Biotransformation of Isoprenoids and Shikimic Acid Derivatives by a Vegetable Enzymatic System

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In biotransformations carried out under similar conditions enzymatic systems from carrot (*Daucus carota* L.), celeriac (*Apium graveolens* L. var. *rapaceum*) and horse-radish (*Armoracia lapathifolia* Gilib.) hydrolyzed the ester bonds of acetates of phenols or alicyclic alcohols. Nevertheless, methyl esters of aromatic acids did not undergo hydrolysis. Alcohols were oxidized to ketones in a reversible reaction.

Key words: Biotransformation, Isoprenoids, Plant Enzymatic System

Introduction

Biotransformations of xenobiotic substrates are the source of many biologically active compounds, *i.e.* drugs, flavourings, fragrant substances and plant protection agents. An advantage of enzymatic reactions over the classic synthesis is that they usually provide a high level of stereoselectivity. Therefore, they may be used for the synthesis of bioactive compounds, as well as chiral synthons, potential valuable reagents for asymmetric synthesis of more complex molecules.

Microorganisms, which are widely used in biotransformations (Farooq *et al.*, 2002), may be successfully replaced with plant enzymatic systems present either in suspension cell cultures (Suga and Hirata, 1990; Hamada *et al.*, 1998), or in cultures of the whole plants (Mironowicz *et al.*, 1994; Pawłowicz *et al.*, 1988) or its fragments – shoots or hairy roots (Mironowicz and Kromer, 1998).

The aim of our study was to check a possibility of using homogenized vegetable (carrot, celeriac and horse-radish) tissue for transformation of isoprenoids and shikimic acid derivatives. We chose monoterpenes, steroids and shikimic acid derivatives as the starting materials for the biotransformations. We expected that such a selection of substrates would afford various types of rearrangements, leading stereoselectively to the products that may be attractive as bioactive compounds or may find an application as chiral synthons in organic synthesis.

Biotransformations by means of plant cultures, which are commonly used as biocatalysts, demand sterile conditions. The method we applied, using homogenized plant roots, tubers, and fruits does not require sterile conditions and the biotransformation is about 20 times more efficient than in the case of the cell cultures of the same plants (Mironowicz, 1998). Additionally, an application of homogenized tissue of roots of mature plants in biotransformations instead of isolated enzymes is possible thanks to the group of enzymes that are capable of accepting xenobiotic substrates.

Experimental

Substrates

trans-Cinnamyl acetate (**7**), testosterone propionate (**10**), androstenolone (**11**), pregnenolone (**12**), (d,l)-menthol (**13**), and ethyl *trans*-cinnamate (**17**) were acquired from Aldrich Chemical Co. (Milwaukee, WI), the other substrates were obtained in our laboratory.

Biocatalysts

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

Analytical methods

GC: Hewlett-Packard 5890, FID, carrier gas H_2 at 2 ml/min, using following Chrompack WCOT capillary columns: HP-5 (30 m × 0.32 mm × 0.5 μm) for androstenolone acetate (**1**), pregneno-

