Pergamon

Tetrahedron: *Asymmetry* 13 (2002) 2299–2302

TETRAHEDRON: *ASYMMETRY*

Enantioselective hydrolysis of 1-aryl ethyl acetates and reduction of aryl methyl ketones using carrot, celeriac and horseradish enzyme systems

Wanda K. Mączka and Agnieszka Mironowicz*

Department of Chemistry, Agricultural University, ul. Norwida 25, 50-375 Wrocław, Poland

Received 26 July 2002; revised 6 September 2002; accepted 27 September 2002

Abstract—Enantioselective hydrolyses of racemic 1-phenyl (or naphthyl) ethyl acetates and the reduction of methylphenyl (or naphthyl) ketones have been conducted using the comminuted roots of carrot (*Daucus carota* L.), celeriac (*Apium graveolens* L., var. *rapaceum*), and horseradish (*Armoracia lapatifolia* Gilib.). (*S*)-(−)-1-(2-Naphthyl)ethanol (100% yield, ee = 100%) has been obtained by reduction of 2-acetonaphthone using the comminuted roots of carrot.

1. Introduction

Optically active alcohols may be obtained either by the well known method of asymmetric reduction of a prochiral ketone, or alternatively, by enantioselective hydrolysis of racemic esters. Plant cell enzymes, like those from microorganisms, are able to catalyse reactions with high regio- and stereospecificity. Hence, both the reduction of acetophenone **4**[1,2] (or other ketones[3]) and the enantioselective hydrolysis of racemic 1-phenylethyl acetate **1**[4] using the enzyme systems of plant cells result in 1-phenylethanol enantiomers **1a** (or other alcohols).[3]

In our previous research, Jerusalem artichoke (*Helianthus tuberosus* L.) and carrot (*Daucus carota* L.) were used (among other vegetables) for the biotransformation of racemic 1-phenylethyl acetate **1**, acetophenone **4** and their analogues. Jerusalem artichoke was used both in the form of cell cultures and comminuted root tissue, while carrot was used in the form of cell cultures only.[5,6] On comparing the reactions of Jerusalem artichoke cell cultures with those where the comminuted tissues of its root were used, the efficiency of the biostransformation was found to be 20 times higher when the comminuted root tissue was used. Hence, we anticipated the same results using the comminuted roots of carrot. As well as carrot cells, celeriac (*Apium graveolens* L., var. *rapaceum*—from the same family *Appiaceae*) and horseradish (*Amroracia lapatifolia* Gilib.) from the taxonomically distant family *Brassicaceae* were selected for the biotransformation.

The application of comminuted tissue of ripe vegetable roots in the biotransformation (which have the advantages of low cost, short reaction times of ca. 48 h) instead of isolated enzymes is possible thanks to the group of enzymes that are able to accept xenobiotic substrates. However, the course of the transformation can become complex because the use of groups of enzymes enables many processes to occur simultaneously in the course of the biotransformation. For example, ester hydrolysis, oxidation of the resulting alcohol into a ketone and its subsequent reduction.

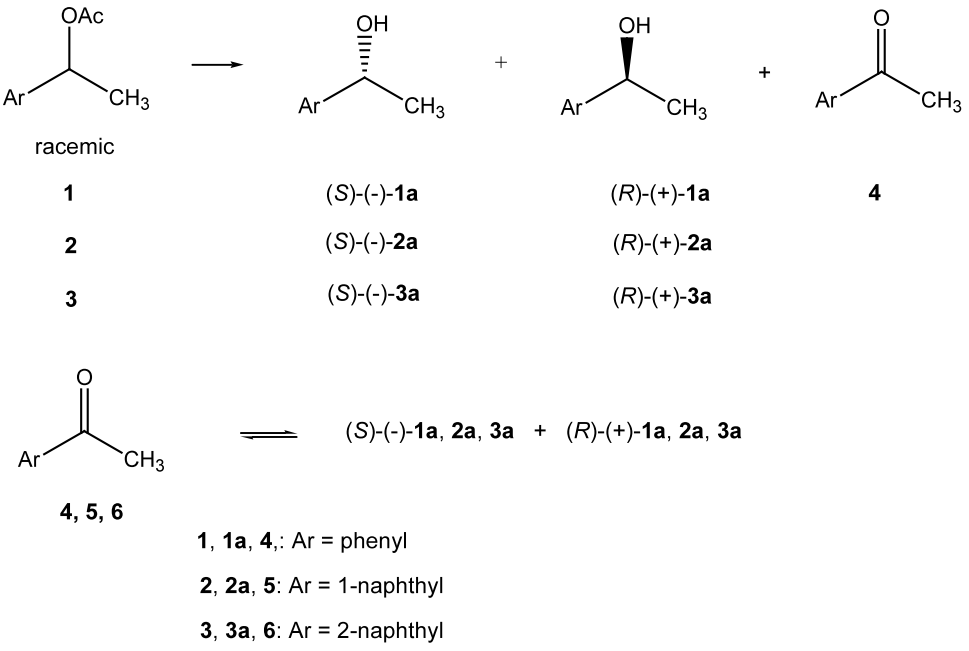
The 1-phenylethyl analogues were selected as substrates for this study due to the utility of these compounds as chiral auxiliaries and synthons when in enantiomerically pure form.

