UDC 541.632:547.787.3:547.514:582.281.21
ISSN 1330-9862

original scientific paper

Differences in the Regio- and Stereoselectivity of the Microbial Hydroxylation of a Derivatized Cyclopentanecarboxylic Acid by Employing Various Mucorales Strains

Michaela Kreiner,[1] *Gerhart Braunegg,*[1*] *Anna de Raadt,*[2] *Herfried Griengl,*[2] *Peter Plachota*[2] *and Hansjörg Weber*[2]

[1]Institute of Biotechnology, Technical University of Graz, Petersgasse 12, 8010 Graz, Austria

[2]Institute of Organic Chemistry, Technical University of Graz, Stremayrgasse 16, 8010 Graz, Austria

Received: December 19, 1996
Accepted: March 21, 1997

Summary

Mucorales strains ***Absidia glauca, Mortierella alpina, Mucor plumbeus, Phycomyces blakesleeanus, Rhizopus oryzae,*** *and* ***Syncephalastrum racemosum*** *were found to be efficient biocatalysts for the biotransformation of derivatized cyclopentanecarboxylic acid into hydroxy and oxo derivatives. Cyclopentanecarboxylic acid was protected against microbial degradation by chemical transformation into 2-cyclopentylbenzoxazole* **1.** *The biotransformation yielded 3-(benzoxazol-2-yl)cyclopentan-1-ol* **3** *as the main product. 2-(Benzoxazol-2yl)cyclopentan-1-ol* **2** *and 3-(benzoxazol-2-yl)cyclopentan-1-one* **4** *were detected as by-products.*

The enantiomeric compositions and optical purities of the main products were determined in order to compare interspecific differences in the regio- and stereoselectivity of the fungal species. The main product **3** *produced by* ***M. plumbeus, R. oryzae,*** *and* ***S. racemosum*** *was predominantly the (1S,3S)-enantiomer, whilst that formed by* ***A. glauca, M. alpina,*** *and* ***P. blakesleeanus*** *was predominantly the (1R,3R)-enantiomer. The results indicate that although related fungi may exhibit similar product spectra, there are differences in regio- and stereoselectivity that are species dependent.*

Keywords: biohydroxylation, mucorales, stereochemistry

Introduction

Biohydroxylation reactions are a powerful tool for the introduction of hydroxyl groups into organic compounds as an intermediate step for any other specific functionalization, with the additional benefit of the usual regio- and stereoselectivity of enzymic reactions *(1,2)*. Filamentous fungi of the order mucorales have been widely used in biocatalytic processes *(1–3)*. Most prominent is their use in the field of steroids and terpenes *(2,4)*. One of the first of these preparatively useful reactions was the hydroxylation of progesterone in the 11α-position by *Rhizopus arrhizus* which made 11α-hydroxyprogesterone available for therapy at a reasonable cost *(5)*. *Mucor plumbeus* was found to be a useful microorganism for the hydroxylation of bicyclic enones *(6)*. In addition, mucorales strains are used as microbial models of mammalian metabolism *(7,8)*.

In the continuation of our work on the application of anchor/protecting groups for selective biohydroxylation, the modulation of the steric outcome of the microbial transformation of 2-cyclopentylbenzoxazole (**1**) as a masked cyclopentanecarboxylic acid was investigated by applying this compound as substrate for various mucorales. 2-substituted benzoxazoles serve as nonpolar substitutes for cycloalkanecarboxylic acids pre-

* corresponding author

venting degradation by the microbial cells *(9)*. Substrate specificities and stereoselectivities of the hydroxylations of various benzoxazoles with *Cunninghamella blakesleeana* DSM 1906 were described by de Raadt *et al. (9)*. The time course of the biotransformations of benzoxazoles by this mucorales strain as well as the influence of its morphological appearance were also reported previously *(10)*.

Material and Methods

Strains and cultivation

Absidia glauca DSM 811, *Phycomyces blakesleeanus* DSM 900, *Rhizopus oryzae* DSM 907, and *Syncephalastrum racemosum* DSM 859 were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM), Braunschweig, Germany, *Mortierella alpina* ATCC 8979 from the American Type Culture Collection (ATCC), Rockeville, USA, and *Mucor plumbeus* CBS 110.16 from Centraalbureau voor Schimmelcultures (CBS), Baarn, The Netherlands. Stock cultures of *P. blakesleeanus* were maintained on modified medium E1 agar slants, all other strains were maintained on potato-dextrose agar slants. The cultures were stored at 4 °C, and subcultured every three weeks at 23 °C (*P. blakesleeanus, R. oryzae, M. plumbeus*) or 30 °C (*A. glauca, M. alpina, S. racemosum*).

