Enantioselective Hydrolysis of Bromo- and Methoxy-Substituted 1-Phenylethanol Acetates Using Carrot and Celeriac Enzymatic Systems

Wanda K. Mączka and Agnieszka Mironowicz*

Department of Chemistry, Wrocław University of Environmental and Life Science, ul. Norwida 25, 50-375 Wrocław, Poland. Fax: (+48 71) 32 841 24.
E-mail: amiron@ozi.ar.wroc.pl

* Author for correspondence and reprint requests

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Dedicated to Prof. Antoni Siewiński on the occasion of his 80th birthday

Enantioselective hydrolysis of bromo- and methoxy-substituted 1-phenylethanol acetates was conducted using comminuted carrot (*Daucus carota* L.) and celeriac (*Apium graveolens* L. var. *rapaceum*) roots. Hydrolysis of the acetates led to alcohols, preferentially to *R*-(+)-enantiomers. Efficiencies of both reactions – hydrolysis of the acetates with an electron-donating methoxy group and oxidation of the resulting alcohols – increased in the following order: *ortho* < *meta* < *para*. The presence of an electron-withdrawing bromine atom in the aromatic ring had the opposite effect. Oxidation of alcohols with both types of substituents in the aromatic ring showed that location of a substituent had stronger impact on the oxidation rate than its electronic properties.

Key words: Vegetable, Hydrolysis, Oxidation

Introduction

Phenolic compounds (aromatic carboxylic acids, alcohols and aldehydes) that are present in plants may be formed via two different biosynthetic pathways: either by aromatization via shikimic acid, cinnamic acid, phenylalanine and tyrosine or via the malonic acid pathway. Apart from their important biological functions in plants, several compounds of this group have found wide application in pharmacotherapy [due to their bactericidal and disinfecting properties and calcium channel blocking effects (Wu, 1998)], and also in food and perfume industries (Lomascolo *et al.*, 1999). Some of the described compounds may find an application as synthons (chirons) in syntheses of complicated spatial structures or as auxiliaries in separation of racemic mixtures. For example, *R*(+)-1-phenylethanol can be used as a synthon in the synthesis of compactin, which is one of the strongest known competitive inhibitors of 3-hydroxy-3-methylglutarylcoenzyme A reductase (HMGR) (Istvan, 2002; Rosen and Heathcock, 1985). Also, this is a substrate for a simple synthesis of 1-ethylphenylamine (Chen *et al.*, 1996). Moreover, enantiopure (*R*)- or (*S*)-1-phenylethanol can be used as a chiral reagent for the determination of the enantiomeric purity of carboxylic acids and for their resolution (Takeuchi *et al.*, 1987).

In the literature over the last decade there is increasing interest in plant biocatalysts in the form of comminuted parts of ripe plants (*e.g.* roots, tubers, fruits), as a replacement for cell cultures, widely used so far (Blanchard and Weghe, 2006 and literature cited therein; Mironowicz, 1998; Mączka and Mironowicz, 2002, 2004a, b; Andrade *et al.*, 2006; Machado *et al.*, 2006; Utsukihara *et al.*, 2006).

In the present work, which is the continuation of our previous research (Mączka and Mironowicz, 2002; Mączka and Mironowicz, 2004a, b), we obtained enantiomeric secondary alcohols – the derivatives of 1-phenylethanol with either bromine or methoxy substituents in the aromatic ring (in *ortho*, *meta* and *para* positions) – via hydrolysis of the corresponding acetates. We were interested in assessing the influence of these substituents on the ratio of the substrate conversion and on the enantioselectivity of the acetate hydrolysis compared to the non-substituted ester – 1-phenylethanol acetate (Mączka and Mironowicz, 2002). We employed comminuted roots of vegetables: carrot (*Daucus carota* L.) and celeriac (*Apium graveolens* L. var. *rapaceum*) as biocatalysts. They are

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cheap and easily accessible. Additionally, the biotransformation is complete in 48 h, and isolation of products is very simple – only extraction is needed.