2. Results and discussion

The racemic acetates 1-phenylethyl **1**, 1-(1-naphthyl)ethyl **2**, 1-(2-naphthyl)ethyl **3** and prochiral aromatic-aliphatic ketones **4**, **5** and **6** were used as substrates to obtain optically active alcohols by biotransformation using the vegetable (celeriac, carrot and horseradish) enzyme systems (Scheme 1).

* Corresponding author. Tel.: (+4871) 3205468; fax: (+4871) 3284124; e-mail: amiron@ozi.ar.wroc.pl

0957-4166/02/$ - see front matter
PII: S0957-4166(02)00635-3



Scheme 1.

Besides the acetates **1**, **2** and **3** and the ketones **4**, **5** and **6**, the racemic alcohols **1a**, **2a** and **3a** were subjected to separate biotransformations. The results from all the transformations (average results from repeated biotransformations) are presented in Table 1.

These results allow comparison of the presently obtained results from biotransformations using the comminuted carrot root tissue enzymatic system with the results achieved previously using a suspension cell culture.[5,7] This comparison shows that using a comminuted catalyst increases the efficiency of the reaction by a factor of 18.

The progress of the reaction and its enantioselectivity were investigated by monitoring (GC) the components in the reaction mixture every few hours. The results of representative biotransformations are presented in Fig. 1.

Following the example of (±)-1-phenylethyl acetate **1**, biotransformation by the celeriac enzymatic system (Table 1, entry 1 and Fig. 1a), it is possible to examine the successive stages of the reaction: hydrolysis, oxidation and reduction. During the first hours of the biotransformation of **1**, the hydrolysis delivers more (*S*)-(−)-alcohol, (*S*)-(−)-**1a**. But at the end of the biotransformation the amount of (*S*)-(−)-**1a** drops significantly because it is the main substance to be oxidized (Table 1, entry 19). Nevertheless the level of (*S*)-(−)-**1a** in the mixture never equals zero because the ketone obtained is rapidly reduced, initially to (*S*)-(−)-**1a**, as confirmed by the results presented in entry 10 of Table 1, and in Fig. 1(c). This reversible reaction: ketone **4** ⇄ alcohol **1a**, leads to the continuous formation of (*S*)-(−)-1-phenylethanol (*S*)-(−)-**1a**, which makes the preparation of pure (*R*)-(+)-alcohol impossible via this method.

Considering the abilities of these biocatalysts to carry out enantioselective processes, it was observed that the carrot enzymatic system exhibits poor enantioselectivity in the hydrolysis of the racemic acetate **1** (Fig. 1b and Table 1, entry 2) to afford the enantiomerically pure alcohols **1a**. However, the same biocatalyst is capable of converting 2-acetonaphthone **6** with 100% efficiency into (*S*)-(−)-alcohol **3a** with ee = 100% (Table 1, entry 17 and Fig. 1d).

In summary, the following aspects of the biotransformations presented are of note.

Hydrolysis of acetates. (a) The carrot enzymatic system effects hydrolysis of the acetates **1**, **2**, and **3** to higher degrees and with greater stereoselectivity than the enzymatic systems of the other vegetables studied here. (b) Oxidation of the alcohols obtained as a result of the hydrolysis cannot be treated as a rule as it depends on the structure of the substrate and the biocatalyst used. (c) The horseradish enzymatic system effects the hydrolysis of acetates **1**, **2** and **3** to afford alcohols of the opposite configuration compared to the reactions completed using the carrot and celeriac enzyme systems.

Reduction of ketones. The performance of the carrot enzymatic system is highly substrate dependent: 2-acetonaphthone **6** is completely reduced but 1-acetonaphthone **5** is not transformed (Table 1, entries 14 and 17). High enantioselectivity was observed in only one case (Table 1, entry 17).