Media

Medium E and modified media E1 and E2 were prepared as described by Kreiner *et al. (10)*. Modified medium E3 consisted (per litre) of 5 g of peptone (Merck), 2 g of yeast extract (Oxoid), 10 g of malt extract (Merck), and 10 g of glucose. Potato-dextrose-broth was obtained from Difco. All other chemicals were obtained from Merck (*p.a.* quality). Media were sterilized at 121 °C for 30 min. In order to avoid precipitates, C- and N-sources were sterilized separately.

Biotransformation procedure

Biotransformations were performed in baffled shake flasks (1 L) containing 250 mL modified medium E1 (*M. alpina*), modified medium E2 (*A. glauca, M. plumbeus, P. blakesleeanus, S. racemosum*), or modified medium E3 (*R. oryzae*) at an agitation of 140 rpm. Incubation temperature was 20 °C for *P. blakesleeanus*, 23 °C for *M. plumbeus* and *R. oryzae* and 30 °C for *A. glauca, M. alpina*, and *S. racemosum*. Each flask was inoculated with a spore suspension (8 mL) made from an agar slant containing a culture grown for two weeks. The spore concentration was 10^8 spores per litre medium. After 44 h of growth, an ethanolic solution (500 μL) of the substrate 2-cyclopentylbenzoxazole (**1**) was added to the flasks yielding a substrate concentration of 300 mg L^{-1} (330 mg L^{-1} with *M. alpina*) in the culture broth.

Analytical methods

Metabolite concentrations were measured by gas chromatography as described previously *(10)*. Enantiomeric excess (e.e.), defined as the percent excess of the major enantiomer in relation to the minor enantiomer, was determined by chiral high-pressure liquid chromatography (HPLC) as described by de Raadt *et al. (9)*. A JASCO system containing an 880-PU pump, an 875-UV-detector and AXXIOM Model 727 chromatography software was used with a chiral column (Chiralpak AD; Daicel). Heptane/2-propanol 95:5 was applied as eluent. For better separation the column was cooled to 10 °C. Detection of benzoxazoles was conducted at 230 nm. The determination of absolute configurations of the products produced by *C. blakesleeana* is also described by de Raadt *et al. (9)*. Absolute configuration of **3** was done by X-ray analysis of the (1*S*)-camphanic acid derivative of the enantiomerically pure alcohol, obtained by lipase catalysed hydrolysis of the corresponding acetate. Cleavage of the microbially produced ketone **4** gave (+)-3-oxocyclopentylcarboxylic acid. The corresponding (–)-enantiomer was already assigned by Azerad as being *S (9)*. Characterization of products formed by the other mucorales strains was performed by GC/MS. Preparation of samples for GC/MS was the same as for GC. Gas chromatography was performed on a HP 6890 Series II Plus gas chromatograph, equipped with an HP-5 analytical column (30 m × 0.25 mm × 0.25 μm). Helium, flowing at 1.0 mL min^{-1}, acted as carrier gas. The temperature profile was identical to the gas chromatographic determination of metabolite concentrations described above *(10)*. Electron impact method (70 eV) was used for ionization. Detection was performed by a mass selective detector (HP 5972A).

Synthesis of substrates and removal of the anchor/protecting group

Synthesis of 2-cycloalkylbenzoxazoles and removal of the anchor/protecting groups have been described by de Raadt *et al. (9)*.

Results and Discussion

All products obtained (Fig. 1) from incubation of 2-cyclopentylbenzoxazole (**1**) with batch cultures of *A. glauca, M. alpina, M. plumbeus, P. blakesleeanus, R. oryzae*, and *S. racemosum* were identified by GC and GC/MS. The retention times (10) and the GC/MS-spectra of the products were identical to those of the metabolites formed with *C. blakesleeana*. These GC/MS data are given below:

trans-**3**: MS(GC/MS,EI): *m/z* (%) = 203 (6), 184 (5), 159 (5), 146 (100), 133 (11), 129 (6), 91 (8), 63 (15), 51 (4), 39 (11), 29 (9).

cis-**3**: MS(GC/MS,EI): *m/z* (%) = 203 (10), 184 (1), 175 (11), 160 (34), 146 (100), 135 (20), 120 (5), 109 (21), 91(13), 77 (3), 63 (10), 39 (10), 29 (10).