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lone acetate (**2**), cholesterol acetate (**3**), acetonide of 21-acetoxy-6α-fluoro-15α,16α-dihydroxy-4-pregnen-3,20-dione (**4**), testosterone propionate (**10**), androstenolone (**11**), pregnenolone (**12**), androstenedione (**18**), androstenediol (**19**), testosterone (**20**), pregnenolone (**21**), acetonide of 6α-fluoro-15α,16α,21-trihydroxy-4-pregnen-3,20-dione (**22**) (column temperature 200 °C – 1 min; gradient 20 °C/min to 260 °C; gradient 10 °C/min to 300 °C – 10 min; injector temperature 200 °C; detector temperature 250 °C), for 1-naphthyl acetate (**5**), 2-naphthyl acetate (**6**), 1-naphthol (**23**), 2-naphthol (**24**) (column temperature 140 °C – 15 min; gradient 30 °C/min to 300 °C – 2 min; injector temperature 200 °C; detector temperature 250 °C), for bornyl acetate (**9**) (column temperature 120 °C – 10 min; gradient 30 °C/min to 300 °C – 2 min; injector temperature 200 °C; detector temperature 250 °C), for methyl acetylvanillate (**15**) and methyl vanillate (**25**) (column temperature 130 °C – 26 min; gradient 30 °C/min to 300 °C – 2 min; injector temperature 200 °C; detector temperature 250 °C), for methyl acetylsalicylate (**16**) and methyl salicylate (**26**) (column temperature 125 °C – 10 min; gradient 30 °C/min to 300 °C – 2 min; injector temperature 200 °C; detector temperature 250 °C); Chirasil-DEX CB (25 m × 0.25 mm × 0.12 μm) for (d,l)-menthyl acetate (**8**), (d,l)-menthol (**13**), and menthone (**14**) (column temperature 80 °C – 1 min; gradient 10 °C/min to 100 °C – 10 min; gradient 30 °C/min to 200 °C – 2 min; injector temperature 200 °C; detector temperature 250 °C); HP-20Mx (25 m × 0.32 mm × 0.3 μm) for *trans*-cinnamyl acetate (**7**), ethyl *trans*-cinnamate (**17**), *trans*-cinnamic acid (**27**), *trans*-cinnamyl alcohol (**28**) (column temperature 130 °C – 1 min; gradient 5 °C/min to 210 °C – 2 min; injector temperature 200 °C; detector temperature 250 °C).

TLC: Merck Kieselgel 60 F_{254} 0.2 mm thick TLC plates were used to check the purity; eluents: *n*-hexane/acetone (2:1 v/v) for **1–6**, **10–12**, **18–24**; *n*-hexane/acetone (8:1 v/v) for **7**, **9**, **17**, **27**, **28**; *n*-hexane/acetone (8:1.5 v/v) for **15**, **16**, **25**, **26**; *n*-hexane/acetone (12:1 v/v) for **8**, **13**, **14**.

Biotransformation conditions

Healthy vegetable roots were homogenized using an electric mixer for 2 min (diameter of biocatalyst pieces ca. 1 mm), then 20 ml of the vegetable pulp (1.0–1.5 g of dry wt, 100 °C, 24 h) was

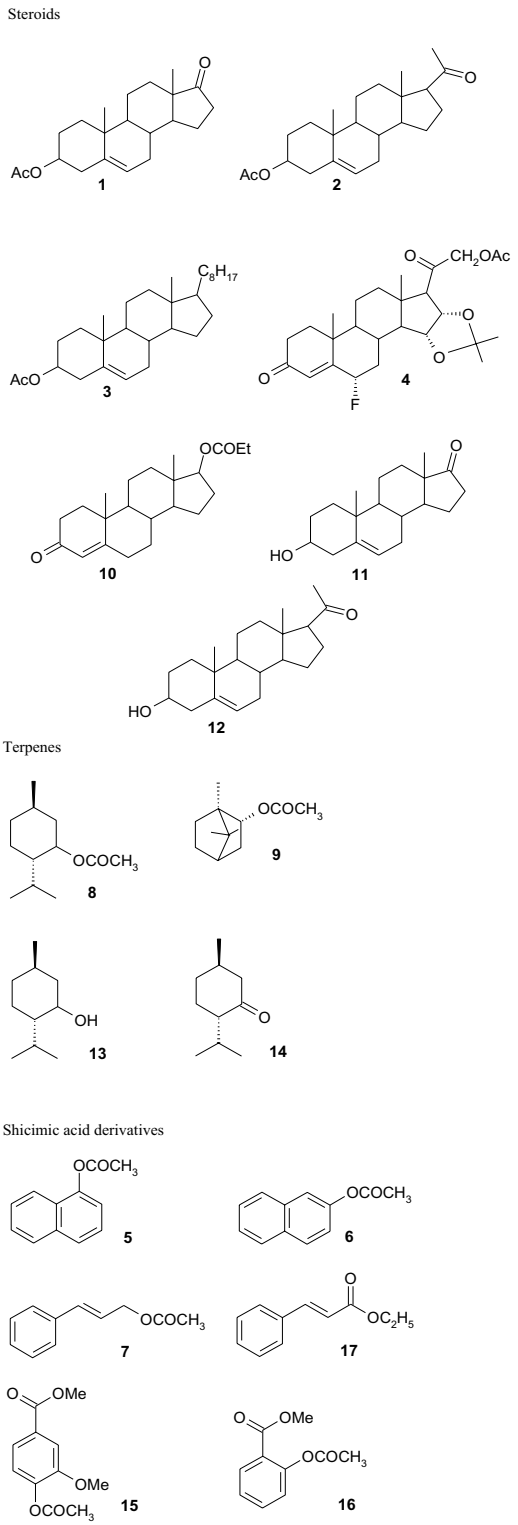

Fig. 1. Structures of substrates.

