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Enantioselective reduction of flavanone and oxidation of *cis*- and *trans*-flavan-4-ol by selected yeast cultures

Tomasz Janeczko*, Monika Dymarska, Monika Siepka, Radosław Gniłka, Agnieszka Leśniak, Jarosław Popłoński, Edyta Kostrzewa-Susłow

Department of Chemistry, Wrocław University of Environmental and Life Sciences, Norwida 25, 50-375 Wrocław, Poland

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ABSTRACT

This research investigated stereochemistry of reduction of racemic flavanone and a concurrent competitive process of oxidation, taking place in cultures of live yeast strains. The results obtained gave us information about capability of tested biocatalysts for enantioselective (with respect to both substrate and product) reduction of flavanone and for enantioselective oxidation of the resulting *cis*- and *trans*-flavan-4-ols. As a result of our experiments we obtained (2*S*,4*S*)-*cis*-flavan-4-ol with 43% of conversion and 96% of enantiomeric excess, and (2*R*,4*S*)-*trans*-flavan-4-ol with 41% of conversion and ee > 99% in the culture of *Rhodotorula rubra*; (2*S*,4*S*)-*cis*-flavan-4-ol (43%, ee = 96%) along with (2*R*,4*R*)-*cis*-flavan-4-ol (44%, ee = 61%) in the culture of *Zygosaccharomyces bailii* KCh 907. Additionally, some of the tested strains demonstrated an excellent capability for enantioselective oxidation of (±)-*cis*-flavan-4-ol and (±)-*trans*-flavan-4-ol, obtained by chemical synthesis. A one-day biotransformation in the culture of *Candida parapsilosis* KCh 909 afforded (*S*)-flavanone (ee = 93%) as 49% of the reaction mixture and 49% of unreacted (2*R*,4*R*)-*cis*-flavan-4-ol with ee = 97%. Racemic *trans*-flavan-4-ol was effectively oxidized in the culture of *Yarrowia lipolytica* KCh 71 – after a three-day biotransformation the reaction mixture contained 52% of (*R*)-flavanone (ee = 85%) and 48% of (2*R*,4*S*)-*trans*-flavan-4-ol with a high enantiomeric excess (ee = 93%).

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1. Introduction

Flavonoids are natural compounds widely present in the plant kingdom, which are biosynthesized by plants from phenylalanine [1,2]. They are naturally found in fruits, vegetables, seeds and plant products, therefore they are an integral part of human diet. Flavonoids proved to be safe for human organism and their daily intake has been estimated to be about 1 g per day [3–5]. Natural flavan-4-ones are homochiral and show levorotation. In isolated from plants flavanones: pinocembrin, pinostrobin, hesperetin and naringenin at the C-2 stereogenic centre there has been (*S*)-configuration assigned [6–9]. Natural flavan-4-ols have also *S*-configuration at C-2, whereas the hydroxyl group at C-4 is situated *trans* to the phenyl group [10,11]. Such an orientation of the substituents at C-2 and C-4, which has been described in literature for luteoforol, apiforol [12,13] and glycosides: abacopterin I, Triphyllin A, and Eruberin B [14,15], means that the hydroxyl group is in a pseudo axial position, and the established absolute configuration at C-4 is *R* [12,15,16].

It has been reported in literature that racemic flavanone (**4**) can be enantiospecifically reduced to (2*R*,4*R*)-*cis*-flavan-4-ol (**5**) with the help of chiral ruthenium complexes [17]. Except for the respective flavan-4-ol (**5**) this method afforded also unreacted (*S*)-flavanone (**4**) with a high enantiomeric excess [17]. (*S*)-flavanone (**4**) and (2*S*,4*S*)-cis-flavan-4-ol (**5**) were also obtained by means of an enantioselective Ru/NHC-catalyzed hydrogenation of flavone [18]. (*S*)-flavanone (**4**) was prepared in six steps, starting from commercially available 3,5-dimethoxyphenol and (+)-ethyl (*R*)-3-hydroxy-3-phenylpropanoate [19]. Optically active (*R*)- and (*S*)-flavanone (**4**) were prepared by the enantioselective enzymatic hydrolysis of (±)-flavanone O-acyl oxime using lipase [20]. Optically active *cis*-flavan-4-ols (**5**) were obtained in two ways: by enzymatic esterification of respective alcohols [21], and by enantioselective hydrolysis of respective esters [22]. Incubation of (±)-flavanone (**4**) in the culture of bakers' yeast gave (+)-(2*S*,4*S*)-*cis*-4-hydroxyflavan (**5**) in 32% yield (83% ee) and optically active (+)-(*R*)-flavanone (**4**) in 51% yield (20% ee) [22].

* Corresponding author. Tel.: +48 71 320 52 52; fax: +48 71 3284124.
E-mail addresses: janeczko13@interia.pl, tomasz.janeczko@up.wroc.pl (T. Janeczko).

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In our previous papers we described biotransformations of (±)-flavanone in the cultures of *Aspergillus* and *Penicillium* strains [23–25]. Except from achiral flavones we obtained mainly the products of hydroxylation of flavanone at C-6 and C-4′ (with the enantiomeric excesses not exceeding 36%) [23]. The obtained products with reduced carbonyl group were racemic [25]. In our article we report an enantiospecific reduction of racemic flavanone (**4**) and an enantioselective oxidation of both (±)-*cis*-flavan-4-ol (**5**) and (±)-*trans*-flavan-4-ol (**7**) by means of selected yeast cultures. The objective of this study was to develop a convenient method of obtaining optically active flavanones and *cis*- and *trans*-flavan-4-ols (**5**,**7**).