2: MS(GC/MS,EI): *m/z* (%) = 203 (3), 185 (3), 175 (10), 160 (6), 146 (6), 133 (12), 120 (4), 91 (9), 77 (8), 63 (22), 55 (11), 39 (21), 28 (53).

4: MS(GC/MS,EI): *m/z* (%) = 201 (23), 186 (1), 173 (23), 158 (12), 145 (100), 133 (12), 117 (3), 91 (7), 78 (3), 63 (29), 55 (11), 39 (17), 27 (12).

The results concerning the transformation of **1** with the various mucorales strains were compared with those obtained with *C. blakesleeana (10,11)* because this strain has been most intensively investigated concerning the influ-

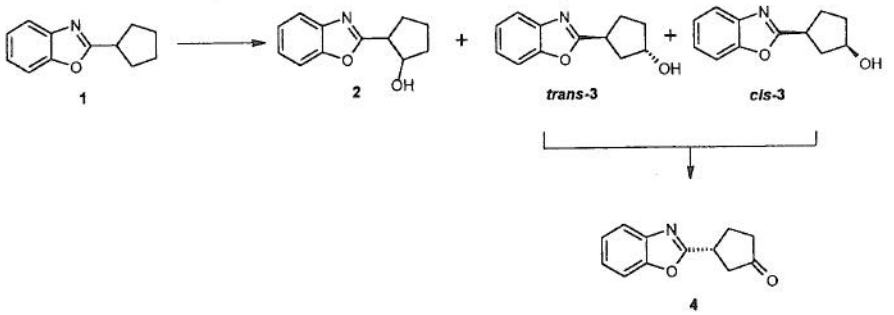

Fig. 1. Transformation of 2-cyclopentylbenzoxazole (**1**) yielding 3-(benzoxazol-2yl)cyclopentan-1-ol (**3**) [*trans:* (1*R*,3*R*)- and (1*S*,3*S*)-enantiomers; *cis:* (1*R*,3*S*)- and (1*S*,3*R*)-enantiomers, drawn are the (1*S*,3*S*)- and the (1*R*,3*S*)-enantiomers], 2-(benzoxazol-2-yl)cyclopentan-1-ol (**2**) and 3-(benzoxazol-2-yl)cyclopentan-1-one [**4**, (*R*)-enantiomer shown] as products.

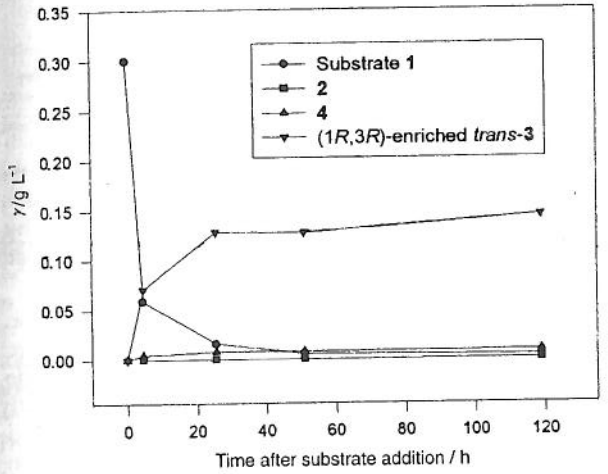

Fig. 2. Transformation of **1** with *Absidia glauca*. Shown are the time courses of substrate consumption and formation of **2**, (1*R*,3*R*)-enriched *trans*-**3**, and **4**.

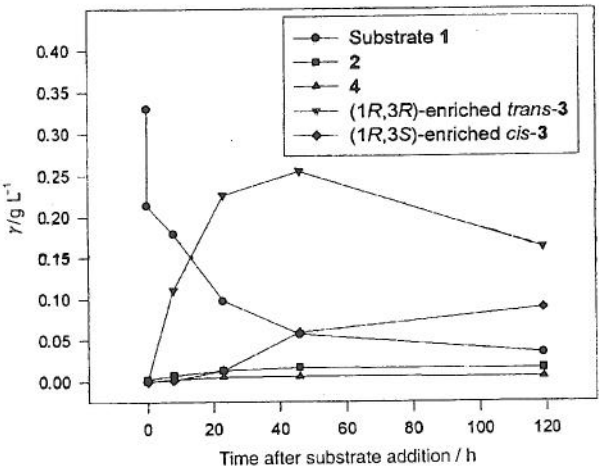

Fig. 3. Transformation of **1** with *Mortierella alpina*. Shown are the time courses of substrate consumption and formation of **2**, (1*R*,3*R*)-enriched *trans*-**3**, (1*R*,3*S*)-enriched *cis*-**3**, and **4**.

ence of culture conditions and morphology on the transformations of derivatized cycloalkanecarboxylic acids.