Experimental

*Synthesis of alcohols **1a–6a***

Alcohols **1a–6a** were obtained from the corresponding ketones using $NaBH_4$ in methanol. The spectroscopic data are in accordance with the literature.

*Synthesis of acetates **1–6***

Acetates **1–6** were obtained from the corresponding alcohols using acetic anhydride in pyridine. The spectroscopic data are in accordance with the literature.

Biocatalysts

Fresh celeriac (*Apium graveolens* L. var. *rapaceum*) and carrot (*Daucus carota* L.) were purchased from local market.

Biotransformation conditions

Healthy vegetable roots were comminuted (cut) for 2 min using an electric mixer. Portions of 20 mL of the vegetable pulp (1.0–1.5 g of dry weight, 100 °C, 24 h) were placed in Erlenmeyer flasks, each containing 50 mL of 0.1 M phosphate buffer [pH = 6.2 (celeriac), 6.5 (carrot)]. Then 20–30 mg of a substrate dissolved in 0.5 mL of acetone were added to this pulp and the mixtures were shaken for 48 h at room temperature. The course of biotransformation was monitored by means of TLC and GC. The biotransformed mixtures were then extracted with $CHCl_3$ (3×50 mL). Enantiomeric compositions of the product mixtures were established by means of GC using chiral columns. All the substrates were stable in buffer solutions under these conditions.

At least three repetitions of each biotransformation experiment were performed. In Table I we present the average results determined on the basis of GC chromatograms.

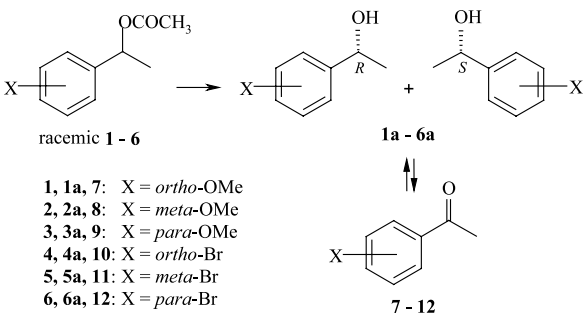
Analytical methods

GC: Hewlett-Packard 5890, FID, carrier gas H_2 at 2 mL min^{-1}, using following Chrompack WCOT capillary columns: Chirasil-Dex CB (25 m × 0.25 mm × 0.25 μm) for **1**, **1a** (column temperature 120 °C – 1 min; gradient 2 °C/min to 140 °C; gradient 30 °C/min to 200 °C – 2 min; injector temperature 200 °C; detector temperature 250 °C); for **2**, **2a** (column temperature 120 °C – 1 min; gradient 2 °C/min to 150 °C, gradient 30 °C/min to 200 °C – 2 min; injector temperature 200 °C; detector temperature 250 °C); for **3**, **3a** (column temperature 119 °C – 1 min; gradient 0.2 °C/min to 125 °C, gradient 30 °C/min to 200 °C – 2 min; injector temperature 200 °C; detector temperature 250 °C); for **4**, **4a** (column temperature 130 °C – 1 min; gradient 1 °C/min to 146 °C, gradient 30 °C/min to 200 °C – 2 min; injector temperature 200 °C; detector temperature 250 °C); for **5**, **5a** (column temperature 126 °C – 1 min; gradient 0.3 °C/min to 133 °C; gradient 30 °C/min to 200 °C – 2 min; injector temperature 200 °C; detector temperature 250 °C); for **6**, **6a** (column temperature 135 °C – 1 min; gradient 0.5 °C/min to 144 °C; gradient 30 °C/min to 200 °C – 2 min; injector temperature 200 °C; detector temperature 250 °C).

TLC: Silica gel 60 F_{254} pre-coated aluminium sheets (layer thickness 0.2 mm, Merck) with *n*-hexane/acetone (5:1 v/v) for **1–3** and **1a–3a** and *n*-hexane/acetone (7:1 v/v) for **4–6** and **4a–6a**.

