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Stereoselective bioreduction for the resolution of racemic mixtures of bicyclo[3.3.1]nonane-2,6-dione using vegetables

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ABSTRACT

Screening of various vegetables as biocatalysts for the stereoselective bioreduction and resolution of racemic bicyclo[3.3.1]nonane-2,6-dione was performed. Vegetables of the family *Apiaceae*, i.e. the roots of parsnip (*Pastinaca sativa*), celery (*Apium graveolens*), parsley (*Petroselinum crispum*) and carrot (*Daucus carota*) were found to be the best. The methodology described here allows the efficient enantioseparation of (+)-enantiomer of bicyclo[3.3.1]nonane-2,6-dione. The reaction time, temperature and the amount of celery roots were optimized and (+)-enantiomer was obtained with the optical purity of 90–98% and the yield of more than 90%.

1. Introduction

In 2013 we will mark the 100th anniversary of the well known publication in what Meerwein and Schürmann described the synthesis of so-called Meerwein's ester and of bicyclo[3.3.1]nonane-2,6-dione which was obtained from the first one [1]. During the last century a lot of various interesting researches have been carried out with bicyclo[3.3.1]nonane and their derivatives in the field of synthetic organic chemistry and stereochemistry [2]. The bicyclo[3.3.1]nonane framework is a common motif amongst natural products, it is also a useful precursor for the synthesis of new compounds displaying a broad spectrum of biological activities [3,4]. For example, the transformation of bicyclo[3.3.1]nonane system into bicyclo[5.3.1]undecane ring system is a substantial step in taxoids synthesis [5]. Undoubtedly, the use of bicyclo[3.3.1]nonane-2,6-dione for the synthesis of chiral compounds and for the research of their chiroptical properties is a topic of special importance because a lot of natural compounds with bicyclo[3.3.1]nonane's moiety are optically active [6–8]. Thus, the separation of enantiomers from racemic bicyclo[3.3.1]nonane-2,6-dione is still actual. Various procedures such as chromatographic means [9], fractional crystallization of diastereomeric derivatives of bicyclo[3.3.1]nonane-2,6-dione [10] are used for the enantiomeric enrichment. Enzymatic methods are also applied because many enzymes exhibit a high degree of enantioselectivity [11–15].

In general, for biotransformations isolated enzymes, microorganisms or cells cultures of higher eukaryotes can be used. Plants are the potential source of various enzymes, and plant cell cultures as well as plant-derived enzymes are involved in the synthesis of important chemical compounds. For that purpose, intact plant materials also can be used because two first mentioned systems have some limitations as high cost, nonrenewable reagents or difficulties associated with plant cells cultivation [16,17]. Among vegetables of the family *Apiaceae*, a root of carrot is the most known chemical reagent. To the best of our knowledge, it was the first plant material used as biocatalyst by Baldassare et al. Racemic 2-methylcyclohexanone and 2-hydroxycyclohexanone were reduced with fresh carrot root and chiral alcohols were obtained [18]. Enantioselective hydrolysis of racemic acetates [19], the preparation of chiral organochalcogeno-α-methylbenzyl alcohols [20], enantioselective reduction of aliphatic and aromatic ketones, cyclic ketones, azidoketones and β-ketoesters [21,22], reduction of cyclic 3-oxo-amines [23], chiral resolution of p- or m-substituted racemic aryl methyl carbinols [24] using carrot root as biocatalyst are described. The use of celery root for the enantioselective hydrolysis of aryl ethyl acetates and the reduction of aryl methyl ketones and bromo- and methoxy-acetophenone derivatives are also described [19,25]. Only one reference was found about the use of roots of parsnip and parsley in the field of synthetic organic chemistry. Whole tissue of celery and parsley was used as biocatalyst for the hydrolysis of acetates and benzoates of 5-hydroxy-4-methyl-3-heptanone [26].

The aim of our study was to screen various vegetables for the enantioseparation of bicyclo[3.3.1]nonane-2,6-dione.

