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Stereoselective Reduction Of Some S-Ketoesters By Brassica Rapa And Daucus Carota Using Plant Roots And Plant Cultured Cells

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Abstract: Asymmetric reduction of 8 prochiral -ketoesers (1a-h) mediated by *Brassica rapa* and *Daucus carota* fresh plant roots (sterile and non-sterile) and cell cultures were studied. Among different method evaluated, all reduced -ketoesers in a Prelog manner. Therefore it is possible to prepare the (S)-secondary alcohols of (1a-h) by reduction of corresponding prochiral ketone with these two biocatalysts. It was observed that reductive capacity of *B.rapa* was higher than *D.carota* generally, since high chemical and optical yields gained. After conducting the biotransformation in sterile condition, it was revealed that in most cases *D.carota* was more efficient in bioreductions than *B.rapa*. For better understanding, the biotransformation was run by cell cultures of these two vegetables. As could be expected no bioreduction was observed with *B.rapa* calli. These facts allow presenting role of microorganism in contribution to biotransformations by plant tissues and the necessity for sterilization prior to running the biotransformation.
Keywords: Stereoselective; Bioreduction; Prochiral ketones; *Brassica rapa*; *Daucus carota.*

Introduction

Chiral alcohols are the key chiral building blocks to many single enantiomer chemicals. These optically active alcohols may be obtained either by use of chiral metal complexes (1) or alternatively, by biocatalysis of corresponding prochiral ketones (2,3). -Keto esters are a well-known group of prochiral ketones and readily reduced to -hydroxyesters, which serve as chiral starting materials for the synthesis of -lactams (4), insect pheromones (5) and carotenoids (6).

To date, few methods have been reported for the synthesis of optically pure -hydroxyesters (7-9).

Plant-mediated biocatalytic transformation of prochiral ketones to chiral alcohols have attracted much attention due to its outstanding enantioselectivity, mild and environmentally friendly reactions (10, 11). Among different

plants used as biocatalyst, carrot (*Daucus carota* L.) showed the broadest substrate scope and the highest enantioselectivity (up to 99%) (12) both as plant growing and cultured cells (13, 14).

Here, in our study to prepare optically pure -hydroxyesters in addition to *Daucus carota* L. we also chose *Brassica rapa* L., with few reports, as plant growing and cultured cells biocatalyst. We also accomplish the bioreduction of our -ketoesters with this two plant roots in two sterile and nonsterile conditions in order to explore the influence of microbes which has few reports in literature (15).

Material and methods

Materials

Optical rotation was measured using a POLAX-2L, ATAGO® polarimeter. Biotransformation reactions were monitored by GC/MS in an Agilent 7890A instrument equipped with a HP-5 capillary column (30m × 0.25 mm; 0.25 µm film thicknesses). Enantiomeric excess were determined by GC (FID) analysis in an Agilent technology 6890N instrument equipped with a CP-Chirasil-DEX CB Varian GC column. Preparative and analytical thin layer chromatography procedures were performed on silica gel 60 GF_{254} (Merck).

All substrate ketones **(Fig. 1.),** 2,4-dichlorophenoxy acetic acid and 1-naphtylic acetic acid were purchased from Merck (Germany). 2-Benzyl aminopurine was purchased from Sigma-Aldrich (USA). Chlorhexidine was prepared from Shahrdarou pharmaceutical company (Iran). Dichloromethane and other reagents were of analytical grade and commercially available.

