Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 15 (2004) 1965–1967

Enantioselective reduction of bromo- and methoxy-acetophenone derivatives using carrot and celeriac enzymatic system

Wanda K. Mączka and Agnieszka Mironowicz*

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

Received 1 April 2004; accepted 25 May 2004

Abstract—The enantioselective reductions of the carbonyl group of bromo- and methoxy-acetophenones have been conducted using comminuted carrot (*Daucus carota* L.) and celeriac (*Apium graveolens* L., var. *rapaceum*) roots. (*S*)-(−)-1-(3-Methoxyphenyl)ethanol (100% yield, ee = 100%) has been obtained by reduction of *meta*-methoxyacetophenone using carrot.

1. Introduction

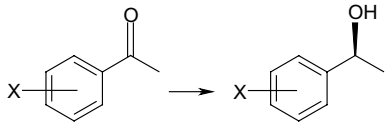
Asymmetric syntheses of chiral alcohols have found wide application in the production of drugs, agrochemicals, flavours and pigments. These alcohols may be obtained by enantioselective reduction of prochiral ketones. Among numerous methods of asymmetric carbonyl group reduction that have been developed over the last 25 years, biochemical methods using higher plants have been being widely used.[1] Acetophenone is a very interesting model xenobiotic substrate for bioreduction, because it may give rise to both enantiomers of 1-phenylethanol. These compounds, as well as their simple derivatives, have been effectively used as building blocks for asymmetric synthesis of drugs.[2–5]

It has been reported that reduction of a carbonyl group may be performed using various plant biocatalysts in different forms: as suspension cell cultures,[6,7] cell cultures,[8] immobilised plant cells[9,10] and also as cuted fresh fruits and vegetables.[11–15]

Our research has been focused on investigations as to the influence of the two types of substituents (bromine atom or methoxyl group) on the aromatic ring of acetophenone on the efficiency and stereoselectivity of the carbonyl group reduction. Comminuted roots of fresh carrot (*Daucus carota* L.) and celeriac (*Apium graveolens* L., var. *rapaceum*) have been employed as biocatalysts. This work is a continuation of our previous research using these two biocatalysts.[16]

2. Results and discussion

Six acetophenone derivatives have been used as the substrates for the biotransformations: *ortho*- **1**, *meta*- **2** and *para*-methoxyacetophenone **3**, and *ortho*- **4**, *meta*- **5** and *para*-bromoacetophenone **6**. The reduction of the carbonyl group by the carrot's and celeriac's enzymatic systems proceeded stereoselectively, following the Prelog's rule, which resulted in formation of (*S*)-alcohols predominantly (Scheme 1).



1 - 6 **1a - 6a**

1, 1a: X = *ortho*-OMe
2, 2a: X = *meta*-OMe
3, 3a: X = *para*-OMe
4, 4a: X = *ortho*-Br
5, 5a: X = *meta*-Br
6, 6a: X = *para*-Br

Scheme 1.

The results of bioreduction of substrates **1**–**6** are presented in Table 1.

* Corresponding author. Tel.: +48-71-3205468; fax: +48-71-3284124; e-mail: amiron@ozi.ar.wroc.pl

0957-4166/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2004.05.033

Table 1. Results of bioreductions of **1**–**6** with carrot and celeriac

Substrate	Entry	Ketone conversion (%)/ee of (*S*)-OH (%)	
		Carrot	Celeriac
1 (*o*-OMe)	1	11/78	
	2		10/87
2 (*m*-OMe)	3	100/100	
	4		100/98
3 (*p*-OMe)	5	12/88	
	6		10/87
4 (*o*-Br)	7	8/100	
	8		27/100
5 (*m*-Br)	9	54/91	
	10		40/86
6 (*p*-Br)	11	41/95	
	12		30/88

The ratios of substrates conversion and configurations of the resulting alcohols were determined by GC analysis of crude extracts.

The results obtained show that the presence of a substituent at the *meta*-position, no matter whether it is electron-withdrawing or electron-donating, gives rise to the highest yields of substrates **2** and **5** using both the biocatalysts described (entries 3, 4 and 9, 10). This observation is in accordance with the results of the bioreduction of *meta*-methoxyacetophenone by means of *Rhizopus arrhizus* cultures,[17] acetone powder of *Geotrichum candidum*[18] and fresh root of carrot, in the case of replacing the oxygen atom in the methoxyl group with the selenium one.[19]

As far as the *para*-substituted acetophenone derivatives are concerned (entries 5, 6, 11 and 12), we have observed that reduction of the substrate containing a bromine atom **6** by means of both biocatalysts proceeds three times faster than for the methoxy-substituted ketone **3**, which is in accordance with the results reported by Akakabe et al.[10] and Nakamura and Matsuda.[18] The highest enantiomeric excess of the alcohols was observed when *meta*-methoxyacetophenone **2** (entries 3 and 4) and *ortho*-bromoacetophenone **4** (entries 7 and 8) were subjected to the reduction. The results obtained indicate that both the enantioselectivity and efficiency of the reduction of bromo- and methoxy-substituted acetophenone derivatives by means of carrot's and celeriac's enzymatic systems depends mainly on the substituent location. The influence of a substituent nature is less important.