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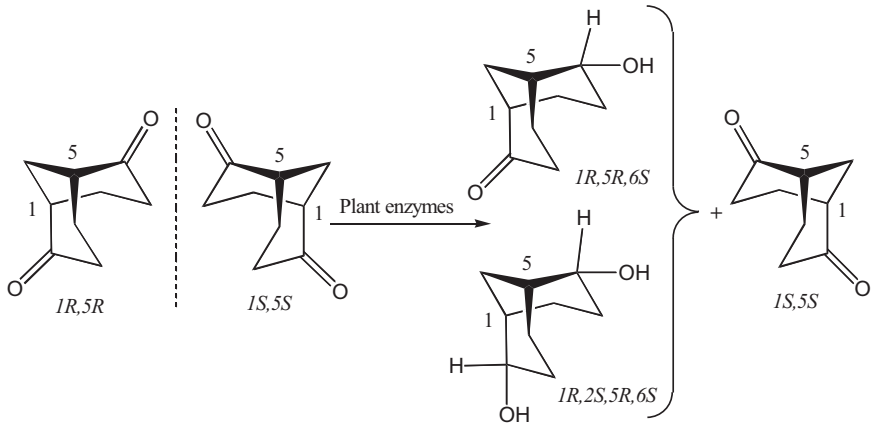

Scheme 1. Reduction of racemic bicyclo[3.3.1]nonane-2,6-dione by vegetables enzymes.

Stereoselective reduction of racemic diketone by plant enzymes was performed. The remaining (+)-enantiomer was extracted from the reaction mixture by organic solvent whereas (−)-enantiomer had undergone an enzymatic reduction, and the reaction product, i.e. 6-hydroxybicyclo[3.3.1]nonane-2-one was found.

2. Experimental

2.1. General

Racemic bicyclo[3.3.1]nonane-2,6-dione (**1**) was synthesized from Meerwein's ester according to the procedure previously described in [15]. All vegetables were purchased in the local market. To increase the contact of substrate with a biocatalyst, vegetables were freshly cut into small thin pieces (approximately 1 cm long slice). Column chromatography was performed on a silica gel 60 (230–400 mesh, Merck) using chloroform/acetone (9/1, v/v) as an eluent. TLC was performed on silica gel covered aluminum plates ($60F_{254}$, Merck). Compounds were detected by spraying with 0.5% $KMnO_4$ solution in water. The optical activity of the final product in chloroform was recorded in a 10 cm path length cuvette using polarimeter Polamat A (Carl Zeiss/Jena). The optical purity was calculated as followed: optical purity = $(\alpha_{observed}/\alpha_{pure\ enantiomer}) \times 100\%$. For pure (+)-**1**, $[\alpha]^{25}_{546} = +205^{\circ}$ was used [6]. Diketone: white crystals, m.p. 134–135 °C. $C_9H_{12}O_2$ (152.19): calcd. C 71.03, H 7.95; found C 70.95, H 7.98. IR (CCl_4): $\nu = 1712\ cm^{-1}$ (C=O). ^1H NMR (400 MHz, $CDCl_3$): δ(ppm) = 2.01–2.14 (m, 4 H, CH_2), 2.20 (m, 2 H, CH_2), 2.39 (quintet, J = 8 Hz, 2 H, CH_2), 2.54–2.62 (m, 2 H, CH_2), 2.73 (m, 2 H, CHCO). ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) = 26.65 (2 C, CH_2), 31.41 (1 C, CH_2), 37.09 (2 C, CH_2), 43.54 (2 C, CH), 212.64 (2 C, C=O).

2.2. Reduction of bicyclo[3.3.1]nonane-2,6-dione

The amount of 50 mg of racemic (**1**) was added to the suspension of vegetables (8 g) in 30 mL of distilled water. The mixture was stirring for 24–72 h at 25 °C, 30 °C or 35 °C temperature. Finally, the suspension was filtered off, and slices of vegetables were washed with distilled water. The aqueous solution was then extracted with chloroform (3 × 20 mL), and the organic phase was dried with anhydrous Na_2SO_4, and then evaporated in a vacuum. Additionally, the reaction product was purified by column chromatography on silica gel as mentioned above.

2.3. UPLC analysis of reaction aqueous solution and of chloroform extract

UPLC analysis was performed on the Acquity UPLC system (Waters) equipped with the Acquity UPLC photodiode array detector using Acquity UPLC HSS T3 (100 mm × 2.1 mm I.D., 1.8 μm) column at 30 °C. A volume of 2 μL was injected using a partial loop injection mode and "needle overfill" as the injection technique. The separation was achieved within 4 min at a flow rate of 0.25 mL/min. A solvent mixture of water/acetonitrile (20/80, v/v) containing 15 mM formic acid was used as the mobile phase.