2. Experimental

2.1. Materials

The substrates: Racemic *cis*-flavan-4-ol (**5**) was prepared by reducing (±)-flavanone (**4**) with sodium borohydride in methanol. Flavanone was obtained from 2′-hydroxychalcone (**3**) according to the previously described method [26]. 10 g of the substrate was dissolved in ethanol (75 ml) with addition of sodium acetate (8 g) and the reaction mixture was refluxed for 48 h. Crystallization from ethanol afforded pure (±)-flavanone. 2′-Hydroxychalcone (**3**) was obtained from 2′-hydroxyacetophenone (**1**) and benzaldehyde (**2**) following the method by Yadav et al. [27]; *trans*-flavan-4-ol (**7**) was prepared by transforming *cis*-flavan-4-ol (**5**) into the the tosylate (**6**), which was then hydrolysed under basic conditions. The microorganisms (twelve strains of yeast) were obtained as follows: *Candida pelliculosa* ZP22 was obtained from the Department of Biotechnology and Food Microbiology of Wrocław University of Environmental and Life Sciences [28], *Yarrowia lipolytica* KCh 71, *Candida parapsilosis* KCh 909, *Candida wiswanathi* KCh 120, *Rhodotorula rubra* KCh 4, *R. rubra* KCh 82, *Rhodotorula glutinis* KCh 242, *Saccharomyces cerevisiae* KCh 464, *Saccharomyces brasiliensis* KCh 905, *Saccharomyces pastorianus* KCh 906, *Zygosaccharomyces bailii* KCh 907, and *Candida sake* KCh 908 were obtained from the Department of Chemistry of Wrocław University of Environmental and Life Sciences. All the strains were cultivated on a Sabouraud agar consisting of aminobac (5 g), peptone K (5 g), glucose (40 g) and agar (15 g) dissolved in 1 l of distilled water, at 28 °C and pH 5.5 and stored in a refrigerator at 4 °C.

2.2. Analytical methods

The course of biotransformation was controlled by means of TLC. Analytical TLC was carried out on silica gel G 60 F_{254} plates (Merck). Chromatograms were developed using hexane/acetone mixture (4:1, v/v) as the eluent. Compounds were detected by spraying the plates with 1% $Ce(SO_4)_2$ and 2% $H_3[P(Mo_3O_{10})_4]$ in 10% H_2SO_4. The products were separated by column chromatography using silica gel (SiO_2, Kieselgel 60, 230-400 mesh, 40–63 μm, Merck) and hexane/acetone mixture (4:1, v/v) as the developing system. Composition of biotransformation mixtures was established by gas chromatography (GC) on Agilent Technologies 7890 A GC instrument, fitted with a flame ionization detector (FID) and a chiral column CYCLOSIL-B 30 m × 0.25 mm × 0.25 μm. To determinate the composition and enantiomeric excesses of the ketone and alcohol mixtures the following temperature program was used: 120 °C/0 min, gradient 1 °C min^{-1} to 194 °C/0 min, gradient 10 °C min^{-1} to 250 °C/5 min; the retention times: (*S*)-flavanone (**4**) – 55.9 min; (*R*)-flavanone (**4**) – 56.5 min, (2*S*,4*R*)-*trans*-flavan-4-ol (**7**) – 61.6 min; (2*R*,4*S*)-*trans*-flavan-4-ol (**7**) – 62.3 min, (2*S*,4*S*)-*cis*-flavan-4-ol (**5**) – 67.1 min; (2*R*,4*R*)-*cis*-flavan-4-ol (**5**) – 67.9 min. Reference samples of the racemic flavanone (**4**), *cis*-flavan-4-ol (**5**) and *trans*-flavan-4-ol (**7**) were prepared according to the procedure described above. NMR spectra were recorded on a DRX 600 MHz Bruker spectrometer and measured in $CDCl_3$. Optical rotations were measured with an Autopol IV automatic polarimeter (Rudolph). Absolute configurations of the products were determined by comparison of their optical rotation values with literature data.

2.3. Screening procedure

Erlenmeyer flasks (300 ml), each containing 100 ml of the sterile medium consisting of 3 g glucose and 1 g aminobac dissolved in water, were inoculated with a suspension of microorganisms and then incubated for 3–7 days at 25 °C on a rotary shaker (190 rpm). After full growth of the culture 20 mg of a substrate dissolved in 1 ml of acetone was added. After 12 h and 1, 3, 6, and 9 days of incubation under the above conditions, portions of 10 ml of the transformation mixture were taken out and extracted with $CHCl_3$ (3× 10 ml). The extracts were dried over $MgSO_4$, concentrated in vacuo and analyzed by GC. All the experiments were repeated three times.

2.4. Preparative biotransformation

The same transformations were performed on the preparative scale in 2000 ml flasks, each containing 500 ml of the sterile cultivation medium. The cultures were incubated under the same conditions and then 100 mg of substrates dissolved in 10 ml of acetone were added to the grown cultures. After incubation the mixtures were extracted with $CHCl_3$ (3× 300 ml), dried ($MgSO_4$) and concentrated in vacuo. The transformation products were separated by column chromatography and analyzed (TLC, GC, ^{1}H NMR and also confirmed by ^{13}C NMR and correlation spectroscopy).

2.5. Spectral data of isolated metabolites

2.5.1. (2*R*,4*R*)-*cis*-flavan-4-ol (**5**)

One-day transformation of (±)-*cis*-flavan-4-ol (**5**) (100 mg) in the culture *C. parapsilosis* KCh 909 yielded 46 mg of (2*R*,4*R*)-*cis*-flavan-4-ol (**5**) (colorless crystals); $[\alpha]_D^{20} = -64.4°$ (*c* = 1.1, $CHCl_3$) (97% ee) (lit. $[\alpha]_D^{25} = +65.7°$ (*c* = 1.49, $CHCl_3$), 92% ee zmieżone dla (2*S*,4*S*)-*cis*-flavan-4-ol (**5**) [21]. ^{1}H NMR (600 MHz) ($CDCl_3$) δ (ppm): 1.73 (d, 1H, *J* = 8.6 Hz, —O*H*); 2.13 (dt, 1H, *J* = 13.0; 11.3 Hz, H-3a); 2.53 (ddd, 1H, *J* = 13.0, 6.3, 1.5 Hz, H-3e); 5.11 (ddd, 1H, *J* = 11.3, 8.6, 6.3 Hz, H-4a); 5.18 (dd, 1H, *J* = 11.3, 1.5 Hz, H-2a); 6.90 (d, 1H, *J* = 8.2 Hz, H-8); 6.99 (t, 1H, *J* = 7.4 Hz, H-6); 7.21 (t, 1H, *J* = 8.0 Hz, H-7); 7.34 (t, 1H, *J* = 7.3 Hz, H-4′); 7.40 (t, 2H, *J* = 7.6 Hz, H-3′ and H-5′); 7.45 (d, 2H, *J* = 7.4 Hz, H-2′ and H-6′); and 7.52 (d, 1H, *J* = 7.7 Hz, H-5).