A. glauca. The time course of the transformation of 2-cyclopentylbenzoxazole (**1**) with *A. glauca* is presented in Fig. 2. The substrate concentration decreased very rapidly. (1*R*,3*R*)-enriched *trans*-**3** was formed up to 128 mg L^{-1} within the first 25 h after substrate addition. Thereafter, the concentration increased only slightly. **4** and **2** were found as by-products: their concentrations remained below 9 mg L^{-1}. Fifty-one hours after substrate addition the e.e. of the (1*R*,3*R*)-enriched *trans*-**3** was 22%. In contrast to *C. blakesleeana*, the e.e. did not increase significantly during the whole course of the experiment (Table 1). In addition, regioselectivity was reduced with

Table 1. Time dependence of the enantiomeric excess (e.e.) of **2**, *trans*-**3** and **4** and diastereomeric excess (d.e.) of **3** formed during the transformation of **1** with various mucorales strains

Strain	Time/h*	e.e.(2)/%	e.e.(*trans*-3)/%	e.e.(4[c])/%	d.e.(*trans*-3)/%
Absidia glauca	51	4	22[b]	–	99
	119	16	24[b]	–	97
*Cunninghamella blakesleeana***	14	–	22[a]	73	–
	58	–	64[a]	68	–
Mortierella alpina	8	–	32[b]	–	96
	76	–	27[b]	–	86
	116	–	13[b]	–	60
Mucor plumbeus	24	15	35[a]	–	99
	120	17	73[a]	–	99
Phycomyces blakesleeanus	120	37	18[b]	–	87
Rhizopus oryzae	115	6	49[a]	–	97
Syncephalastrum racemosum	23	65	23[a]	21	98
	127	51	52[a]	35	93

* time after substrate addition

** culture cultivated in stirred tank reactor *(10)*

[a] (1*S*,3*S*)-enriched **3**, [b] (1*R*,3*R*)-enriched **3**, [c] (1*R*)-enriched **4**.

A. glauca. The ratio of **2** to **3** was 1 : 15 (1 : 40 with *C. blakesleeana*).

M. alpina. As can be seen in Fig. 3, 2-cyclopentylbenzoxazole (**1**) was hydroxylated by *M. alpina* predominantly at carbon atom 3 of the cyclopentane system. Four stereoisomers of **3** could be detected, the concentration of the predominantly formed (1*R*,3*R*)-enriched *trans*-**3** increased within the first 46 h after substrate addition yielding 255 mg L^{-1} (yield of 71 mol%). After this point its concentration declined.

As presented in Table 1, the e.e. of the (1*R*,3*R*)-enriched *trans*-**3** decreased from 32% (8 h after substrate addition) to 13% at the end of the experiment (116 h). *M. alpina* demonstrated the strongest tendency of all tested mucorales strains to form the (1*R*,3*S*)-enriched *cis*-**3**. This can be seen with a decrease of the diastereomeric excess (d.e.), defined as the percent excess of the major pair of enantiomers in relation to the minor diastereomeric pair of enantiomers, from 96% after eight hours to 60% at the end of the experiment (Table 1). In comparison with the main pair of enantiomers, the production of the (1*R*,3*S*)-enriched *cis*-**3** was delayed (Fig. 3) but still continued when the concentration of (1*R*,3*R*)-enriched *trans*-**3** had already decreased. Traces of **3** were further oxidized yielding 6 mg L^{-1} of the corresponding ketone **4**. The hydroxylation of 2-cyclopentylbenzoxazole (**1**) was not totally regioselective, since **2** was also formed with a yield of 5 mol%.

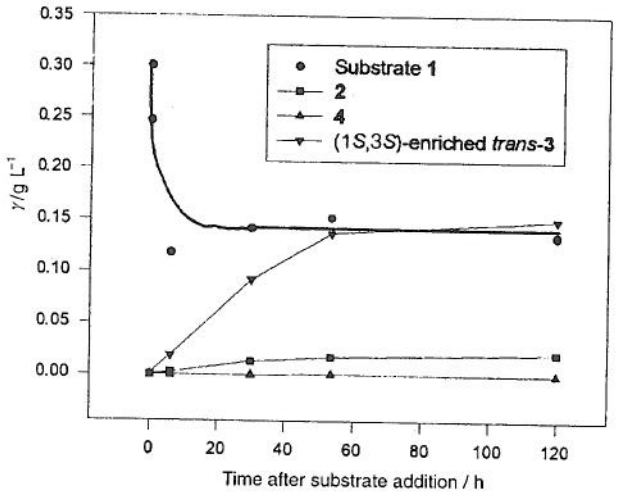

Fig. 4. Transformation of **1** with *Mucor plumbeus*. Shown are the time courses of substrate consumption and formation of **2**, (1*S*,3*S*)-enriched *trans*-**3**, and **4**.

