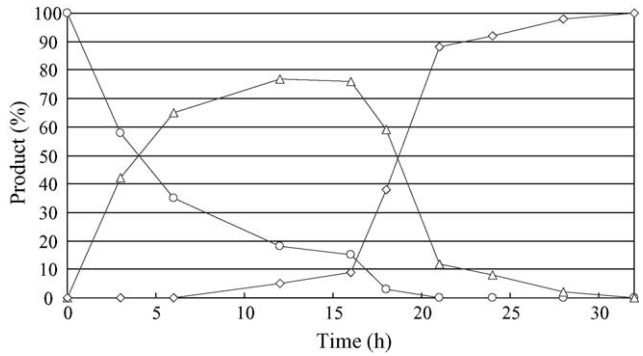


Fig. 3. Reduction of 1,2-cyclohexanedione (**1c**) by carrot.

Table 6
Reduction of 2-methylcyclohexanone (**1d**) by various vegetables[a]

Run	Substrate	Vegetable	Product	Yield (%)[b]	*cis*-Alcohol[c] [ratio/e.e. (%)]	*trans* -Alcohol[c] [ratio/e.e. (%)]
1	**1d**	Carrot (*Ducus carota*)	**2d**	67	48/100 (1*S*, 2*R*)	52/100 (1*S*, 2*S*)
2	**1d**	Potato (*Soanum tuberosum*)	**2d**	38	8	92/77 (1*S*, 2*S*)
3	**1d**	Sweet potato (*Ipomoea batatas*)	**2d**	61	70/37 (1*S*, 2*R*)	30/90 (1*S*, 2*S*)
4	**1d**	Apple (*Malus pumila*)	**2d**	18	77/95 (1*S*, 2*R*)	23/50 (1*S*, 2*S*)
5	**1d**	Radish (*Raphanus sativus*)	**2d**	14	36/77 (1*S*, 2*R*)	64/73 (1*S*, 2*S*)
6	**1d**	Cucumber (*Cucumis sativus*)	**2d**	15	24/75 (1*S*, 2*R*)	76/81 (1*S*, 2*S*)
7	**1d**	Burdock (*Arctium lappa*)	**2d**	66	53/70 (1*S*, 2*R*)	47/88 (1*S*, 2*S*)
8	**1d**	Onion (*Allium cepa*)	**2d**	49	39/80 (1*S*, 2*R*)	61/90 (1*S*, 2*S*)

[a] Reaction conditions: substrate (20 mg), water (70 ml) and vegetables (10 g) were cultivated with shaking at 25 °C for 48 h.
[b] GLC yields.
[c] GLC peak area.

Here, we report on the asymmetric reduction of 1,2-cyclohexanedione (**1c**) using various vegetables (Scheme 3).

Biotransformation of 1,2-cyclohexanedione (**1c**) using various vegetables reduced to product *trans*-1,2-cyclohexanediol in high selectivity. These results are shown in Table 5. From these results, it was found that only diols were obtained and hydroxy ketones were not afforded. 1,2-Cyclohexanediol was produced by reduction of hydroxyketone compound (Fig. 3). The enantioselective reduction of **1c** using burdock resulted in 91% enantiomeric excess of (1*S*, 2*S*)-*trans*-1,2-cyclohexanediol (**3c**) at full conversion. Moreover, carrot, apple and onion were provided **3c** in high enantiomeric excess. On the contrary, sweet potato and radish were obtained (1*R*, 2*R*)-*trans*-1,2-cyclohexanediol (**3c**) as major products. In the case of radish, the diastereomeric excess of the *trans*-1,2-cyclohexanediol ((*R*, *R*)-**3**) was 80% at complete conversion. On the contrary, burdock provided *trans*-1,2-cyclohexanediol in good diastereomeric excess (60%).