^{13}C NMR (151 MHz, $CDCl_3$) δ = 40.1 (C-3), 65.8 (C-4), 76.8 (C-2), 116.8 (C-8), 121.0 (C-6), 125.7 (C-4a), 126.1 (C-2′ and C-6′), 127.0 (C-5), 128.2 (C-4′), 128.7 (C-3′ and C-5′), 129.2 (C-7), 140.5 (C-1′), and 154.5 (C-8a).

In the same biotransformation we isolated also (*S*)-flavanone (**4**) (42 mg) $[\alpha]_D^{20} = -46.1°$ (*c* = 1.0, $CHCl_3$) (93% ee) (lit. $[\alpha]_D^{25} = -56.2°$ (*c* = 0.50, $CHCl_3$), 92% ee [22]. Oxidation of (2*R*,4*R*)-*cis*-flavan-4-ol (**5**) with Jones reagent was carried out in the same way as described previously [29], to give (*R*)-flavanone (**4**) (31 mg) $[\alpha]_D^{20} = -61.6°$ (*c* = 0.5, $CHCl_3$) (97% ee). ^{1}H NMR (600 MHz) ($CDCl_3$) δ (ppm): 2.91 (dd, 1H, *J* = 16.9; 3.0 Hz, H-3e); 3.11 (dd, 1H, *J* = 16.9, 13.2 Hz, H-3a); 5.49 (dd, 1H, *J* = 13.2; 3.0 Hz, H-2a); 7.05–7.08 (m, 2H, H-6 and H-8), 7.34–7.59 (m, 6H, H-7, H-2′, H-3′, H-4′, H-5′and H-6′), and 7.95 (dd, 1H, *J* = 8.1; 1.8 Hz, H-5).

^{13}C NMR (151 MHz, $CDCl_3$) δ = 44.7 (C-3), 75.6 (C-2), 118.1 (C-8), 120.0 (C-4a), 121.6 (C-6), 126.1 (C-2′ and C-6′), 127.1 (C-5), 128.7 (C-4′), 128.9 (C-3′ and C-5′), 136.1 (C-7), 138.8 (C-1′), 161.6 (C-8a), and 191.8 (C-4).

*2.5.2. trans-flavan-4-ol tosylate (**6**)*

Colorless crystals; ^{1}H NMR (600 MHz) ($CDCl_3$) δ (ppm): 2.29 (s, 3H, —C*H*$_3$ from tosyl group), 2.61 (d, 1H, *J* = 15.5 Hz, H-3e), 2.72 (dd, 1H, *J* = 15.5, 11.9, 4.7 Hz, H-3a), 4.86 (d, 1H, *J* = 11.9 Hz, H-2a), 6.66 (s, 1H, H-4e), 6.99 (t, 1H, *J* = 7.4 Hz, H-6), 7.05 (d, 2H, *J* = 7.5 Hz, H-3″ and H-5″ from tosyl group), 7.07 (d, 1H, *J* = 7.5 Hz, H-8), 7.21 (d, 1H, *J* = 7.7 Hz, H-5), 7.29 (t, 1H, *J* = 7.6 Hz, H-4′), 7.39–7.31 (m, 4H, H-2′, H-3′, H-5′ and H-6′), 7.41 (t, 1H, *J* = 7.4 Hz, H-7), and 7.69 (d, 2H, *J* = 7.5 Hz, H-2″ and H-6″ from tosyl group).

^{13}C NMR (151 MHz, $CDCl_3$) δ = 21.26 (—*C*H$_3$ from tosyl group), 37.21 (C-3), 65.18 (C-4), 71.96 (C-2), 113.29 (C-4a), 118.39 (C-8), 122.43 (C-6), 128.89 (C-2″ and C-6″ from tosyl group), 126.40 (C-2′ and C-6′), 128.63 (C-3″ and C-5″ from tosyl group), 128.73 (C-3′ and C-5′), 128.74 (C-4′), 131.19 (C-5), 132.57 (C-7), 138.32 (C-4″ from tosyl group), 139.23 (C-1″ from tosyl group), 143.79 (C-1′), and 156.45 (C-8a).

*2.5.3. (2S,4R)-trans-flavan-4-ol (**7**)*

Three-day transformation of (±)-*trans*-flavan-4-ol (**7**) (100 mg) in the culture of *Y. lipolytica* KCh 71 yielded 41 mg of (2*S*,4*R*)-*trans*-flavan-4-ol (**7**) (colorless crystals); $[\alpha]_D^{20} = +12.4^\circ$ (*c* = 0.7, $CHCl_3$) (94% ee). ^{1}H NMR (600 MHz) ($CDCl_3$) δ (ppm): 2.12 (ddd, 1H, *J* = 14.4; 12.2; 3.3 Hz, H-3a); 2.28 (ddd, 1H, *J* = 14.4, 2.5; 1.7 Hz, H-3e); 4.85 (dd, 1H, *J* = 3.3; 2.5 Hz, H-4e); 5.28 (dd, 1H, *J* = 12.2; 1.7 Hz, H-2a); 6.98 (t, 1H, *J* = 7.8 Hz, H-6), 6.99 (d, 1H, *J* = 8.1 Hz, H-8), 7.27 (t, 1H, *J* = 7.6 Hz, H-7), 7.37 (t, 1H, *J* = 7.6 Hz, H-4′), 7.38 (d, 1H, *J* = 7.6 Hz, H-5), 7.43 (t, 2H, *J* = 7.3 Hz, H-3′ and H-5′), and 7.50 (d, 2H, *J* = 7.7 Hz, H-2′ and H-6′).