Table I. Results (GC) of biotransformation of steroid compounds **1**–**4**, **10**–**12**.

Compound	Products[a,b]/Biocatalysts		
	A. graveolens	*D. carota*	*A. lapathifolia*
1	**11**[c] (16%)	**11**[c] (10%)	**11**[c] (12%) **18**[d] (35%)
2	**21**[c] (12%)	**21**[c] (11%)	
3	–	–	–
4	–	–	**22**[c] (2%)
10	**20**[c] (7%) **19**[e] (9%)	**20**[c] (3%)	**20**[c] (4%) **19**[e] (7%)
11	**18**[d] (2%)	**19**[d] (5%)	**18**[d] (11%)
12	–	–	–

[a] **18**: Androstenedione; **19**: androstenediol; **20**: testosterone; **21**: pregnenolone; **22**: acetonide of 6α-fluoro-15α,16α,21-trihydroxy-4-pregnen-3,20-dione.
[b] Yields are given in parentheses.
[c] Hydrolysis.
[d] Oxidation.
[e] Reduction.

Table II. Results (GC) of biotransformation of shikimic acid derivatives **5**–**7**, **15**–**17** and terpenic compounds **8**, **9**, **13**, **14**.

Compound	Products[a,b]/Biocatalysts		
	A. graveolens	*D. carota*	*A. lapathifolia*
Shikimic acid derivatives			
5	**23** (100%)	**23** (100%)	**23** (100%)
6	**24** (87%)	**24** (78%)	**24** (72%)
7	**28** (100%)	**28** (100%)	**28** (81%)
15	**25**[c] (100%)	**25**[c] (100%)	**25**[c] (20%)
16	**26**[c] (100%)	**26**[c] (100%)	**26**[c] (100%)
17	**27** (100%)	**27** (100%)	**27** (38%)
Terpenes			
(d,l)-**8**	**13** 1*S*(+), 16.5% ee (78%)	**13** 1*R*(−), 25% ee (57%)	**13** (2%)
9	–	–	–
(d,l)-**13**	–	–	–
14	**13** (2%)	**13** (2%)	**13** 1*S*(+), 22% ee (11%)

[a] **23**: 1-Naphthol; **24**: 2-naphthol; **25**: methyl vanillate; **26**: methyl salicylate; **27**: *trans*-cinnamic acid; **28**: *trans*-cinnamyl alcohol.
[b] Yields are given in parentheses.
[c] Hydrolysis of OAc group only.

placed in Erlenmeyer flasks (250 ml) together with 50 ml of 0.1 M phosphate buffer (celeriac: pH 6.2; carrot: pH 6.5; horse-radish: pH 4.5). To this pulp, 0.1 mmol of the substrate dissolved in 0.5 ml acetone was added and the mixture was shaken for 48 h. The biotransformed mixtures were then extracted with 3×50 ml of $CHCl_3$ (steroids and aromatic compounds) or 3×50 ml of Et_2O (terpenes). The course of biotransformation was followed by means of TLC and GC. All substrates proved stable in the buffer solution without biocatalysts, under the same conditions as for biotransformation. The reaction conditions were not optimized.

Results and Discussion

Results of biotransformations

The following substrates were used for biotransformations: acetates of androstenolone acetate (**1**), pregnenolone acetate (**2**), cholesterol acetate (**3**), acetonide of 21-acetoxy-6α-fluoro-15α,16α-dihydroxy-4-pregnen-3,20-dione (**4**), 1-naphthyl acetate (**5**), 2-naphthyl acetate (**6**), *trans*-cinnamyl acetate (**7**), d,l-menthyl acetate (**8**), bornyl acetate (**9**), testosterone propionate (**10**), androstenolone (**11**), pregnenolone (**12**), d,l-menthol (**13**), menthone (**14**), methyl acetylvanilliate (**15**), methyl acetylsalicylate (**16**) and ethyl *trans*-cinnamate (**17**).