3. Experimental

3.1. Substrates **1**–**6** were obtained from Fluka.

3.2. Biocatalysts

Fresh celeriac (*A. graveolens* L. var. *rapaceum*) and carrot (*D. carota* L.) were purchased in a local market.

3.3. Biotransformation conditions

Healthy vegetable roots were comminuted (cuted) using an electric mixer for 2 min and 20 mL of vegetable pulp (1.0–1.5 g of dry wt, 100 °C, 24 h) was placed in Erlenmayer flasks with 50 mL of 0.1 M phosphate buffer [pH = 6.2 (celeriac), pH = 6.5 (carrot)]. This pulp with 20–30 mg of the substrate dissolved in 0.5 mL acetone was shaken for 48 h. The course of biotransformation was controlled by means of TLC and GC. Biotransformed mixtures were extracted with $CHCl_3$. The enantiomeric composition of product mixture was established by GC by application of chiral columns. 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, using following Chrompack WCOT capillary columns: Chirasil-Dex CB (25 m × 0.25 mm × 0.25 μm) for **1**, **1a** (column temp 120 °C/1 min; gradient 2 °C/min to 140 °C, gradient 30 °C/min to 200 °C–2 min; injector temp 200 °C, detector temp 250 °C); for **2**, **2a** (column temp 120 °C/1 min; gradient 2 °C/min to 150 °C, gradient 30 °C/min to 200 °C–2 min; injector temp 200 °C, detector temp 250 °C); for **3**, **3a** (column temp 119 °C/1 min; gradient 0.2 °C/min to 125 °C, gradient 30 °C/min to 200 °C–2 min; injector temp 200 °C, detector temp 250 °C); for **4**, **4a** (column temp 130 °C/1 min; gradient 1 °C/min to 146 °C, gradient 30 °C/min to 200 °C–2 min; injector temp 200 °C, detector temp 250 °C); for **5**, **5a** (column temp 126 °C/1 min; gradient 0.3 °C/min to 133 °C, gradient 30 °C/min to 200 °C–2 min; injector temp 200 °C, detector temp 250 °C); for **6**, **6a** (column temp 135 °C/1 min; gradient 0.5 °C/min to 144 °C, gradient 30 °C/min to 200 °C–2 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–acetone (5:1) for **1**–**3** and **1a**–**3a** and *n*-hexane–acetone (7:1) for **4**–**6** and **4a**–**6a**.

Acknowledgements

We thank the State Committee for Scientific Research (KBN) for support (Grant No. 3 PO6 030 24).

References and notes

1. Ishihara, K.; Hamada, H.; Hirata, T.; Nakajima, N. *J. Mol. Catal. B: Enzym.* **2003**, *23*, 145–170.
2. Chen, C.-P.; Prasad, K.; Repic, O. *Tetrahedron Lett.* **1991**, *32*(49), 7175–7178.
3. King, L. A.; Poortman-van der Meer, A. J.; Huizer, H. *Forensic Sci. Int.* **1996**, *77*, 141–149.
4. Rosen, T.; Heathcock, C. H. *J. Am. Chem. Soc.* **1985**, *107*, 3731–3733.
5. Yadav, J. S.; Reddy, P. T.; Nanda, S.; Rao, A. B. *Tetrahedron: Asymmetry* **2001**, *12*, 3381–3385.

6. Naoshima, Y.; Akakabe, Y. *Phytochemistry* **1991**, *30*, 3595–3597.
7. Villa, R.; Molinari, F.; Levati, M.; Aragozzini, F. *Biotechnol. Lett.* **1998**, *20*, 1105–1108.
8. Mironowicz, A.; Kukułczanka, K.; Krasiński, K.; Siewiński, A. *Phytochemistry* **1987**, *26*, 1959–1960.
9. Akakabe, Y.; Naoshima, Y. *Phytochemistry* **1991**, *35*, 661–664.
10. Akakabe, Y.; Takahashi, M.; Kamezawa, M.; Kikuchi, K. *J. Chem. Soc., Perkin Trans. 1* **1995**, *10*, 1295–1298.
11. Chan, N. G.; Procházka, Ž. *Collect. Czech. Chem. Commun.* **1973**, *38*, 227–234.
12. Mironowicz, A. *Acta Soc. Bot. Poloniae* **1997**, *66*, 325–328.
13. Mironowicz, A. *Phytochemistry* **1998**, *47*, 1531–1534.
14. Bruni, R.; Fantin, G.; Medici, A.; Pedrini, P.; Sacchetti, G. *Tetrahedron Lett.* **2002**, *43*, 3377–3379.
15. Baldassarre, F.; Bertoni, G.; Chiappe, C.; Marioni, F. *J. Mol. Catal. B: Enzym.* **2000**, *11*, 55–58.
16. Mączka, W. K.; Mironowicz, A. *Tetrahedron: Asymmetry* **2002**, *13*, 2299–2302.
17. Salvi, N. A.; Patil, P. N.; Udupa, S. R.; Banerji, A. *Tetrahedron: Asymmetry* **1995**, *6*, 2287–2290.
18. Nakamura, K.; Matsuda, T. *J. Org. Chem.* **1998**, *63*, 8957–8964.
19. Comasseto, J. V.; Omori, A. T.; Porto, A. L. M.; Andrade, L. H. *Tetrahedron Lett.* **2004**, *45*, 473–476.