^{13}C NMR (151 MHz, $CDCl_3$) δ = 38.34 (C-3), 63.73 (C-4), 73.11 (C-2), 117.45 (C-8), 120.80 (C-6), 123.67 (C-4a), 126.29 (C-2′ and C-6′), 128.04 (C-4′), 128.60 (C-3′ and C-5′), 129.93 (C-7), 130.14 (C-5), 141.04 (C-1′), and 154.90 (C-8a). In the same biotransformation we isolated also (*R*)-flavanone (**4**) (38 mg) $[\alpha]_D^{20} = +54.8^\circ$ (*c* = 0.8, $CHCl_3$) (85% ee) (lit. $[\alpha]_D^{25} = +66.5^\circ$ (*c* = 0.48, $CHCl_3$), 98% ee [22]. Oxidation of (2*S*,4*R*)-*trans*-flavan-4-ol (**7**) with Jones reagent was carried out in the same way as described previously [29], to give (*S*)-flavanone (**4**) (28 mg) $[\alpha]_D^{20} = -62.0^\circ$ (*c* = 0.5, $CHCl_3$) (94% ee).

Preparative biotransformation of (±)-flavanone (**4**) in the culture of *C. wiswanathi* KCh 120 was also carried out. The three-day biotransformation using 100 mg of the substrate afforded 45 mg of (2*S*,4*S*)-*cis*-flavan-4-ol (**5**) $[\alpha]_D^{20} = +59.8^\circ$ (*c* = 1.2, $CHCl_3$) (95% ee) and 32 mg of (2*R*,4*S*)-*trans*-flavan-4-ol (**7**) $[\alpha]_D^{20} = -14.8^\circ$ (*c* = 0.9, $CHCl_3$) (92% ee).

3. Results and discussion

We performed biotransformations of racemic flavanone (**4**) and *cis*- and *trans*-flavan-4-ols (**5**,**7**), which were prepared in a few step synthesis (Scheme 1). For the biotransformations we used cultures of twelve yeast strains. The selected microorganism comprised: yeast of the genus *Rhodotorula: R. rubra* KCh 4, *R. rubra* KCh 82, and *R. glutinis* KCh 242; of the genus *Candida*: *C. wiswanathi* KCh 120, *C. parapsilosis* KCh 909, *C. sake* KCh 908, and *C. pelliculosa* ZP22; of the genus *Saccharomyces*: *S. cerevisiae* KCh 464, *S. brasiliensis* KCh 905, and *S. pastorianus* KCh 906 and two other yeast strains: *Y. lipolytica* KCh 71 and *Z. bailii* KCh 907. The biocatalysts were selected based on their biocatalytic properties, such as fast growth rate, capability for effective enantioselective reduction of aliphatic-aromatic ketones and oxidation of the respective alcohols [30–33]. Moreover, unlike in the case of filamentous fungi, in the cultures of yeasts competitive reactions such as hydroxylation, dehydratation and degradation rarely occur [34,35].

In the case of biotransformation of flavanone (**4**) we observed that depending on the yeast species used as a biocatalyst, there were considerable differences in enantioselectivity of the process with respect to the *S*- or *R*-enantiomer of **4** preference and spatial position of the hydroxyl group formed by reduction of the carbonyl group of the substrate (Table 1). In the cultures of most of the strains (*R. rubra* KCh 4 and KCh 82, *R. glutinis* KCh 242, *Z. bailii* KCh 907, *C. sake* KCh 908 and *S. pastorianus* KCh 906) reduction of (*R*)-flavanone was significantly faster than of the *S*-enantiomer. As opposed to this, the strain *C. pelliculosa* ZP22 demonstrated enantioselectivity towards (*S*)-flavanone, whereas, in the culture of the strain *C. wiswanathi* KCh 120 at first (*S*)-flavanone was more effectively reduced, but after 3 days we observed lower concentration of the *R*-enantiomer compared to the *S*-one.

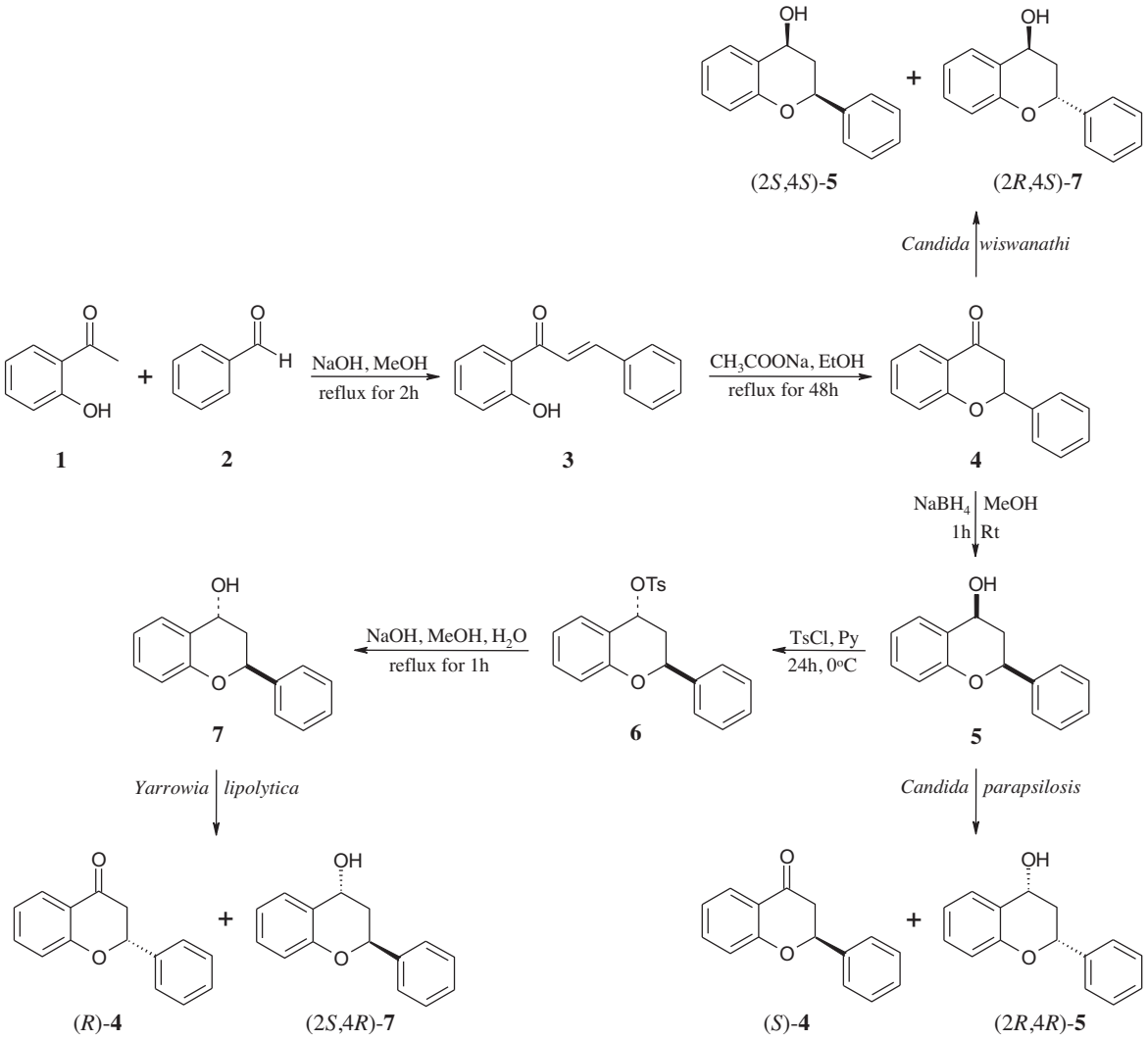
In all the cultures of the genus *Rhodotorula*, i.e. *R. rubra* KCh 4, *R. rubra* KCh 82, and *R. glutinis* KCh 242 a very high *S*-selectivity of the reduction of substrate **4** was observed. After one day of the transformation (2*R*,4*S*)-*trans*-flavan-4-ol (**7**) was identified in the reaction mixtures (41% for *R. rubra* KCh 4 and *R. rubra* KCh 82; 13% for *R. glutinis* KCh 242) with the enantiomeric excess of over 99%. At the first stage the reduction proceeded also with a very high diastereoselectivity – after 12 h de = 85% for the strain *R. rubra* KCh 4; 81% for *R. rubra* KCh 82 and 94% for *R. glutinis* KCh 242. However, between the first and the third day of the transformation we observed a significant increase in concentration of the *cis*-alcohol. Determination of the absolute configuration of both stereogenic centres of this product as (2*S*,4*S*)-*cis*-flavan-4-ol (**5**) showed that it was formed by the reduction of (*S*)-flavanone (**4**).