Oxidation of alcohols. The celeriac enzymatic system is highly sensitive to the substrate structure (Table 1, entries 22 and 25). The oxidation of ethanols with a naphthyl substituent by the carrot enzymatic system did

Table 1. Results from transformations of (±)-**1**, (±)-**2**, (±)-**3**, **4**, **5**, **6**, (±)-**1a**, (±)-**2a** and (±)-**3a** with celeriac, carrot and horseradish. (The contents of the reaction mixture was determined by gas chromatographic analysis of the crude extracts)

Entry substrate		Vegetable	Contents of the reaction mixture after biotransformation				
			Alcohol		Ketone (%)	Unreacted substrate	
			Quantity (%)	ee (%)		Quantity (%)	ee (%)
	Acetates						
1		Celeriac*	61	47 (*R*)-(+)	39	0	–
2	(±)-**1**	Carrot*	91	11 (*R*)-(+)	0	9	67 (*S*)-(−)
3		Horseradish	62	41 (*S*)-(−)	0	38	66 (*R*)-(+)
4		Celeriac	48	68 (*R*)-(+)	0	52	71 (*S*)-(−)
5	(±)-**2**	Carrot	64	75 (*R*)-(+)	0	36	82 (*S*)-(−)
6		Horseradish	20	53 (*S*)-(−)	0	80	70 (*R*)-(+)
7		Celeriac	55	33 (*R*)-(+)	0	45	32 (*S*)-(−)
8	(±)-**3**	Carrot	74	50 (*R*)-(+)	0	26	91 (*S*)-(−)
9		Horseradish	14	0	0	86	0
	Ketones						
10		Celeriac*	20	85 (*S*)-(−)		80	
11	**4**	Carrot	4	82 (*S*)-(−)		96	
12		Horseradish	5	88 (*S*)-(−)		95	
13		Celeriac	8	0		92	
14	**5**	Carrot	0	–		100	
15		Horseradish	0	–		100	
16		Celeriac	19	72 (*S*)-(−)		81	
17	**6**	Carrot*	100	100 (*S*)-(−)		0	
18		Horseradish	0	–		100	
	Alcohols						
19		Celeriac			29	71	69 (*R*)-(+)
20	(±)-**1a**	Carrot			11	89	0
21		Horseradish			Trace		–
22		Celeriac			0	100	–
23	(±)-**2a**	Carrot			0	100	–
24		Horseradish			0	100	–
25		Celeriac			60	40	35 (*S*)-(−)
26	(±)-**3a**	Carrot			0	100	–
27		Horseradish			0	100	–

* Reaction enantioselectivity see Fig. 1.

not proceed in our hands (Table 1, entries 23 and 26); the presence of a phenyl substituent in the substrate allows the oxidation to occur more readily with all three biocatalysts (Table 1, entries 19, 20 and 21).

On comparing the transformations of 1- and 2-naphthalene derivatives it can be said that the β-position is more suitable for enzymatic reaction. Additionally, substrates with one aromatic ring are transformed more easily and with higher enantioselectivity than molecules containing biaryl systems.

3. Experimental

3.1. Substrates 1a, 4, 5 and 6

These substrates were purchased from Aldrich Chemical Co., the others were obtained in our laboratory.

3.2. Biocatalysts

Fresh celeriac (*Apium graveolens* L. var. *rapaceum)*, carrot (*Daucus carota* L.) and horseradish (*Armoracia lapathifolia* Gilib.) were purchased from a local market.

3.3. Biotransformation conditions

Healthy vegetable roots were comminuted using an electric mixer for 2 min and vegetable pulp (20 ml, 1.0–1.5 g of dry wt, 100°C, 24 h) was placed in Erlenmayer flasks with phosphate buffer (0.1 M, 50 ml, pH 6.2 (celeriac), pH 6.5 (carrot), pH 4.5 (horseradish)). This pulp was mixed with a solution of the substrate (20–30 mg) in acetone (0.5 mL) and the resulting mixture was shaken for 48 h. The course of the biotransformation was monitored by means of TLC and GC analysis. Biotransformed mixtures were extracted with $CHCl_3$. The enantiomeric composition of the product

