Universität Hannover

Stereospecific allylic oxidation of limonenes:

A route to pure *S*-(+)-and *R*-(-)-carvone

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Introduction

Carvone enantiomers, known as impact compounds of mint and caraway flavour, are two of the most sought-after flavour compounds. Based on the different odour impressions the production of enantiopure carvone is a matter of particular interest to the flavour industry. Apart from the traditional extracting of plant raw materials, biotechnology opens access to natural carvon [1]. However, with the exception of a *Rhodococcus* strain which hydroxylated *R*-(+)-limonene stereospecifically to (+)-trans-carveol [2], all of the previous investigations on limonene biotransformations including plant cell cultures were found to be nonspecific.

Experimental

Fungal strains were obtained from DSMZ (Braunschweig, Germany) and cultivated on standard nutrient medium based on yeast extract. Formation of volatiles was monitored by a miniaturised extraction method, and dried extracts were analysed by HRGC and HRGC-MS [3]. Isomeric ratios of carveol and carvone were measured by chiral gaschromatography and their absolute configuration was determined using authentic standards.

Results and discussion

Optimised feeding of *Pleurotus sapidus* either with *S*- or *R*-limonene resulted in the formation of all four possible stereoisomers of carveol. An enantiomeric distribution of 67 % *R*-(+)- to 33 % *S*-(-)-carvone was observed, regardless of the limonene enantiomer fed. A two step mechanism of a monooxygenase catalysed allylic hydroxylation was proposed *via* an intermediate carbocation or radical, which rearranges along the double bound with a loss of stereochemical information at C-4.

In screenings of fungal strains, ascomycetes of the genus *Chaetomium* performed a highly enantiospecific hydroxylation of *R*-(+)-limonene to *cis*-(+) carveol with ee-values > 95 % in 4 days (figure 1). *Fusarium proliferatum* was found particularly promising, because *R*-limonene was transformed to enantiopure *cis*-(+)-carveol (ee > 99 %) yielding more than 35 mg L^{-1} in 24 hours. This points to an enzymatic mechanism different from that of *Pleurotus sapidus*. *Fusarium proliferatum* accepted *S*-(-)-limonene as well; this was predominantly converted to *trans*-(-)-carveol (figure 2).

Further oxidation of these enantiopure terpene alcohols using *Pleurotus sapidus* or *Rhodococcus erythropolis*, for instance, opens direct access to *R*-(-)-carvone from *trans*-(-)-carveol, and *S*-(+)-carvone from *cis*-(+)-carveol (figure 3).

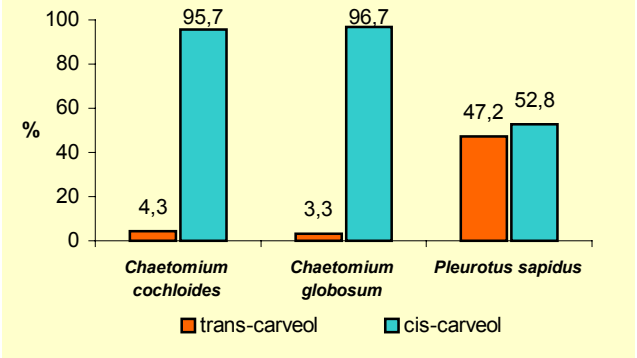


Figure 1. Stereoselectivity of carveol biogenesis by selected fungi.

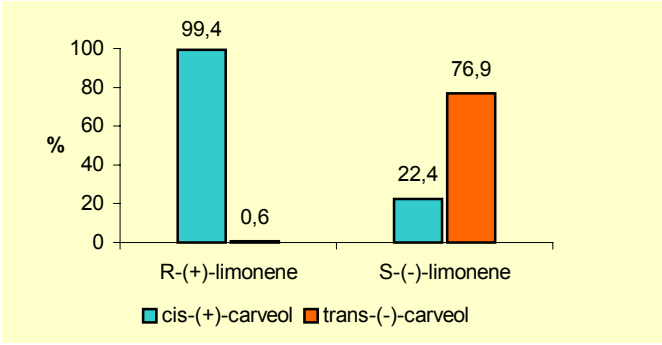


Figure 2. Stereoselectivity of carveol biogenesis by *Fusarium proliferatum* fed with pure limonene enantiomers.

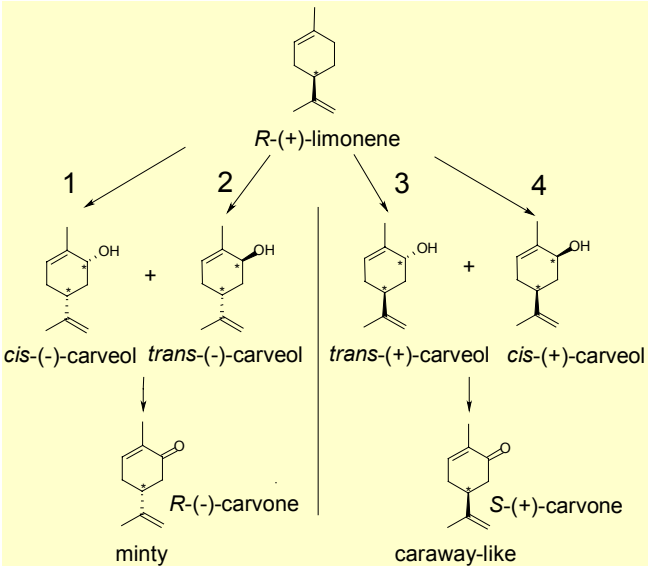


Figure 3. Carveol biogenesis from R-(+)-limonene by *Pleurotus sapidus* (1,2,3, and 4), *Rhodococcus opacus* (3) and *Fusarium proliferatum* (4, and supplemented with S-(-)-limonene, 2).

Acknowledgement. This project was supported by the Federal Minister of Education and Research (BMBF 0330062) and is part of the joint initiative project "Biologisch aktive Naturstoffe – Chemische Diversität" at the University of Hannover.

[1] Schrader, J. & Berger, R.G. (2001) In: Biotechnology Vol. 10: Special Processes, Rehm, H.J. (ed.), Wiley-VCH, Weinheim, Chapter 13, 374-422, [2] Duetz, W. A., *et al*. (2001). Appl. Environ. Microbiol. 67: 2829-2832, [3] Onken, J. & Berger, R. G. (1999) J. Biotechnol. 69: 163-168.