Certain similarities were noticed for biotransformation of racemic flavanone (**4**) by the strains *C. wiswanathi* KCh 120 and *C. pelliculosa* ZP22. Both more effectively reduced (*S*)-flavanone than the *R*-one. The reduction, like in the case of the strains of the genus *Rhodotorula*, proceeded with a high *S*-specificity to give the respective (2*S*,4*S*)-*cis*-flavan-4-ol (**5**) (43% with ee = 96% after 1 day in the culture of *C. wiswanathi* KCh 120 and 33% with ee = 75% after 3 days in the culture of *C. pelliculosa* ZP22). In the culture of the strain *C. wiswanathi* KCh 120 an increase in amount of the *trans*-alcohol with the reaction time was also observed – 51% of (2*R*,4*S*)-*trans*-flavan-4-ol (**7**) with ee = 92% after 3 days of the biotransformation. After six days we noticed a considerable increase in concentration of flavanone (**4**). This might be due to increase of oxidative properties of this strain during the biotransformation.

In the culture of the strain *S. brasiliensis* KCh 905 we observed a drop in (*S*)-flavanone (**4**) concentration during the biotransformation, however we identified the product of *R*-specific dehydrogenase activity – (2*S*,4*R*)-*trans*-flavan-4-ol (**7**) with ee > 90%. The *R*-selectivity of the reduction was also observed for the strain *S. pastorianus* KCh 906. We identified both *cis*- (**5**) and *trans*-alcohol (**7**) with the *R*-configuration at C-4. In the culture of *C. sake* KCh 908 we observed a very high selectivity of the reduction towards *R*-flavanone, but low enantioselectivity with respect to the product. Both products: *cis*- and *trans*-flavan-4-ols were formed by the reduction of the *R*-ketone. The strain *Z. bailii* KCh 907 demonstrated high enantioselectivity towards both the substrate and the product. After three days we identified in the reaction mixture 42% of (2*S*,4*R*)-*trans*-flavan-4-ol (**7**) with ee = 62% and 44% of (2*R*,4*R*)-*cis*-flavan-4-ol (**5**) with ee = 61% and de = 53%.

Because of the observed changes in enantiomeric excesses of compounds in biotransformation mixtures and the increase in amount of the substrate in some of the cultures we subjected to the biotransformation racemic mixtures of both *cis*- and *trans*-flavan-4-ol (**5**,**7**) (Tables 2 and 3).

In Table 2 we did not present the results for four strains: *S. brasiliensis* KCh 905, *S. pastorianus* KCh 906, *S. cerevisiae* KCh 464 and *Z. bailii* KCh 907, for which substrate conversion was less than 5%, even after ten days of incubation. All the strains omitted in Table 2 belong to the genus *Saccharomyces*, including the strain of *S. cerevisiae*, which is commonly used for biocatalysis. In the cultures of the genus *Rhodotorula* we observed a drop in



Scheme 1. Chemoenzymatic synthesis of optically active flavanone (**4**) and *cis*- and *trans*-flavan-4-ols (**5**,**7**).

concentration of (2*R*,4*R*)-*cis*-flavan-4-ol (**5**) with the reaction time, along with an increase in (2*R*,4*S*)-*trans*-flavan-4-ol (**7**) concentration. This was due to the enantioselective oxidation of (2*R*,4*R*)-*cis*-flavan-4-ol (**5**) to (*R*)-flavanone (**4**), followed by the stereoselective reduction of this enatiomer of **4** to (2*R*,4*S*)-*trans*-flavan-4-ol (**7**). The process was the most effective in the culture of *R. glutinis* KCh 242. After ten days the reaction mixture contained 68% of (2*R*,4*S*)-*trans*-flavan-4-ol (**7**) with ee = 52% and 27% of (*R*)-flavanone (**4**) with ee = 94%. In the cultures of the strains *C. wiswanathi* KCh 120 and *Candida sake* KCh 908 an enantioselective oxidation of (2*S*,4*S*)-*cis*-flavan-4-ol (**5**) took place. For these strains substrate conversion was low (11 and 19% of (*S*)-flavanone (**4**), respectively, was observed in the reaction mixture), but the enantioselectivity was high (Table 2). Surprisingly, *C. parapsilosis* KCh 909 proved to be the most effective biocatalyst in this part of our study. After one day of incubation of (±)-*cis*-flavan-4-ol (**5**) in

Table 1
Microbial transformation of racemic flavanone (**4**).