2.4. Mass spectrometry analysis

The mass spectrometry analysis was carried out on a quadrupole, time-of-flight mass spectrometer (micrOTOF-Q II,

Table 1
Results of the enantioseparation of racemic bicyclo[3.3.1]nonane-2,6-dione by some vegetables.[a,b,c]

No	Vegetables	$[\alpha]^{25}_{546}$ (°)	Overall yield of diketone (%)	Optical purity (%)
1	Roots of *Daucus carota*	+(38 ± 18)	50 ± 16	59 ± 4
2	Roots of *Apium graveolens*	+(82 ± 44)	43 ± 18	70 ± 11
3	Stems of *Allium porrum*	+(25 ± 7)	53 ± 10	56 ± 2
4	Leaves of *Brassica oleracea*	+(9 ± 2)	51 ± 12	52 ± 1
5	Cores of *Brassica oleracea*	+(27 ± 9)	51 ± 19	57 ± 2
6	Rootstocks of *Zingiber officinale*	+(46 ± 26)	34 ± 15	61 ± 6
7	Roots of *Raphanus sativus var. niger*	+(4 ± 3)	60 ± 20	51 ± 1
8	Roots of *Beta vulgaris atrorubra*	+(23 ± 3)	71 ± 12	56 ± 1
9	Roots of *Petroselinum crispum*	+(52 ± 2)	46 ± 12	63 ± 1
10	Roots of *Pastinaca sativa*	+(91 ± 24)	36 ± 9	72 ± 6

[a] The experimental data are presented as mean values ± standard deviations of 3–6 parallel experiments.
[b] Reaction was carried out at 30 °C for 72 h.
[c] Entries 1, 2, 9, 10; entry 3; entries 4, 5, 7; entry 6 and entry 8 belong to the families *Apiaceae*, *Amaryllidaceae*, *Brassicaceae*, *Zingiberaceae* and *Amarathaceae*, respectively.

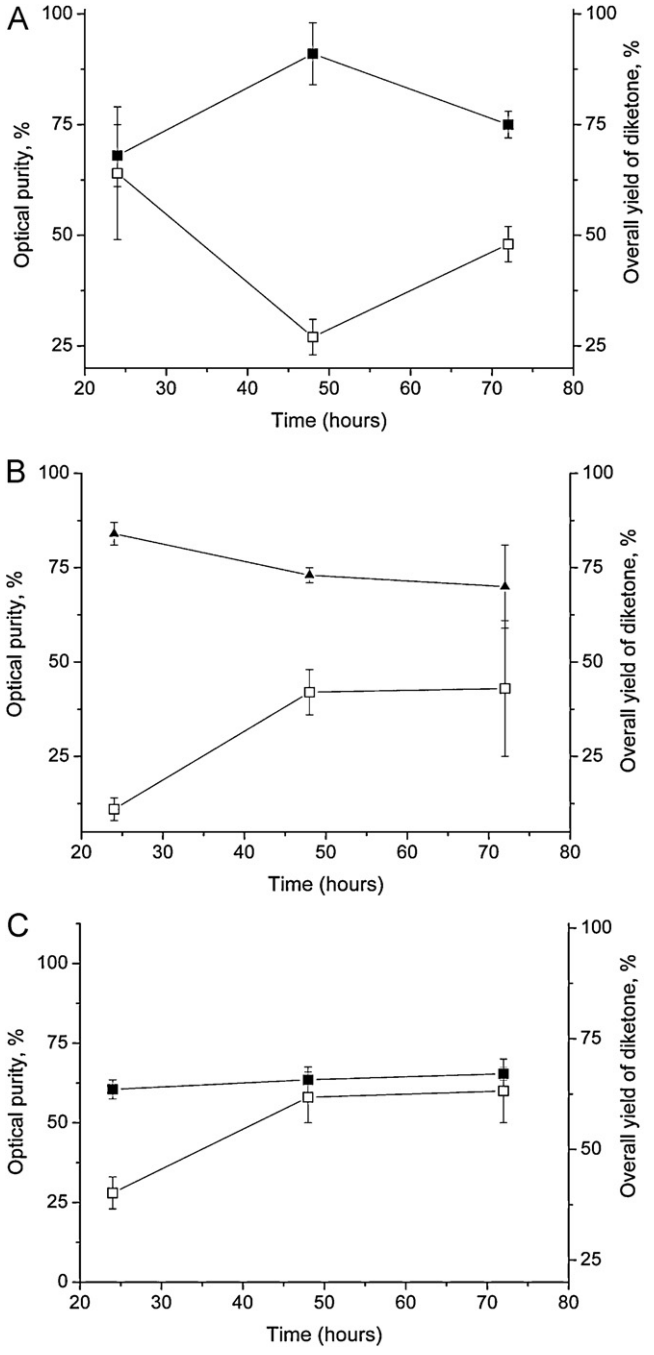

Fig. 1. Dependence of overall diketone yield (□) and optical purity (■) on the reaction duration at 25 °C (A), 30 °C (B) and 35 °C (C). Reaction mixture contained 50 mg of (**1**) and 8 g of celery in 30 mL of water.