M. plumbeus. This organism also hydroxylated 2-cyclopentylbenzoxazole (**1**) in positions 2 and 3 of the carbon ring (Fig. 4). The main product (1*S*,3*S*)-enriched *trans*-**3** and the by-product **2** were formed within the first 50 h after substrate addition. In contrast to *C. blakesleeana*, *M. plumbeus* showed a lower regioselectivity in the hydroxylation reaction. The ratio of the two alcohols was 1 : 8 (1: 40 with *C. blakesleeana*). The ketone **4** was only found in trace amounts. The e.e. of the (1*S*,3*S*)--enriched *trans*-**3** increased from 35% (24 h after substrate addition) to 73% at the end of the experiment (Table 1). **2** was produced with a low e.e. of 17%.

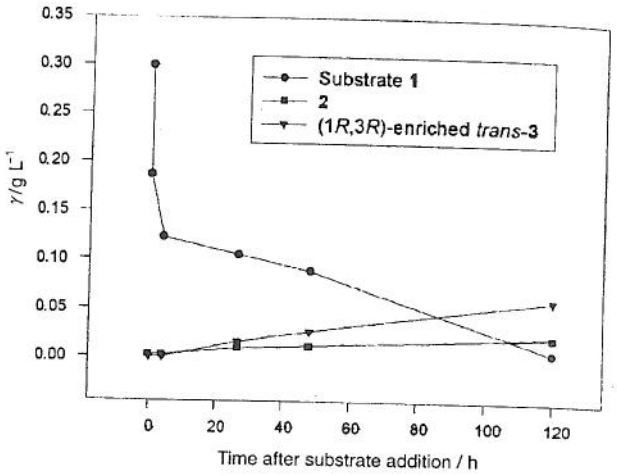

Fig. 5. Transformation of **1** with *Phycomyces blakesleeanus*. Shown are the time courses of substrate consumption and formation of **2**, and (1*R*,3*R*)-enriched *trans*-**3**.

Cultivation temperature was of importance for the hydroxylation activity of *M. plumbeus*. Whereas transformations at 30 °C afforded only small amounts of hydroxylated products, at 23 °C 52 mol% of the substrate was converted into hydroxylated compounds.

P. blakesleeanus. The time course of substrate consumption and product formation during transformation of 2-cyclopentylbenzoxazole (**1**) with *P. blakesleeanus* is depicted in Fig. 5. Substrate concentration decreased rapidly to 123 mg L^{-1} within the first four hours after substrate addition. Afterwards the consumption rate declined. The concentrations of the main products (1*R*,3*R*)-enriched *trans*–**3** and **2** increased in a linear manner. The yields were low (19 and 7 mol%, respectively). As can be seen from these data, hydroxylation performed with this fungus shows a low regioselectivity. The ketone **4** was only found in trace amounts and the e.e. of (1*R*,3*R*)-enriched *trans*–**3** (18%) was also low. In addition, diastereomers were formed and the d.e. was 87% after a transformation time of five days.

As with *M. plumbeus*, the cultivation temperature was of importance for the hydroxylation activity of *P. blakesleeanus*. Temperature had to be kept below 30 °C in order to achieve any hydroxylation. Although medium E2 was found to minimize the pellet size (diameter of about 2 mm) of *C. blakesleeana* without a loss of hydroxylation activity *(11), P. blakesleeanus* formed pellets with a diameter up to 5 mm in this medium. The large pellets could be an explanation for the slow hydroxylation observed with this fungus. The fact that product concentrations increased in a linear manner during the whole experiment supports this assumption.

R. oryzae. This organism hydroxylated 2-cyclopentylbenzoxazole (**1**) in positions 2 and 3 of the carbon ring (Fig. 6). The main product (1*S*,3*S*)-enriched *trans*-**3** was formed with a rate of 2 mg L^{-1} h^{-1} within the first 42 h after substrate addition. Subsequently, the rate of product formation declined and a final yield of 33 mol% was obtained. The (1*S*,3*S*)-enriched *trans*-**3** had an e.e. of 49% 115 h after substrate addition (Table 1). The regioselectivity of *R. oryzae* can be compared with that of *M. plumbeus*: 4 mol% of substrate **1** was transformed to **2**. The ketone **4** was found in trace amounts only.