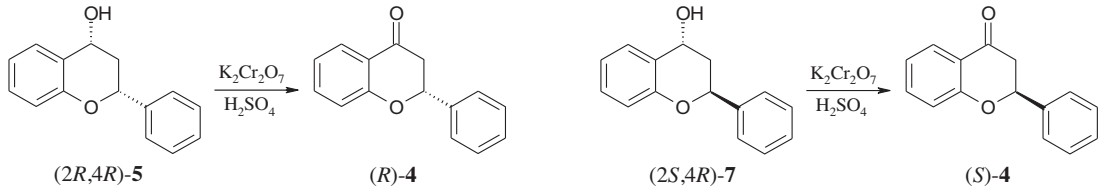
Microorganism	*t* [days]	Flavanone (**4**)			*trans*-flavan-4-ol (**7**)			*cis*-flavan-4-ol (**5**)		
		%	ee	Config.	%	ee	Config.	%	ee	Config.
R. rubra KCh 4	1	51	53	*S*	41	>99	2*R*,4*S*	8	46	2*S*,4*S*
R. rubra KCh 82	1	50	59	*S*	41	>99	2*R*,4*S*	9	40	2*S*,4*S*
R. glutinis KCh 242	3	28	56	*S*	42	98	2*R*,4*S*	29	91	2*S*,4*S*
C. wiswanathi KCh 120	3	11	54	*S*	51	92	2*R*,4*S*	38	95	2*S*,4*S*
C. sake KCh 908	6	73	32	*S*	17	93	2*R*,4*S*	11	75	2*R*,4*R*
C. parapsilosis KCh 909	6	87	0	–	9	15	2*S*,4*R*	4	53	2*R*,4*R*
C. pelliculosa ZP22	3	55	33	*R*	12	40	2*R*,4*S*	33	75	2*S*,4*S*
S. cerevisiae KCh 464	6	90	0		6	12	2*R*,4*S*	4	27	2*S*,4*S*
S. brasiliensis KCh 905	6	81	6	*R*	12	91	2*S*,4*R*	7	22	2*R*,4*R*
S. pastorianus KCh 906	6	78	7	*S*	6	84	2*S*,4*R*	16	66	2*R*,4*R*
Y. lipolytica KCh 71	6	100	0	–	0	–	–	0	–	–
Z. bailii KCh 907	3	42	62	*S*	14	36	2*R*,4*S*	44	61	2*R*,4*R*

Table 2
Microbial transformation of *cis*-flavan-4-ol (**5**).

Microorganism	*t* [days]	*cis*-flavan-4-ol (**5**)			Flavanone (**4**)			*trans*-flavan-4-ol (**7**)		
		%	ee	Config.	%	ee	Config.	%	ee	Config.
R. rubra KCh 4	6	74	22	2*S*,4*S*	7	52	*S*	19	93	2*R*,4*S*
R. rubra KCh 82	6	86	15	2*S*,4*S*	4	42	*S*	10	86	2*R*,4*S*
R. glutinis KCh 242	6	66	40	2*S*,4*S*	11	59	*S*	23	88	2*R*,4*S*
C. wiswanathi KCh 120	6	83	17	2*R*,4*R*	11	70	*S*	6	21	2*S*,4*R*
C. sake KCh 908	6	76	17	2*R*,4*R*	19	75	*S*	5	7	2*R*,4*S*
C. parapsilosis KCh 909	1	49	97	2*R*,4*R*	49	93	*S*	2	56	2*S*,4*R*
C. pelliculosa ZP22	6	94	8	2*R*,4*R*	4	52	*S*	2	28	2*S*,4*R*
Y. lipolytica KCh 71	3	75	10	2*S*,4*S*	24	35	*R*	1	27	2*S*,4*R*

Table 3
Microbial transformation of *trans*-flavan-4-ol (**7**).

Microorganism	*t* [days]	*trans*-flavan-4-ol (**7**)			Flavanone (**4**)			*cis*-flavan-4-ol (**5**)		
		%	ee	Config.	%	ee	Config.	%	ee	Config.
R. rubra KCh 82	6	87	6	2*S*,4*R*	6	31	*R*	7	30	2*R*,4*R*
R. glutinis KCh 242	6	84	14	2*S*,4*R*	12	30	*R*	4	31	2*R*,4*R*
C. wiswanathi KCh 120	6	84	16	2*S*,4*R*	16	43	*R*	0	–	
C. parapsilosis KCh 909	6	74	14	2*S*,4*R*	26	41	*R*	0	–	
Y. lipolytica KCh 71	3	48	93	2*S*,4*R*	52	85	*R*	0	–	



(2*R*,4*R*)-**5** (*R*)-**4** (2*S*,4*R*)-**7** (*S*)-**4**

Scheme 2. Oxidation of (2*R*,4*R*)-*cis*-flavan-4-ol (**5**) and (2*S*,4*R*)-*trans*-flavan-4-ol (**7**) recovered from the bioreduction mixture.

the culture of this strain we observed almost complete oxidation of (2*S*,4*S*)-enantiomer of the substrate to (*S*)-flavanone (**4**) (ee = 93%). This highly enantioselective oxidation allowed us to obtain (2*R*,4*R*)-*cis*-flavan-4-ol (**5**) with the enantiomeric excess of over 97%. The capability of the *C. parapsilosis* strains for enantioselective oxidation of *S*-alcohols was reported earlier for racemic 1-arylethanols and allylic alcohols, which were deracemised to the (*R*)-enantiomer (ee up to >99%) by whole cells of *C. parapsilosis* ATCC 7330 culture [36–38].*trans*-Flavan-4-ol (**7**) underwent oxidation at much lower rate compared to the *cis*-alcohol in the cultures of almost all tested biocatalysts. In Table 3 we presented the results for five strains that were able to transform *trans*-flavan-4-ol (**7**). For the other seven species the substrate conversion was below 5%, even after 10 days of incubation. The only strain that performed effective oxidation of *trans*-flavan-4-ol (**7**) was *Y. lipolytica* KCh 71. In the culture of this biocatalyst we observed the effective enantiospecific oxidation of (2*R*,4*S*)-*trans*-flavan-4-ol (**7**) to (*R*)-flavanone (**4**) (ee = 85% after three days). Due to the high enantioselectivity of oxidation by this strain, after six days of biotransformation we identified also (2*S*,4*R*)-*trans*-flavan-4-ol (**7**) with ee = 97%. This result was astonishing, because when (±)-*cis*-flavan-4-ol (**5**) was subjected to biotransformation by this strain, the selectivity of oxidation process was low.

