APPLES AS ENANTIOSPECIFIC BIOREAGENTS IN THE HYDROLYSIS OF RACEMIC ESTERS AND OXYGENATION OF ALCOHOLS OBTAINED DURING THE PROCESS

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ABSTRACT

Biotransformation carried out by apples (*Malus silvestris*) resulted in enantiospecific hydrolysis of racemic acetates, the derivatives of secondary aromatic-aliphatic or terpenic alcohols. The alcohols are oxygenated to ketones (reversible reaction). The differences in the rate of the hydrolysis of enantiomeric esters enable isolation of pure, unreacted R-acetates.

KEY WORDS: *Malus silvestris*, fruit pulp, biotransformation, enantiospecific hydrolysis of esters, enantio-specific oxidation of alcohols, enantiospecific reduction of ketones.

INTRODUCTION

The paper presents the results of biocatalysis for the hydrolysis of racemic esters.

The results of our earlier investigation (Mironowicz et al. 1987, 1993, 1994, 1995) provide information about biotransformations of various esters, the derivatives of aliphatic-aromatic, steroid or terpenic alcohols. In those biotransformations different forms of higher plants were used as biocatalyzers, i.e.: tissue cultures (orchid), cell suspensions (carrot, petunia, topinambur), whole, intact plants (fern, duckweed), the fruit (apple), or tubers (potato, topinambur). As expected (Berlin J. 1988), the tissue and cell cultures of which the enzyme system is poorer than that of whole, ripe plants carried out the transformations giving lower yields as compared to whole plants.

The enzymes present in higher plants (used in various forms) are capable of carrying out enantiospecific transformations (Suga et al. 1988, Naoshima et al. 1989, 1991).

The purpose of the present investigation was to assess the ability of whole plant fragments to carry out enantiospecific transformations of racemic substrates. The apples of 'Gloucester' and 'Golden' varieties were used in the experiment.

MATERIALS AND METHODS

Bioreagents

Apples (*Malus silvestris*) of 'Gloucester' and 'Golden' varieties received from the Institute of Pomology, Wróblowice near Wrocław were subject to the procedures described by Mironowicz et al. (1995).

Substrates

Esters, alcohols and ketones used for biotransformations were obtained from P.O.Ch. (Gliwice, Poland), Aldrich and Fluka.

Biotransformations

The transformations were carried out according to Mironowicz et al., (1995). The biomass used in one trial was 20 cm³, i.e. 1.7g of apple pulp in conversion to dry mass (after drying at 105°C for 12 h). The mixture of the apple pulp and the substrate (20-30 mg in 50 cm³ of phosphate buffer at pH=4.5) was shaken for two days.

The transformation mixture was extracted with chloroform into which both the transformation products and unreacted substrates and some compounds present in the plant mass have passed. The course of biotransformation was controlled by means of TLC: silica gel 60 F₂₅₄ pre-coated (layer thickness 0.2 mm, Merck) with n-hexane-EtOAc (8:1) for **1, 5, 9** and **2, 6, 10**, n-hexane-Et₂O (5:1) for **3, 7, 11** and benzene-EtOAc (10:3) for **4, 8, 12**.

Yields of biotransformations were determined by means of gas chromatography: Hewlett Packard 5890, FID, carrier gas – hydrogen; HP-5 column, 30 m, inside diam. 0.32 mm, film thickness 0.5 µm (column temp. 70°/3 min.; 40°/min.; 300°/3 min., injection temp. 250°C for **2, 6, 10** and column temp. 80°/1 min.; 10°/min.; 140°/0 min.; 40° min.; 300°/3 min., injection temp. 250 °C for **4, 8, 12**) and HP-20M (Carbowax 20 M) column, 25 m, inside diam. 0.32 mm, film thickness 0.3 µm (column temp. 140°/2.5 min.; 30°/min.; 220°/3 min., injection temp. 250°C for **1, 5, 9** and column temp. 120°/1 min.; 5°/min.; 220°/3 min., injection temp. 250 °C for **3, 7, 11**).

The substrates used for biotransformation were racemates. The quantitative ratio of enantiomers (both the products and unreacted substrates) was determined using gas chromatography: Chrompack WCOT Fused Silica, Chirasil-Val (0.25

*) Biotransformations XLII. For part XLI see A. Mironowicz, Phytochemistry, in press.

TABLE 1. Biotransformations of acetates (±)-1, (±)-2, (±)-3, (±)-4, alcohols (±)-5, (±)-6, (±)-7, (±)-8 and ketones 9, 10, 11, 12 by means of apples Gloucester and Golden.