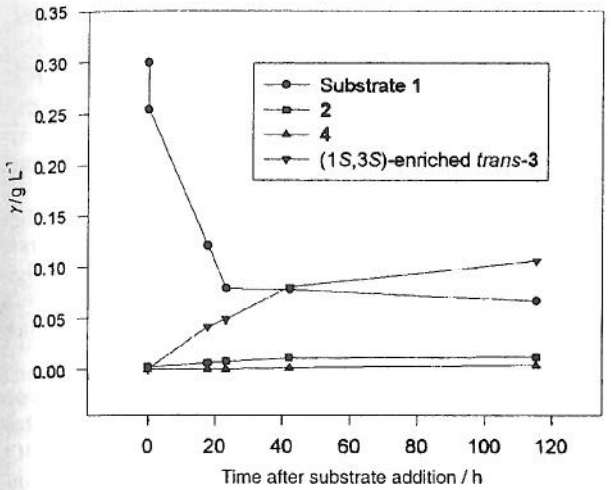

Fig. 6. Transformation of **1** with *Rhizopus oryzae*. Shown are the time courses of substrate consumption and formation of **2**, (1*S*,3*S*)-enriched *trans*-**3**, and **4**.

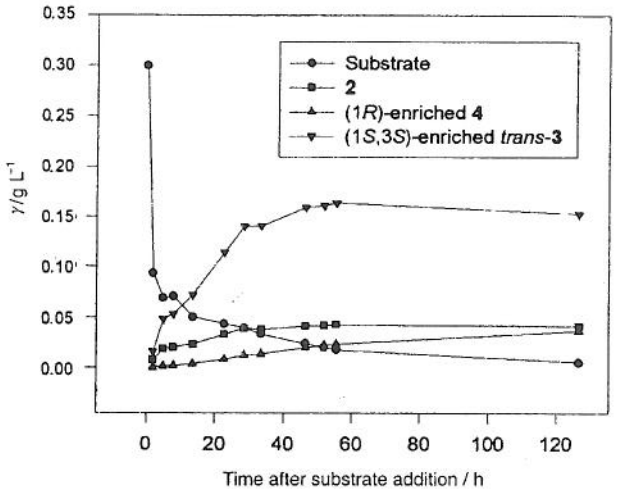

Fig. 7. Transformation of **1** with *Syncephalastrum racemosum*. Shown are the time courses of substrate consumption and formation of **2**, (1*S*,3*S*)-enriched *trans*-**3**, and (1*R*)-enriched **4**.

S. racemosum. The time course of substrate consumption and product formation during the transformation of 2-cyclopentylbenzoxazole (**1**) with *S. racemosum* is shown in Fig. 7. The substrate concentration decreased rapidly to 93 mg L^{-1} within the first two hours after substrate addition. The concentrations of (1*S*,3*S*)-enriched *trans*-**3** and **2** increased within the first 48 h after substrate addition resulting in a yield of 50 and 13 mol% respectively. The ketone **4** was produced in a yield of 11 mol%. The e.e. of (1*S*,3*S*)-enriched *trans*-**3** increased from 23% (23 h after substrate addition) to 52% (after 127 h) due to the stereoselective oxidation of *trans*-**3** to the corresponding (1*R*)-enriched ketone **4** (Table 1). In addition to the *trans*--diastereomers, the *cis*-diastereomers were found in increasing amounts as also observed with *M. alpina*. The d.e. of *trans*-**3** was reduced from 98% (23 h after substrate addition) to 93% at the end of the experiment (Table 1).

Table 2 summarizes the products formed during the biotransformation of 2-cyclopentyl-*trans*-3-benzoxazole (**1**) and Fig. 8 presents an overview of the stereochemistry of the main product 3-(benzoxazol-2-yl)cyclopentan-1-ol (*trans*-**3**) obtained with the tested fungi of the order mucorales. *M. alpina*, *A. glauca*, and *P. blakesleeanus* formed (1*R*,3*R*)-enriched *trans*-**3**. All the other tested strains yielded predominantly (1*S*,3*S*)-**3**. Yields of hydroxylated products formed by *M. alpina* and *S. racemosum* were comparable to those furnished during transformation with *C. blakesleeana (10)*. *A. glauca* and *M. plumbeus* yielded about 50% of hydroxylated products, determined as the sum of **2**, *cis*/*trans*-**3** and **4**. *R. oryzae* and *P. blakesleeanus* showed the lowest hydroxylation activity with 2-cyclopentylbenzoxazole (**1**) as substrate. None of the tested strains performed the biotransformation as regioselectively as *C. blakesleeana*. Especially *S. racemosum* produced comparably high amounts (13 mol%) of **2**. *C. blakesleeana (10)* and *S. racemosum* were the only strains that oxidized considerable amounts of *trans*-**3** to the corresponding ketone **4**. The optical purity of *trans*-**3** ranged from 20 to 50%. Exceptions