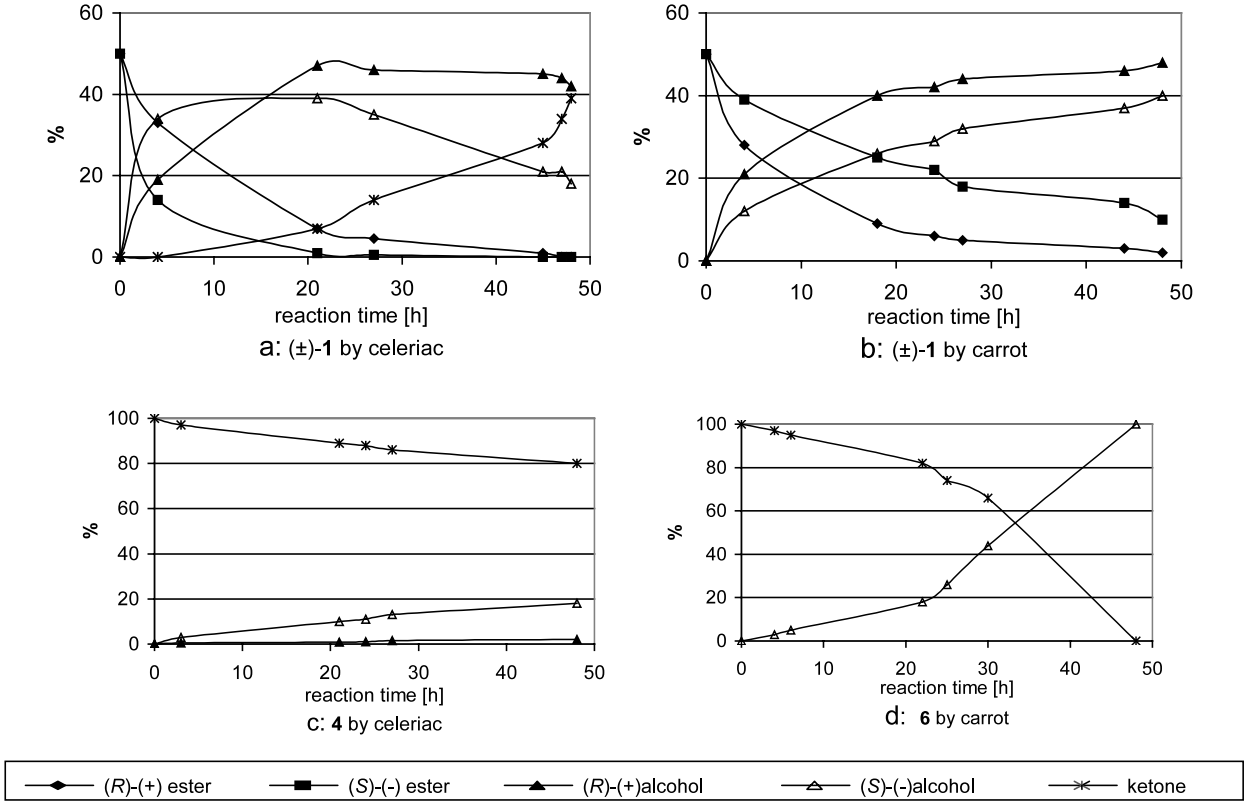


Figure 1. Changes of the reaction mixture contents during the biotransformation: (±)-1-phenylethyl acetate (±)-**1** by celeriac (a) and carrot (b); acetophenone **4** by celeriac (c) and 2-acetonaphthone **6** by carrot (d). The contents of the reaction mixture was determined by gas chromatographic analysis of the crude extracts.

mixture was established by GC by application of chiral columns, as mentioned in Section 3.4. All substrates in the buffer solution were stable under these conditions.

3.4. Analytical methods

GC: Hewlett–Packard 5890, FID, carrier gas—H_2 at 2 ml min^{-1}, using following Chrompack WCOT capillary columns: Chirasil-L-Val (25 m×0.25 mm×0.12 μm) for **2**, **2a**, **3**, **3a**, **5**, **6** (column temp. 120°C/30 min; gradient 20°C/min, injector temp. 200°C, detector temp. 250°C), Chirasil Dex CB (25 m×0.25 mm×0.12 μm) for **1**, **1a**, and **4** (column temp. 125°C/10 min; gradient 15°C/min, injector temp. 200°C, detector temp. 250°C); TLC: silica gel 60 F_{254} pre-coated aluminium sheets (layer thickness 0.2 mm, Merck) with *n*-hexane–Et_2O (5:1) for **2**, **2a**, **3**, **3a**, **5**, **6** and *n*-hexane–acetone (14:1) for **1**, **1a** and **4**.

References

1. Akakabe, Y.; Naoshima, Y. *Phytochemistry* **1994**, *35*, 661–664.
2. Villa, R.; Molinari, F.; Levati, M.; Aragozzini, F. *Biotechnol. Lett.* **1998**, *20*, 1105–1108.
3. Naoshima, Y.; Akakabe, Y. *J. Org. Chem.* **1989**, *54*, 4237–4239.
4. Mironowicz, A.; Kromer, K. *Collect. Czech. Chem. Commun.* **1998**, 1655–1662.
5. Mironowicz, A.; Jarosz, B.; Siewinski, A. *Acta Soc. Bot. Poloniae.* **1995**, *64*, 281–285.
6. Mironowicz, A. *Phytochemistry* **1998**, *47*, 1531–1534.
7. Mironowicz, A.; Kromer, K.; Pawłowicz, P.; Siewinski, A. *Acta Soc. Bot. Poloniae.* **1994**, *63*, 43–48.