Substrates	Contents of the reaction mixture after biotransformation									
	Apple Gloucester					Apple Golden				
	Alcohol		Ketone	Unreacted substrate		Alcohol		Ketone	Unreacted substrate	
	yield; [%]	ee [%]	yield; [%]	yield; [%]	ee [%]	yield; [%]	ee [%]	yield; [%]	yield; [%]	ee [%]
(±)-1	59	40 (S)-5	15 (9)	26	100 (R)-1	72	16 (S)-5	15 (9)	13	100 (R)-1
(±)-2	58	73 (S)-6	0 (10)	42	96 (R)-2	64	62 (S)-6	0 (10)	36	90 (R)-2
(±)-3	86	14 (S)-7	1 (11)	13	100 (R)-3	88	2 (S)-7	3 (11)	9	100 (R)-3
(±)-4	16	90 (1R)-8	trace 12	84	15 (1S)-4	15	92 (1R)-8	trace 12	85	17 (1S)-4
(±)-5			1 (9)	99	3 (S)-5			1 (9)	99	3 (S)-5
(±)-6			< 1 (10)	99	6 (R)-6			2 (10)	98	6 (R)-6
(±)-7			2 (11)	98	5 (R)-7			1 (11)	99	5 (R)-7
(±)-8			1 (12)	99	5 (1R)-8			1 (12)	99	3 (1R)-8
9	2	10 (R)-5		98		1	12 (R)-5		99	
10	1	100 (S)-6		99		1	100 (S)-6		99	
11	trace	18 (S)-7		99		1	100 (S)-7		99	
12	3	40 (1R)-8		97		3	40 (1R)-8		97

Fig. 1. Substrates and products of biotransformation by means of apples.

Fig. 2. Gas chromatograms of chloroform extracts after biotransformations.

Biotransformation by means of: a) apple 'Gloucester', b) apple 'Golden'. Biotransformation I: ($\pm$)-1; II: ($\pm$)-2; III: ($\pm$)-3; IV: ($\pm$)-4.

mm x 25 m) capillary column for **1**, **5** (column temp. 125° const., injection temp. 250°C), for **2**, **6** and **3**, **7** (column temp. 100°/5 min; 0.5°/1 min; 116° const., injection temp. 250°C) and Chirasil-DEX CB (0.25 mm x 25 m) for **4**, **8** (column temp. 80°/1 min; 1°/1 min; 110° const., injection temp. 250°C).

The interpretation of gas chromatograms obtained by means of chiral columns required comparison with enantiomer standards. The only standard available in our laboratory was 1R(-)-menthol ((-)-**8**); the information about R_t of the enantiomers of the other products and unreacted substrates was obtained by the method described below. After biotransformation the products and unreacted substrates with prevalence of one of enantiomers (gas chromatography with chiral column) were isolated by means of column chromatography: SiO₂, 0.2-0.5 mm, Merck; hexane and acetone mixtures (20:1 ratio for isolation of ($\pm$)-1-phenylethyl acetate (($\pm$)-**1**) and ($\pm$)-1-phenylethanol (($\pm$)-**5**), 30:1 ratio for ($\pm$)-1-(1-naphthylethyl) acetate (($\pm$)-**2**) and ($\pm$)-1-(1-naphthyl)ethanol (($\pm$)-**6**) and 35:1 ratio for ($\pm$)-1-(2-naphthylethyl) acetate (($\pm$)-**3**) and ($\pm$)-1-(2-naphthyl)ethanol (($\pm$)-**7**) were used as eluents).

Specific optical rotation of the isolated compounds (with prevalence of one of enantiomers) was measured using a Polamat A (Karl Zeiss, Jena), at wavelength = 546 nm. The sign ((+) or (-)) determined this way allowed the identification (GC) of the peak larger in area. It was found that S(-)-aceta-

tes (-)-**1**, (-)-**2**, (-)-**3** and R-(+)-alcohols (+)-**5**, (+)-**6** (+)-**7** in gas chromatography, using Chirasil-Val-L column, had a longer retention time than their opposite enantiomers. Longer retention time of 1S-(+)-acetate (+)-**4** and alcohols 1R(-)-**8** than in their opposite enantiomers was observed in gas chromatography with Chirasil-DEX CB column.

Stability of each substrate in the buffer solution had been examined prior to the biotransformations and it was found that all the substrates were stable under these conditions. The biotransformation (20-30 mg of substrate, 1.7 g apple pulp as a dry mass in 50 cm³ of buffer solution) was performed in several (3-8) replications for confirmation of the results and/or isolation of the adequate quantities of the compound for further measurements.

RESULTS AND DISCUSSION

The following racemic acetates were used for biotransformations: ($\pm$)-1-phenylethyl (($\pm$)-**1**), ($\pm$)-1-(1-naphthylethyl) (($\pm$)-**2**), ($\pm$)-1-(2-naphthylethyl) (($\pm$)-**3**) and ($\pm$)-menthyl (($\pm$)-**4**). Each acetate used for the transformation was hydrolysed, and the alcohols obtained in this way were oxygenated to ketones (Fig. 1).