In our previous research with carrot cell culture, its ability to perform the transformation of esters, alcohols and ketones was observed (Mironowicz and Kromer, 1998). Presently, the application of homogenized carrot root tissue, instead of the suspension cell culture, resulted in *ca.* 18 times higher reaction efficiency. In this context, homogenized root of celeriac, belonging to the same family Apiaceae, and horse-radish from another, taxonomically distant family Brassicaceae were selected for biotransformations along with the carrot.

The structures of applied substrates contain: a) secondary hydroxyl group, b) carbonyl group and c) ester groups of two types: acetates of alicyclic alcohols (steroids, monoterpenoids) or phenols (Ar-O-CO-CH_3), and methyl (or ethyl) esters of acids with an aromatic or an aromatic-aliphatic system (Ar-CO-OR, where Ar means either naphthyl or a substituted phenyl ring).

The results of these transformations are presented in Table I and II [the yield and enantiomeric excess (ee) values represent average results of 3 to 6 repetitions]. The transformation results illustrate that all the examined acetates [with the exception of cholesterol (**3**) and bornyl (**9**) acetate] undergo hydrolysis to a different degree: acetates of flat, aromatic one- and two-ring compounds are hydrolyzed easier than the steroid three-dimensional ones. Among the steroid esters – acetates and propionate – the latter one was hydrolyzed with less efficiency.

The hydrolysis of shikimic acid analogues **15** and **16** proceeded selectively: the ester bond in phenyl acetates was hydrolyzed while the ester bond in methyl esters of aromatic acid failed to undergo the hydrolysis. Nevertheless, both, acetate **7** as well as the ethyl ester **17**, were hydrolyzed (100%) by means of carrot and celeriac roots.

In the course of biotransformation neither reduction of the C=C bond nor hydroxylation of the steroid skeleton was observed. Oxidation of the secondary alcohols and reduction of the carbonyl group proceeded with low efficiency. The enantioselectivity of the biocatalysts applied is the subject of a separate study.

In conclusion, roots of carrot (*Daucus carota* L.), celeriac (*Apium graveolens* L. var. *rapaceum*) and horse-radish (*Armoracia lapathifolia* Gilib.) can be used as biocatalysts in transformations of isoprenic compounds and shikimic acid derivatives.

Farooq A., Tahara S., Choudhary M. I., Atta-ur-Rahman, Achmad Z., Baser K. H. C., and Demirci F. (2002), Biotransformation of (–)-α-pinene by *Botrytis cinerea.* Z. Naturforsch. **57c**, 303–306.

Hamada H., Miyamoto Y., Nakajima N., and Furuya T. (1998), Highly selective transformation by plant catalysts. J. Mol. Catal. B: Enzymatic **5**, 187–189.

Mironowicz A. (1998), Biotransformations of racemic acetates by potato and topinambur tubers. Phytochemistry **47**, 1531–1534.

Mironowicz A. and Kromer K. (1998), Apple-tree shoots and transformed carrot and apple roots used as biocatalysts in enantioselective acetate hydrolysis, alcohol oxidation and ketone reduction. Coll. Czech. Chem. Commun. **63**, 1655–1662.

Mironowicz A., Kromer K., Pawłowicz P., and Siewiński A. (1994), Abilities of some higher plants to hydrolyze the acetates of phenols and aromatic-aliphatic alcohols. Acta Soc. Bot. Poloniae **63**, 43–48.

Pawłowicz P., Piątkowski K., Siewiński A. (1988), Enantiospecific hydrolysis of acetates of racemic monoterpenic alcohols by *Spirodela oligorrhiza*. Phytochemistry **27**, 2809–2811.

Suga T. and Hirata T. (1990), Biotransformation of exogenous substrates by plant cell cultures. Phytochemistry **29**, 2303–2406.