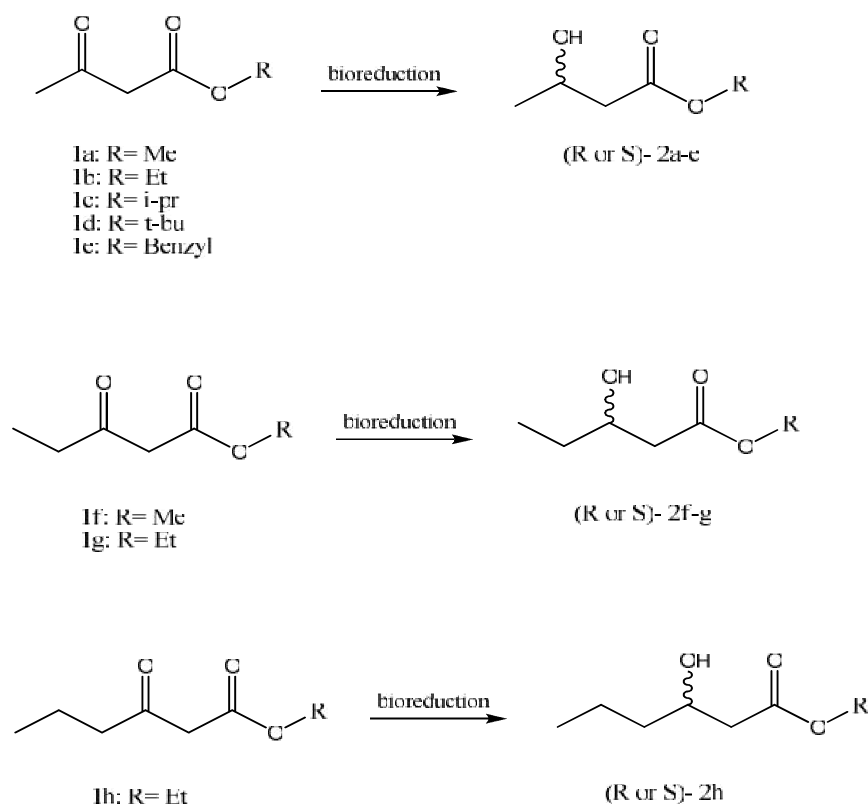


Figure 1. Bioreduction of -ketoesters **1a-h** catalyzed by plants

Biotransformation methods

Biotransformation with plant root

Fresh plants of *Daucus carota* and *Brassica rapa* were obtained from a local market. The external layer of the plants was removed and the rest was carefully minced into small thin pieces. A certain amount of substrate **1a-h** (100 µl) (Fig 1.) solved in 1 ml acetone, was added to a suspension of freshly cut plant tissue (100 g) in

phosphate buffer (0.1 M, 150 ml, pH 6.5) in Erlenmeyer flasks (500 ml). The resulting mixture kept under vigorous stirring (150 rpm) for 96 hour in a shaker incubator at 25˚C. The progress of the biotransformation was monitored by means of GC-MS at 48 and 96 hours. The 75 ml samples for analysis were collected at 2 and 4 day of the reaction. The suspension was filtered and the media was extracted with CH_2Cl_2 (×3), dried over anhydrous sodium sulphate and evaporated in vacuum.

For sterile biotransformation condition the roots were washed thoroughly with water and surface disinfected with EtOH 70% for 5 min, chlorhexidine 10% for 15 min. In addition, sterilization of instruments was performed by means of autoclave at 121˚C for 20 min. The procedures were also carried out under a horizontal laminar flow hood to ensure the sterile condition. To check the sterility of the reaction a culture of samples were prepared after the end of the biotansformation and no microorganisms were observed in the media.

Biotransformation with undifferentiated cells (calli)

The plant cell calli used in this research were initiated from seeds and maintained on Murashige Skoog agar supplemented with sucrose (30 g/ l) and auxins and cytokinins at different ratios. The calli were mechanically disrupted and placed in flasks (5g per flasks) containing 25 ml of liquid MS media with 0.5 mg/L 2,4-dichlorophenoxy acetic acid, 0.1 mg/L 1-naphtylic acetic acid and 0.2 mg/L 2-benzyl aminopurine. After that 100 µl of substrate dissolved in 1 ml acetone were added to each flask and incubated in an orbital shaker at 150 rpm and 25˚C. The suspension was filtered after 2 and 4 days of incubation and the media was extracted with CH_2Cl_2 (×3), dried over anhydrous Na_2SO_4 and evaporated in vacuo.

Assignment of the absolute configurations and enantiomeric excess for the alcohols (RS)-2a-h

The enantiomeric excess of these -hydroxyesters was determined by chiral GC analysis. All of chiral alcohols reported here have been prepared previously as single enantiomers and the absolute configurations were assigned by comparison of the optical rotation data with literature value (16,17).

Results and discussion

In order to evaluate the catalytic potential of different ways reported in the publications for bioreduction of prochiral ketone, we decided to use plant roots as well as calli of *D.carota* and *Brassica rapa* as biocatalysts in our study. We also tried to investigate the role of endophytic microorganisms in bioreduction by proceeding with conducting the bioreductions in two sterile and non sterile media.

Among the different methods tested, all reduced **1a-h** in a Prelog manner (18) to produce S-form of 2a-h, but with different conversion yields and with different stereoselection (Table 1, 2).