Table 1 shows results of biotransformations of ($\pm$)-**1**, ($\pm$)-**2**, ($\pm$)-**3**, ($\pm$)-**4** acetates, alcohols: ($\pm$)-1-phenylethanol (($\pm$)-**5**), ($\pm$)-1-(1-naphthyl)ethanol (($\pm$)-**6**), ($\pm$)-1-(2-naphthyl)ethanol (($\pm$)-**7**), ($\pm$)-menthol (($\pm$)-**8**) and acetophenone (**9**), methyl-(1-naphthyl)ketone (**10**), methyl-(2-naphthyl)ketone (**11**) and menthone (**12**).

Comparison of the results obtained in the present study with those obtained earlier (Mironowicz et al. 1986, 1987, 1993, 1994, 1995) shows that the transforming capability of apple pulp is comparable with that of whole intact plants, whereas the ability of plant-tissue cultures and cell suspensions to carry out biotransformations is markedly weaker. Besides, the ability of the enzyme system present in apples to carry out enantiospecific biotransformations is much higher than that of whole plants, tissue cultures or cell suspensions. This proves that enzyme system present in a whole, intact plant (fern, duckweed) or its fruit (apple), or tuber (potato, topinambur) is richer than that of cell or tissue cultures.

The presence of ketones in the reaction mixture after transformation of acetates (**1**, **2**, **3** and **4**) proves that the enzymes present in apples are able to oxygenate secondary alcohols (**5**, **6**, **7** and **8**). For confirmation, the alcohols ($\pm$)-**5**, ($\pm$)-**6**, ($\pm$)-**7** and ($\pm$)-**8** were subject to biotransformations carried out by apples and it was found that both the oxygenation yield and enantiospecificity of the reaction were low. No deracemization of ($\pm$)-1-phenylethanol (($\pm$)-**5**) as described by Nakamura (1995) was observed with apples used as bioreagents.

Also ketones **9**, **10**, **11** and **12** were subject to transformations in the same conditions. Ketone reduction with apples was negligible, but the enantiospecificity of the reaction was increased (Table 1).

Low efficiency of alcohol oxygenation and ketone reduction with apples used as biocatalysers indicates that these reactions did not affect the quantitative composition and enantiospecificity of acetates ($\pm$)-**1**, ($\pm$)-**2**, ($\pm$)-**3** and ($\pm$)-**4** hydrolysis products under the conditions of the present investigation.

The hydrolysis of racemic esters ($\pm$)-**1**, ($\pm$)-**2**, ($\pm$)-**3** and ($\pm$)-**4** shows that alcohols **5**, **6**, **7** with flat, aromatic substituents are produced in larger quantities than non-flat terpene alcohol (**8**). The comparison of ee of acetates **2** and **3** (non-hydrolysed parts of them during biotransformations) containing aro-

matic naphthyl substituents (-naphthyl and -naphthyl) shows that acetate S(-)-**3**, spatially less hindered hydrolysed faster than its analogue S(-)-**2**.

Figure 2 shows gas chromatograms of chloroform extracts after acetates (±)-**1**, (±)-**2**, (±)-**3** and (±)-**4** biotransformation. Non-hydrolysed enantiomers of R(+)-**1** (Fig. 2, Ia, b) and R(+)-**3** (Fig. 2, IIIa, b) acetates (ee=100%) can be obtained in preparative amount by means of biotransformations carried out by apples of the two varieties.

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JABŁKA JAKO ENANCJOSPECYFICZNE BIOREAGENTY W HYDROLIZIE RACEMICZNYCH ESTRÓW I W UTLENIANIU POWSTAJĄCYCH NA TEJ DRODZE ALKOHOLI

STRESZCZENIE

Jabłka (*Malus silvestris*) użyte do transformacji powodują enancjospetyczną hydrolizę racemicznych octanów będących pochodnymi drugorzędowych alkoholi aromatyczno-alifatycznych lub alkoholi terpenowych. Alkohole utworzone w wyniku hydrolizy octanów podlegają odwracalnej reakcji utlenienia do ketonów. Różnica w szybkości hydrolizy enancjomerycznych estrów pozwala na wyodrębnienie nieprze-reagowanych czystych R-(+)-octanów.

SŁOWA KLUCZOWE: *Malus silvestris*, rozdrobniony miąższ owoców, biotransformacje, enancjospetyczna hydroliza estrów, enancjospetyczne utlenianie alkoholi, enancjospetyczna redukcja ketonów.