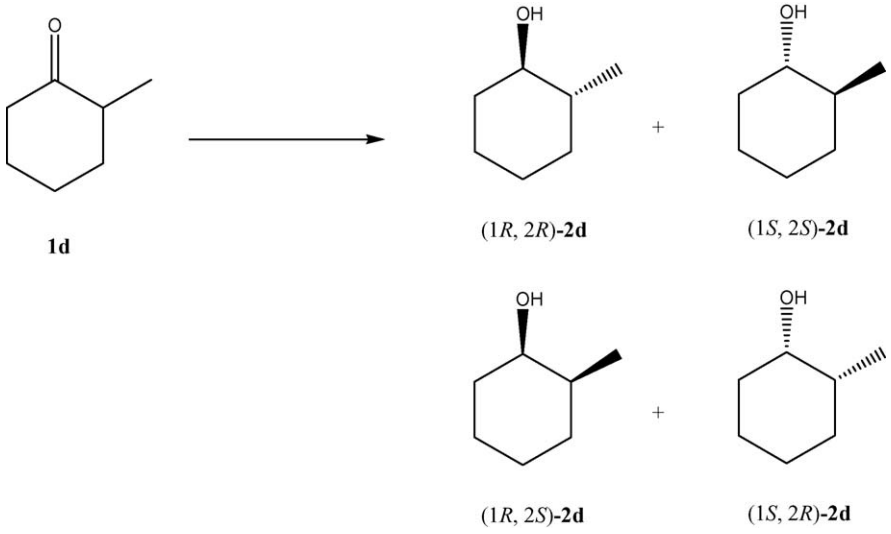
3.5. Reduction of 2-methylcyclohexanone (*1d*) by various vegetables

The biotransformation of acetophenone by immobilized plant cell cultured of tobacco, gardenia and carrot have been investigated [29]. In particular, in the case of immobilized carrot cell cultures, excellent enantioselectivity has been demonstrated in the reduction of prochiral ketone substrates [30,31]. However, immobilized cell cultures need a long time for cultivation and careful growing operations and difficult large scale set. Furthermore, unlike the purified enzyme, the biotransformation carried out with vegetables is economical and convenient method. In order to avoid the time consuming, we have been investigating the possibility to direct use part of vegetable as biocatalysts for the reduction of prochiral ketones. In this case, the starting material used for the preparations of cell cultures is directly obtained from a portion of the vegetable. In this paper, the reduction of **1d** with various vegetables are described.

2-Methylcyclohexanone (**1d**) was reduced to give *trans*- and *cis*-2-methycyclohexanol (**2d**). These results are shown in Table 6. In the case of the potato, the reduction was high diastereomeric excess (*trans*-2-methylcyclohexanol, 84%) (Scheme 4).

3.6. Preparative scale reduction

Finally, preparative scale reduction of (+)-camphorquinone (**1a**) was carried out by reduction of subjecting 1.0 g using burdock (100 g) and water (700 ml) at 25 °C (Table 7, entry 6).



Scheme 4. Reduction of 2-methylcyclohexanone (**1d**) by various vegetables.

Table 7
Reduction of (+)-camphorquinone (**1b**) on a preparative scale[a]

Run	Substrate	Substrate/burdock (w/w)	Yield (%)[b]	Product ratio (%)[c]			
1	**1a**	0.02/10	86	**2a** (−)	**3a** (−)	**4a** (100)	**5a** (−)
2	**1a**	0.04/10	84	**2a** (−)	**3a** (−)	**4a** (95)	**5a** (5)
3	**1a**	0.06/10	85	**2a** (−)	**3a** (−)	**4a** (90)	**5a** (10)
4	**1a**	0.08/10	91	**2a** (−)	**3a** (−)	**4a** (91)	**5a** (9)
5	**1a**	0.10/10	90	**2a** (−)	**3a** (−)	**4a** (93)	**5a** (7)
6	**1a**	1.0/100	90	**2a** (−)	**3a** (−)	**4a** (94)	**5a** (6)

[a] Reaction conditions: substrate (20–100 mg), water (70 ml) and vegetable (10 g) were cultivated with shaking at 25 °C for 48 h.
[b] Isolated yields.
[c] Relative intensities by ^{1}H NMR peak area.

After 48 h, 0.902 g of α-keto alcohols were obtained in 90% yield and (−)-3*S*-*exo*-hydroxycamphor (**4a**) in 94% selectivity. The result indicates that reduction of (+)-camphorquinone (**1a**) yielded (−)-3*S*-*exo*-hydroxycamphor (**4a**) with high stereoselectivity in preparation scale.

4. Conclusion

In summation, we have established a convenient and simple procedure for the preparation of α-hydroxycamphor from (+)- and (−)-camphorquinones (**1a**, **1b**) with various vegetables using the reduction process, under mild conditions. In particular, reduction of (+)-camphorquinone (**1a**) with burdock gave (−)-3*S*-*exo*-hydroxycamphor (**4a**) with higher stereoselectivity than previous biotransformation of **1a** [2–6]. The availability of the enzymatic system using various vegetables is low cost, with ease of work-up and eco-friendly system for all advantages.

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