It is also worth noting, that when (±)-*trans*-flavan-4-ol (**7**) served as a substrate for the strains *C. wiswanathi* KCh 120, *C. parapsilosis* KCh 909 and the strains of the genus *Rhodotorula*, oxidation of (2*R*,4*S*)-*trans*-flavan-4-ol (**7**) was observed. Moreover, in the cultures of the *Rhodotorula* strains concentration of (2*R*,4*R*)-*cis*-flavan-4-ol (**5**) increased, as a result of reduction of (*R*)-flavanone (**4**) (Scheme 2).

(2*R*,4*R*)-*cis*-Flavan-4-ol (**5**) and (2*S*,4*R*)-*trans*-flavan-4-ol (**7**) recovered from the preparative biotransformation mixture were oxidized with Jones reagent to give (*R*)-flavanone and (*S*)-flavanone, respectively. Enantiomeric excesses of the starting alcohols and the obtained ketones were identical.

During biotransformation of *trans*-flavan-4-ol we observed a selective oxidation of the alcohol with *S* configuration at C-4 (CH-OH). The same selectivity was observed in deracemization of 1-aryl ethanol in the cultures of *C. parapsilosis* ATCC 7330 [36], *Candida albicans* CCT 0776 [37] and some bacterial strains [39,40], which proceeded via enantioselective oxidation of the (*S*)-enantiomer. In our previous research concerning bioreduction of propiophenone we observed that during incubation of propiophenone in the cultures of all tested microorganisms, longer biotransformation time resulted in a drop in a percent content of the *S*-alcohol, along with an increase in a percent content of the *R*-alcohol. This time-dependence of products concentration was observed for microorganisms that possessed either *S*-specific or *R*-specific dehydrogenases. Employment of racemic 1-phenylpropan-1-ol as a substrate confirmed these findings [41]. The ability to perform enantioselective oxidation of *R*-alcohol was also observed for some plant biocatalysts (*Arracacia xanthorrhiza*, *Beta vulgaris*, *Zingiber officinale*) [42] and few strains of filamentous fungi (*Aspergillus terreus* CCT 4083) [43]. Such an opposite enantioselectivity we found also in the case of oxidation of racemic *cis*-flavan-4-ol (**5**) in the cultures of *Rhodotorula* strains.

4. Conclusions

Twelve yeast strains were tested with respect to their capability for enantioselective reduction of flavanone (**4**). The majority of the strains demonstrated high enantioselectivity towards this substrate. In the cultures of *R. rubra* KCh 4, *R. rubra* KCh 82, *R. glutinis* KCh 242, *Z. bailii* KCh 907, and *Candida sake* KCh 908 the

reduction rate was higher for the *R*-enantiomer of the substrate, whereas in the culture of *Candida pelliculosa* ZP22 (*S*)-flavanone was reduced faster. Reduction of the carbonyl group proceeded with a very high enantioselectivity, leading to formation of (2*R*,4*S*)-*trans*-flavan-4-ol (**7**) with ee > 99% in the culture of *R. rubra* (41% of the reaction mixture after one day of biotransformation); with ee = 98% in the culture of *R. glutinis* KCh 242 (42% after three days); and with ee = 92% in the culture of *C. wiswanathi* KCh 120 (51% after three days). Additionally, in the culture of the last strain after one day of biotransformation we obtained also (2*S*,4*S*)-*cis*-flavan-4-ol (**5**) (43%) with the enantiomeric excess of 96%. The same compound was also identified in biotransformation with *Candida pelliculosa* ZP22 (33% after three days with ee = 75%). Only in the culture of *Z. bailii* KCh 907 (2*R*,4*R*)-*cis*-flavan-4-ol (**5**) was obtained in a decent amount (44% after three days of substrate incubation, ee = 61%).

The strain *C. parapsilosis* KCh 909 was proven to be the catalyst capable to separate (±)-*cis*-flavan-4-ol (**5**). Due to its enzymatic system activity, after a one-day biotransformation it is possible to obtain (*S*)-flavanone (**4**) with 49% of conversion and ee = 93%, along with 49% of unreacted (2*R*,4*R*)-*cis*-flavan-4-ol (**5**) with ee = 97%. Whereas, the strain *Y. lipolytica* KCh 71 was the only one which effectively and enantioselectively oxidized (±)-*trans*-flavan-4-ol (**7**). After three days of transformation we identified (*R*)-flavanone (**4**) (ee = 85%), and after six days (2*S*,4*R*)-*trans*-flavan-4-ol (**7**) (45%) with a high enantiomeric excess (ee = 97%). The results obtained prove that *C. parapsilosis* KCh 909 and *Y. lipolytica* KCh 71 may find application for separation of racemic mixtures of synthetic flavanols.