Results

Hydrolysis of the actates **1–6** using carrot and celeriac enzymatic systems proceeded as shown in Scheme 1.



Scheme 1.

In order to obtain more detailed information about the course of reaction, not only the acetates **1–6**, but also the respective alcohols **1a–6a** (in separate experiments) were subjected to the biotransformations. The results of biotransformations

Table I. Results of biotransformations of acetates **1**–**6** and alcohols **1a**–**6a** with carrot and celeriac. The ratios of substrate conversion and configurations of the resulting alcohols were determined by GC analysis of the crude extracts.

Substrate	Entry	Biocatalyst[a]	Composition of the product mixture (%)		
			Ketone	Alcohol Yield (%)/ee(%) of *R*(+)-OH	Unreacted substrate Yield (%)/ee of *S*(−)-OAc or yield (%)/ee of *R*(+)-OH
(±)**1** (*o*-OMe)	1	*A. g.*	0	88/13	12/57
	2	*D. c.*	0	90/18	10/99
(±)**2** (*m*-OMe)	3	*A. g.*	0	90/5	0
	4	*D. c.*	0	74/26	26/99
(±)**3** (*p*-OMe)	5	*A. g.*	47	53/51	0
	6	*D. c.*	12	88/20	0
(±)**4** (*o*-Br)	7	*A. g.*	10	100/0	0
	8	*D. c.*	0	98/0	2/0
(±)**5** (*m*-Br)	9	*A. g.*	5	95/0	0
	10	*D. c.*	0	85/23	15/99
(±)**6** (*p*-Br)	11	*A. g.*	27	64/56	9/63
	12	*D. c.*	0	70/44	30/67
(±)1-Phenyl-ethanol acetate[b]		*A. g.*	39	61/47	0
		D. c.	0	91/11	9/67
(±)**1a** (*o*-OMe)	13	*A. g.*	0	–	100
	14	*D. c.*	0	–	100
(±)**2a**(*m*-OMe)	15	*A. g.*	9	–	91/4
	16	*D. c.*	trace	–	~100
(±)**3a** (*p*-OMe)	17	*A. g.*	32	–	68/42
	18	*D. c.*	5	–	95/4
(±)**4a** (*o*-Br)	19	*A. g.*	0	–	100
	20	*D. c.*	0	–	100
(±)**5a** (*m*-Br)	21	*A. g.*	6	–	94/4
	22	*D. c.*	trace	–	~100
(±)**6a** (*p*-Br)	23	*A. g.*	24	–	76/37
	24	*D. c.*	3	–	97/3
(±)1-Phenylethanol[b]		*A. g.*	29	–	71/69
		D. c.	11	–	89/0

[a] Biocatalyst to substrate ratio (dry weight) was 33–75; *A. g.*, *Apium graveolens*; *D. c., Daucus carota.*
[b] Mączka and Mironowicz, 2002.

of substrates **1**–**6** and **1a**–**6a** are presented in Table I.

After the standard biotransformation time (48 h) the ratio of acetate hydrolysis has already been high, which did not allow us to draw any conclusions about the stereoselectivity of the reactions and about the mechanism of oxidation of the resulting alcohols. Therefore, in order to monitor the course of biotransformation, the samples of the reaction mixture after 2, 4, 21, 24, 27, 43, 46 and 48 h of the biotransformation process were taken out. The results obtained are presented in Fig. 1.

Discussion

Ratio of substrate conversion

Both tested biocatalysts proved to be suitable for the hydrolysis of acetates **1**–**6** (the ratio of conversion was 70–100%). Oxidation of alcohols **2a**, **3a**, **5a** and **6a** was more efficient when using celeriac (6–32%) than carrot (3–5%). Biotransformations of acetates **3**, **5** and **6**, which led to both alcohols and ketones, proved that the celeriac enzymatic system is capable of not only hydrolysis but also of the reversible alcohol-ketone reaction. The carrot enzymatic system performed hydrolysis followed by oxidation of the resulting alcohol only in the case of substrate **3** (*p*-OMe).