The results obtained from biotransformation in non sterile condition, allowed comparison of the plant roots biotransformation of substrate **1a-h** with *D.carota* and *B.rapa*. We observe that *Brassica rapa* was generally a better biocatalyst since high chemical and optical yields gained (Table 1). Only in one case (**1d**) a significant increase in yield was observed by carrot root. For another compound (**1h)** no transformation was observed with either plant (Table 1). In addition no bioreduction products were detected for (**1f)** and (**1g)** with *D. carota*.

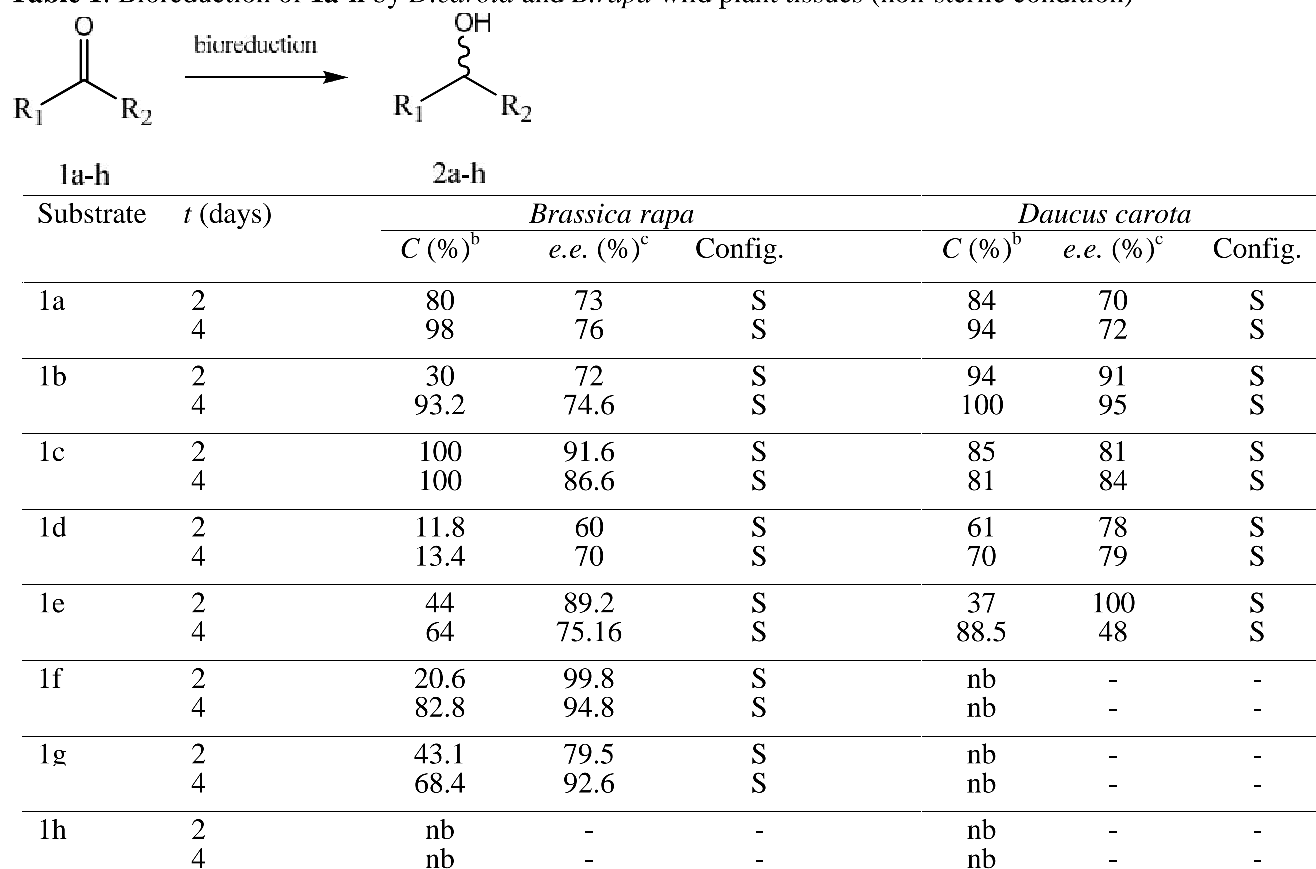
Furthermore for evaluation of the influence of endophytic microorganisms, the reactions were done in sterile condition. As it can be seen from table 2, the yields of conversions decrease in *B.rapa* dramatically. So we presumed that *B. rapa* is more efficient biocatalyst in nonsterile condition, although, in sterile condition *D. carota* exhibited better conversion yields.