Bruker Daltonik GmbH). The instrument was modified for medium masses (50–1500 amu). The following instrumental parameters were used: transfer RF1, 200 Vpp; RF2, 300 Vpp; hexapole RF, 300 Vpp; ion energy, 5.0 eV; low mass, 100 Vpp; collision energy, 8.0 eV; collision RF, 200 Vpp; transfer time, 80.0 μs; pre pulse storage, 5.0 μs. The ion source was operated in the positive mode on the following set parameters: capillary voltage, 4.5 kV; nebulizer, 1.2 bar; flow rate, 7 mL/min; dry temperature, 180 °C. All mass spectra were calibrated externally using the low concentration tuning mix (ESI-L, Agilent Technologies) and were processed with DataAnalysis 4.0 software (Bruker Daltonik GmbH).

3. Results and discussion

Previously, racemic mixtures of bicyclo[3.3.1]nonane-2,6-dione were separated using a horse liver alcohol dehydrogenase catalyzed reduction coupled with the regeneration of the coenzyme NAD by dithionite or ethanol [13]. For the stereospecific reduction and the resolution of the enantiomers of that compound, baker's yeast were also applied [11,15]. Thus, having in mind previous papers we could expect that biocatalytic enantioselective reduction would take place (Scheme 1). In this sense, preliminary screening revealed that vegetables of the family *Apiaceae*, i.e. the roots of parsnip (*Pastinaca sativa*), celery (*Apium graveolens*), parsley (*Petroselinum crispum*) and carrot (*Daucus carota*) showed the best results in the enantioseparation of racemic (**1**) (Table 1). The final product, i.e. diketone extracted with chloroform was enriched by (+)-enantiomer of (**1**). The results obtained with rootstocks of ginger (*Zingiber officinale*), which belongs to another plant family of *Zingiberaceae*, were similar as for carrots. Roots of celery were chosen for more detailed investigation and optimization of reaction conditions (Fig. 1). Additional experiments showed that the highest optical purity of (+)-(**1**) can be reached at 25 °C after 48 h (Fig. 1A). Moreover, the enantioseparation of racemic mixture was performed under those conditions using four-fold higher amount of (**1**), i.e. 200 mg of (**1**) was added to the suspension of vegetables (20 g) in 140 mL of water. (+)-Enantiomer was obtained in the yield of 50–54% and with the excellent optical purity of 90–98%. The 1.5-fold increase of celery amount, i.e. 30 g of vegetables for 200 mg of racemic diketone had no effect on (+)-**1** purity, but lower amount of celery (8 g for 200 mg of diketone) was not enough, and the optical purity of (+)-**1** was only 59%. Under reaction conditions mentioned above (200 mg of diketone, 20 g of vegetables, 25 °C, 48 h) we also obtained (+)-enantiomer in the yield of 40–45% and with optical purity of 92–96% using roots of parsnip (*P. sativa*). To increase the yield of (+)-**1**, we changed the procedure of diketone extraction previously mentioned in Section 2.2. When the suspension had been filtered off the slices of vegetables (roots of celery) were ground, and water was poured over the mash. After centrifugation both water fractions were joined and extracted with chloroform as mentioned in Section 2.2. This procedure increased the yield of pure (+)-**1** up to more than 90%.

As can be seen in Table 1, entry 7 (*Raphanus sativus var. niger*) has no enzymatic activity for (**1**) biotransformation or an enzyme does not exhibit enantioselectivity (Table 1). The same conclusion could be drawn in regard to Table 1 entries 3 (*Allium porrum*), 4 (*Brassica oleracea*) and 5 (*B. oleracea*). About 50% of diketone amount was lost, but the optical purity increased only slightly. The prolongation of reaction time (Fig. 1A) or the increase of reaction temperature (Fig. 1B and C) did not increase the optical purity. It is possible that a reverse reaction takes place or there are two enzymes of different enantioselectivity, i.e. one for (+)-**1** and another for (−)-**1** enantiomer, and the rates of their catalyzed reaction are different. Thus, the product of slower reaction decreases the optical purity

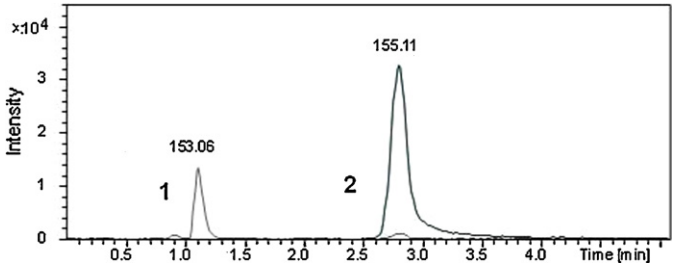

Fig. 2. Analysis of reaction aqueous solution using Acquity UPLC HSS T3 (100 mm × 2.1 mm I.D., 1.8 μm) column.