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References

[1] B. Winkel-Shirley, Curr. Opin. Plant Biol. 5 (2002) 218–223.
[2] S. Das, J.P.N. Rosazza, J. Nat. Prod. 69 (2006) 499–508.
[3] N.C. Cook, S. Samman, J. Nutr. Biochem. 7 (1996) 66–76.
[4] B.H. Havsteen, Pharmacol. Ther. 96 (2002) 67–202.
[5] P.C.H. Hollman, M.B. Katan, Food Chem. Toxicol. 37 (1999) 937–942.
[6] H. Arakawa, M. Nakazaki, Chem. Ind. 12 (1960) 73.
[7] E. Hardegger, H. Braunschweiger, Helv. Chim. Acta 44 (1961) 1413–1417.
[8] M. González-Cortazar, A.M. Maldonado-Abarca, E. Jiménez-Ferrer, S. Marquina, E. Ventura-Zapata, A. Zamilpa, J. Tortoriello, M. Herrera-Ruiz, Molecules 18 (2013) 13260–13270.
[9] T. Morikawa, K. Funakoshi, K. Ninomiya, D. Yasuda, K. Miyagawa, H. Matsuda, M. Yoshikawa, Chem. Pharm. Bull. 56 (2008) 956–962.
[10] H. Halbwirth, S. Kahl, W. Jaeger, G. Reznicek, G. Forkmann, K. Stich, Plant Sci. 170 (2006) 587–595.
[11] H. Halbwirth, Int. J. Mol. Sci. 11 (2010) 595–621.
[12] E.C. Bate-Smith, Phytochemistry 8 (1969) 1803–1810.
[13] F. Spinelli, J.-B. Speakman, W. Rademacher, H. Halbwirth, K. Stich, G. Costa, Eur. J. Plant Pathol. 112 (2005) 133–142.
[14] H. Wei, G. Wu, D. Shi, S. Song, X. Zhang, Y. Lei, J. Ruan, Food Chem. 134 (2012) 1959–1966.
[15] J. Jiang, L. Tian, L. Wang, Y. Liu, Y. Chen, Biochem. Syst. Ecol. 50 (2013) 136–138.
[16] Z. Zhao, J. Jin, J. Ruan, C. Zhu, C. Lin, W. Fang, Y. Cai, J. Nat. Prod. 70 (2007) 1683–1686.
[17] M.-K. Lemke, P. Schwab, P. Fischer, S. Tischer, M. Witt, L. Noehringer, V. Rogachev, A. Jäger, O. Kataeva, R. Fröhlich, P. Metz, Angew. Chem. Int. Ed. 52 (2013) 11651–11655.
[18] D. Zhao, B. Beiring, F. Glorius, Angew. Chem. Int. Ed. 52 (2013) 8454–8458.
[19] K.J. Hodgetts, Tetrahedron Lett. 42 (2001) 3763–3766.
[20] T. Izumi, K. Suenaga, J. Heterocyclic Chem. 34 (1997) 1535–1538.
[21] S. Ramadas, G.L.D. Krupadanam, Tetrahedron-Asymmetry 15 (2004) 3381–3391.
[22] T. Izumi, T. Hino, A. Kasahara, J. Chem. Soc. Perkin Trans. 1 (1992) 1265–1267.
[23] E. Kostrzewa-Susłow, J. Dmochowska-Gładysz, A. Białońska, Z. Ciunik, J. Mol. Catal. B-Enzym. 52–53 (2008) 34–39.
[24] E. Kostrzewa-Susłow, J. Dmochowska-Gładysz, T. Janeczko, K. Środa, K. Michalak, A. Palko, Z. Naturforsch. 67c (2012) 411–417.
[25] E. Kostrzewa-Susłow, J. Dmochowska-Gładysz, A. Białońska, Z. Ciunik, W. Rymowicz, J. Mol. Catal. B-Enzym. 39 (2006) 18–23.
[26] J.-E. Yeom, M.R. Kumar, S. Lee, J.-B. Lee, H.-R. Park, Bull. Korean Chem. Soc. 32 (2011) 4092–4094.
[27] N. Yadav, S.K. Dixit, A. Bhattacharya, L.C. Mishra, M. Sharma, S.K. Awasthi, V.K. Bhasin, Chem. Biol. Drug Des. 80 (2012) 340–347.
[28] I. Zaleska, M. Piegza, Acta Sci. Pol. Biotechnol. 7 (2008) 27–43.
[29] T. Janeczko, J. Dmochowska-Gładysz, E. Kostrzewa-Susłow, World J. Microb. Biol. 26 (2010) 2047–2051.
[30] L. Cappaert, C. Larroche, Biocatal. Biotransform. 22 (2004) 291–296.
[31] R.S. Martins, D.S. Zampieri, J.A.R. Rodrigues, P.O. Carvalho, P.J.S. Moran, ChemCatChem 3 (2011) 1469–1473.
[32] T. Janeczko, W. Gładkowski, E. Kostrzewa-Susłowa, J. Mol. Catal. B-Enzym. 98 (2013) 55–61.
[33] M.R.B. Chaves, P.J.S. Moran, J.A.R. Rodrigues, J. Mol. Catal. B-Enzym. 98 (2013) 73–77.
[34] E. Kostrzewa-Susłow, T. Janeczko, Molecules 17 (2012) 14810–14820.
[35] E. Kostrzewa-Susłow, T. Janeczko, Sci. World J. (2012), ID 254929, 8 p.
[36] T. Kaliaperumal, S.N. Gummadi, A. Chadha, Biocatal. Biotransform. 29 (2011) 262–270.
[37] S.M. Amrutkar, L. Banoth, U.C. Banerjee, Tetrahedron Lett. 54 (2013) 3274–3277.
[38] D. Titu, A. Chadha, Tetrahedron-Asymmetry 19 (2008) 1698–1701.
[39] S.M. Mantovani, C.F.F. Angolini, A.J. Marsaioli, Tetrahedron-Asymmetry 20 (2009) 2635–2638.
[40] L.S. Araújo, E. Kagohara, T.P. Garcia, V.H. Pellizari, L.H. Andrade, Mar. Drugs 9 (2011) 889–905.
[41] T. Janeczko, E. Kostrzewa-Susłowa, Tetrahedron-Asymmetry (2014), http://dx.doi.org/10.1016/j.tetasy.2014.08.006 (in press).
[42] L.H. Andrade, R.S. Utsunomiya, A.T. Omori, A.L.M. Porto, J.V. Comasseto, J. Mol. Catal. B-Enzym. 38 (2006) 84–90.
[43] J.V. Comasseto, L.H. Andrade, Á.T. Omori, L.F. Assis, A.L.M. Porto, J. Mol. Catal. B-Enzym. 29 (2004) 55–61.