Inspired by these results, we decided to run the biotransformations by cell cultures of *B.rapa* and *D.carota* so we could verify our assumption. We observed that *B.rapa* calli didn't reduce any of our substrate. In turn, *D.carota* calli biotransformed **1c**, **1b** and **1d** of our substrate mostly in high yield (Table 3).

All of these data support the hypothesis that endophytic microorganisms are important and involve in the biotransformation, especially for *B.rapa*. Thus, the higher conversion yields of biotransformation with plant root of *B.rapa* may attribute to the contribution of microbes in the biotransformation reactions. Based on the results were reported in this study, it can be concluded that both endophytic microorganisms and whole plant enzymes contributed in the bioreduction.

Therefore it seems that sterilization prior to run the biotransformation with plants is important for exclusion the effect of endophytic microorganisms as well as microbial contamination.

Table 1. Bioreduction of **1a-h** by *D.carota* and *B.rapa* wild plant tissues (non-sterile condition)[a]



Substrate	*t* (days)	*Brassica rapa*			*Daucus carota*		
		C (%)[b]	*e.e.* (%)[c]	Config.	*C* (%)[b]	*e.e.* (%)[c]	Config.
1a	2	80	73	S	84	70	S
	4	98	76	S	94	72	S
1b	2	30	72	S	94	91	S
	4	93.2	74.6	S	100	95	S
1c	2	100	91.6	S	85	81	S
	4	100	86.6	S	81	84	S
1d	2	11.8	60	S	61	78	S
	4	13.4	70	S	70	79	S
1e	2	44	89.2	S	37	100	S
	4	64	75.16	S	88.5	48	S
1f	2	20.6	99.8	S	nb	-	-
	4	82.8	94.8	S	nb	-	-
1g	2	43.1	79.5	S	nb	-	-
	4	68.4	92.6	S	nb	-	-
1h	2	nb	-	-	nb	-	-
	4	nb	-	-	nb	-	-

C: conversion percentage; e.e: enantiomeric excess; Config: absolute configuration; nb: no bioreduction products detected

a. Reaction conditions: Biocatalyst, 100 g (plant root); substrate, 100 µl; temperature, 25 ˚C; shaker speed, 150 rpm; time, 4 day.

b. Method: GC/MS

c. Method: Chiral GC

Table 2. Bioreduction of **1a-h** by *D.carota* and *B.rapa* wild plant tissues (sterile condition)[a]

Substrate (days)	*t*	*Brassica rapa*			*Daucus carota*		
		C (%)	*e.e.* (%)	Config.	*C* (%)	*e.e.* (%)	Config.
1a	2	30	81	S	nb	-	-
	4	47	80	S	nb	-	-
1b	2	nb	-	-	26	90	S
	4	nb	-	-	47	70	S
1c	2	15.5	-	-	45	46	S
	4	19.5	-	-	58	14	S
1d	2	31	88	S	55	88	S
	4	34	91	S	69	88	S
1e	2	nb	-	-	91	38	S
	4	nb	-	-	93	70	S

c: conversion percentage; e.e: enantiomeric excess; Config: absolute configuration; nb: no bioreduction products detected

a. Reaction conditions: Biocatalyst, 100 g (plant root) sterile with EtOH 70% & chlorhexidine 10%; substrate, 100 µl; temperature, 25 ˚C; shaker speed, 150 rpm; time, 4 day.

b. Method: GC/MS

c. Method: Chiral GC

Table 3. Bioreduction of **1a-h** by *D.carota* and *B.rapa* cell cultures.[a]

Substrate	*t* (days)	*Brassica rapa*			*Daucus carota*		
		C (%)	*e.e.* (%)	Config.	*C* (%)	*e.e.* (%)	Config.
1b	2	nb	-	-	100	78	S
	4	nb	-	-	100	70	S
1c	2	nb	-	-	100	76	S
	4	nb	-	-	100	68	S
1d	2	nb	-	-	65	82	S
	4	nb	-	-	74	44	S

c: conversion percentage; e.e: enantiomeric excess; Config: absolute configuration; nb: no bioreduction products detected
a. Reaction conditions: Biocatalyst, 5 g (callus); substrate, 100 µl; temperature, 25 ˚C; shaker speed, 150 rpm; time, 4 day.
b. Method: GC/MS
c. Method: Chiral GC

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Declaration of Interest

There is no conflict of interest.

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