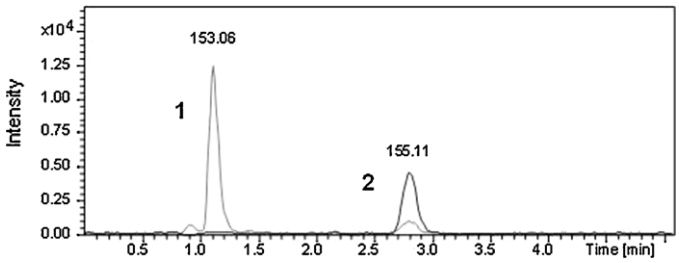

Fig. 3. Analysis of chloroform extract using Acquity UPLC HSS T3 (100 mm × 2.1 mm I.D., 1.8 μm) column.

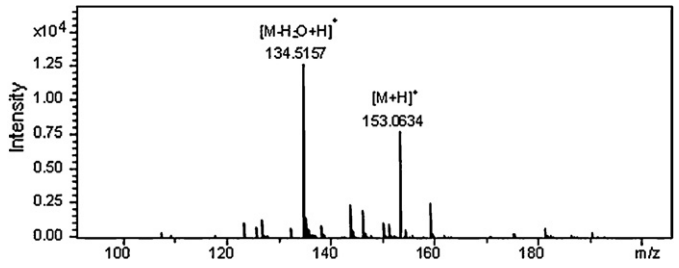

Fig. 4. Mass spectrometry analysis of peak 1 from Figs. 2 and 3.

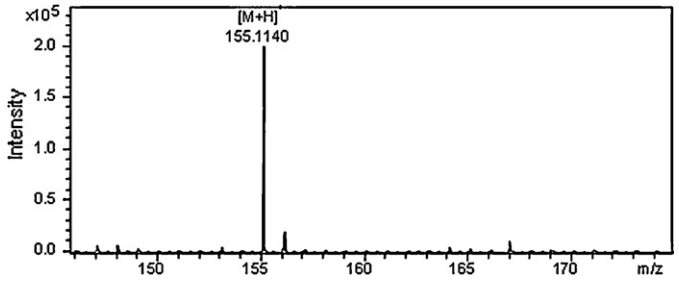

Fig. 5. Mass spectrometry analysis of peak 2 from Figs. 2 and 3.

with the prolongation of reaction time. At higher temperature this effect is observable in a shorter time, and at 35 °C the optical purity of (**1**) is almost the same after 24 and 72 h.

For the identification of reaction products, UPLC separation and identification of compounds by mass spectrometry analysis were applied. Two peaks are seen on the chromatograms of both reaction mixture in water (Fig. 2) and of chloroform extract (Fig. 3). Mass spectrometry analysis shows that bicyclo[3.3.1]nonane-2,6-dione is eluted first and the reduction product, i.e. 6-hydroxybicyclo[3.3.1]nonane-2-one is eluted as the second peak (Figs. 4 and 5). It is noteworthy that under the reaction conditions used in the present work bicyclo[3.3.1]nonane-2,6-diol was not found. It should be mentioned that (+)-**1** is the remaining enantiomer in our reaction as well as in yeast-catalyzed reduction [15]. On contrary, a horse liver alcohol dehydrogenase catalyzes the reduction of (+)-**1** and (−)-**1** is the remaining enantiomer [13].

4. Conclusions

Vegetables of the family *Apiaceae* are promising biocatalysts for the stereoselective bioreduction and enantioseparation of racemic bicyclo[3.3.1]nonane-2,6-dione. This methodology allows to recover the (+)-enantiomer with a very good enantiomeric purity. This biotechnological process is ecofriendly and cheap, the isolation of the final product is easy and vegetables as biocatalysts are easy available. The method for the enantioseparation of bicyclo[3.3.1]nonane-2,6-dione described in this work has some advantages as compared with other enzymatic methods published previously. First, it is more cheaper than the use of horse liver alcohol dehydrogenase and does not require additional experiments for cofactor regeneration. Second, the use of vegetables is very simple as compared with the enantioseparation of bicyclo[3.3.1]nonane-2,6-dione by baker's yeast.

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