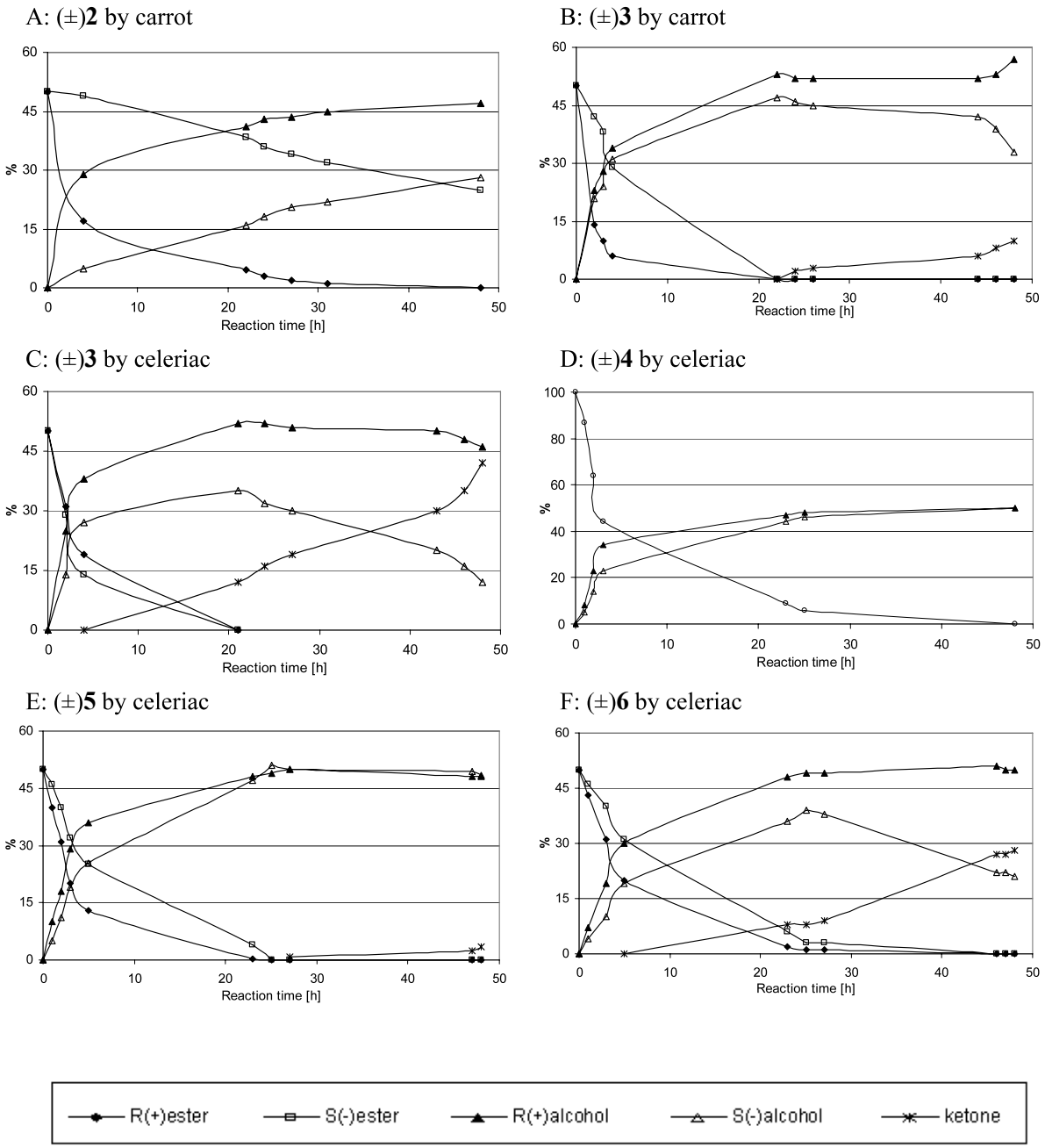


Fig. 1. Progress of biotransformation over time.