Table 2. Transformations of **1** with various mucorales strains

Shown are maximum analytical yields (mol%) of *trans*-**3**, **2**, **4**, and *cis*-**3**. As the maximum yields for each of the products are achieved at different fermentation times and in some cases the yields decline due to further reactions the sum of the yields given is more than 100% in some of the experiments shown. Also given are the preferred configurations of **3** and **4** as well as the morphological appearance of the fungi in the respective medium.

Strain	Yield/% (time/h)				Configuration		Morphological
	trans-**3**	**2**	**4**	*cis*-**3**	**3**	**4**	appearance (diameter)
Absidia glauca	45 *(119)*	3 *(25)*	3 *(119)*	1 *(119)*	1*R*,3*R*	–	flocs
*Cunninghamella blakesleeana**	76 *(58)*	3 *(98)*	22 *(109)*	2 *(98)*	1*S*,3*S*	1*R*	pellets (2 mm)
*Cunninghamella blakesleeana***	86 *(14)*	2 *(14)*	21 *(58)*	1 *(58)*	1*S*,3*S*	1*R*	pellets (1–2 mm)
Mortierella alpina	71 *(46)*	5 *(46)*	2 *(46)*	25 *(119)*	1*R*,3*R*	–	flocs/pellets
Mucor plumbeus	46 *(120)*	6 *(120)*	<1 *(120)*	<1 *(120)*	1*S*,3*S*	–	flocs
Phycomyces blakesleeanus	19 *(120)*	7 *(120)*	<1 *(120)*	1 *(120)*	1*R*,3*R*	–	pellets (4–5 mm)
Rhizopus oryzae	33 *(115)*	4 *(115)*	1 *(115)*	1 *(115)*	1*S*,3*S*	–	flocs
Syncephalastrum racemosum	50 *(55)*	13 *(55)*	11 *(126)*	2 *(126)*	1*S*,3*S*	1*R*	pellets (2 mm)

* shake flask cultures, optimized pellet size *(11). Modified medium E2 was inoculated with a spore suspension made from an agar slant containing a culture grown for two weeks (10^8 spores per litre medium).*

** culture cultivated in a stirred tank reactor *(10). The inoculum was prepared by adding a spore suspension (8 mL) from an agar slant containing a culture grown for four days to a 1-litre shake flask containing Czapek-Dox medium (250 mL). After 24 h of growth at 30 °C, 1 litre was used as inoculum for the fermentor (11 litre medium E).*

were *M. plumbeus*, yielding an e.e. of 73%, *C. blakesleeana (10)* as well as *S. racemosum*, which were capable of a further stereoselective oxidation which increased the optical purity of the main product.

Adsorption of the hydrophobic substrate, as previously found with *C. blakesleeana (10)*, seems to be a common phenomenon. With all the tested strains, the broth concentration of 2-cyclopentyl-benzoxazole (**1**) decreased rapidly immediately after addition, whereas the products were formed gradually at fermentation times when the substrate concentration in the medium remained almost constant (Figs. 2–7).

In submerged culture *A. glauca*, *R. oryzae*, and *M. plumbeus* grew in flocs, *C. blakesleeana*, *S. racemosum*, as well as *P. blakesleeanus* showed pelletial growth, and *M. alpina* appeared in the form of flocs as well as pellets under the given culture conditions (Table 2).

A major advantage of pelletial growth is a significant decrease of the culture broth viscosity in comparison to a filamentous suspension. Small pellets are desired *(12,13)* in order to minimize diffusional limitations of oxygen or other nutrients into the interior of the pellets and to maximize the active surface area. In medium E2, *S. racemosum* and *C. blakesleeana (11)* formed a large number of pellets with a diameter of about 2 mm, whereas *P. blakesleeanus* formed larger pellets (4–5 mm) in lower numbers. Pellet formation and size depend on, among other factors, the fungal strain, the medium employed, the agitation speed, and the inoculum *(14)*. As shown for *C. blakesleeana* in particular, morphology can be influenced by inoculum size and medium composition as well as by cultivation in shake flasks vs. bioreactor *(11)*. A change from shake flask cultures (pellet diameter of about 2 mm) to batch cultivation in a stirred tank reactor (pellet diameter of 1–2 mm) resulted in an acceleration of hydroxylation and further oxidation, reducing the period for achieving maximum amounts of the main product from 58 to 14 h. It can be assumed that similar factors would be found to be important with the other strains tested allowing an optimization of product production rate and yield.