In case of bromine-substituted acetates **4**–**6** the rate of conversion by means of both biocatalysts depended on the bromine location: The greater distance between the bromine atom and the asymmetric carbon atom, the faster was the reaction. The rate of hydrolysis of *para*-bromo-substituted acetate **6** was similar to that of non-substitued 1-phenylethanol acetate (Mączka and Mironowicz, 2002).

The biotransformation time in both cases – hydrolysis of acetates and oxidation of alcohols –

was only 48 h, which was shorter than observed by Andrade and co-workers (Andrade *et al.*, 2006), who performed the oxidation of 4-bromophenylethanol using twelve different higher plants. Only two of these plants oxidized a secondary hydroxy to a carbonyl group with the conversion exceeding 14% after 3–6 days.

Enantioselectivity of the reaction

Hydrolysis of the acetates (Table I, entries 1–6 and 10–12) led preferentially to *R*-(+)-enantiomers of the alcohols, which is in accordance with the Kazlauskas rule (Kazlauskas *et al.,* 1991). Similar correlation was observed during the biotransformation of (±)-(1-phenylethyl)acetate using cell suspension cultures of bryophytes (Speicher and Roeser, 2002) and comminuted potato and topinambur tubers (Mironowicz, 1998). Hydrolysis of acetates **1**, **2**, **5** and **6** using carrot (Table I, entries 2, 4, 10, 12) (when the ketones were not formed) left the unracted *S*-acetates in a very high enantiomeric excess. The carrot enzymatic system is more stereoselective with respect to hydrolysis of acetates **1**–**5** than the celeriac enzymatic system. The higher enantiomeric excess of the alcohol obtained in hydrolysis by acetate **6** in the presence of celeriac (entry 11) arises from its following oxidation to the ketone, which is not performed by the enzymatic system of carrot.

The progress of the selected acetate biotransformations over time presented in Fig. 1 indicates that in all the cases when a ketone was formed (Figs. 1A, C, D, F, which correspond to entries 5, 6, 9 and 11 in Table I), the amount of the ketone has been increasing during the whole time of the biotransformation. That means that the rate of oxidation of alcohols is higher than the rate of the reduction of the resulting ketones. This observation is in accordance with our previous research (Mączka and Mironowicz, 2004a), where we observed highly stereoselective reduction of bromo and methoxy derivatives of acetophenone leading to *S*-OH alcohols.

The rate of the oxidation process was higher for the *S*-OH enantiomer of the alcohols (Table I, entries 15, 17, 18, 21, 23 and 24), however, the predominance of the *R*-OH enantiomer in the product mixture was not strong (3–42%).

Substitution of the aromatic ring with either a methoxy group or bromine atom did not considerably increase the optical purity of the alcohols (obtained by the hydrolysis of acetates **1**–**6**) compared to the non-substituted 1-phenylethanol acetate (Mączka and Mironowicz, 2002).

Effect of type and location of a substituent in the substrate

For acetates with an electron-donating methoxy group (**1**–**3**) both the substrate conversion ratio and the susceptibility of the resulting alcohols to oxidation increase in the order: *ortho* < *meta* < *para*. The electron-withdrawing bromine atom in the aromatic ring of a substrate (**4**–**6**) has the opposite effect – the more distant it is from the reacting group, the lower is the hydrolysis rate.

We have observed that the rate of oxidation of alcohols **1a**–**6a** (with both types of substituents in the aromatic ring) increased when the substituent was more distant from the hydroxy group. This leads to the conclusion that these substituents (*ortho* and *meta)* inhibit the oxidation process and that the location of a substituent has greater impact on the rate of this reaction than the type of a substituent. Alcohols with *para* substituents (entries 17, 18 and 23, 24) are oxidized faster than the *meta*-substituted ones (entries 15, 16 and 21, 22), whereas *ortho*-substituted alcohols do not undergo the oxidation at all.

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