Conclusions

Mucorales species are generally recognized as versatile and rapidly growing fungi, thus providing high amounts of biocatalyst within a short period. All the mucorales strains tested showed the ability to hydroxylate derivatized cyclopentanecarboxylic acid (Fig. 8).

In addition to *C. blakesleeana*, which had been previously reported to be an appropriate system for the hydroxylation of derivatized cyclic carboxylic acids *(9,10)*, *M. alpina* and *S. racemosum* gave good yields. *M. plumbeus* was found to show the highest stereoselectivity of this hydroxylation. It might be possible to increase the optical purity of the (1*S*,3*S*)-enriched *trans*-**3** formed by *S. racemosum* by allowing an increased oxidation to the corresponding ketone as was already shown for *C. blakesleeana (10,11)*.

The (1*S*,3*S*)-enriched *trans*-**3** is produced by *C. blakesleeana (10)*, *M. plumbeus*, *R. oryzae*, and *S. racemosum*. On the contrary, the (1*R*,3*R*)-enantiomer is predominantly formed by *A. glauca*, *M. alpina*, and *P. blakesleeanus*. These results suggest that there is interspecies variability among fungi with respect to the regio- and stereoselectivity of cytochromes P-450, which are supposed to be involved in hydroxylation processes of non-activated carbon atoms *(2,3)*. For example, in the order mucorales, the 11α-hydroxylase of *R. nigricans* was shown to be similar to mammalian steroid hydroxylases and to involve a cytochrome P-450 system *(15)*. Although there are hints for *C. blakesleeana* and *M. alpina* that these enzymes are involved in the particular reac-

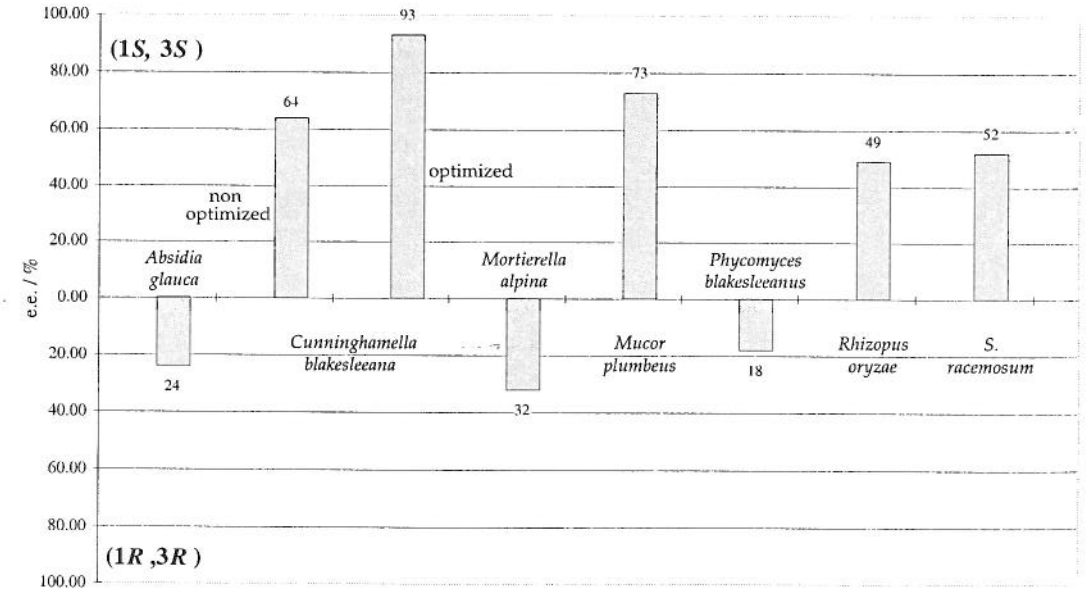

Fig. 8. Hydroxylation of **1** with various mucorales strains. Shown are the stereopreferences and the obtained enantiomeric excess (e.e.) of the main product *trans*-**3**.

tion investigated in this work *(16)*, the possibility of other mechanisms cannot be excluded. It can be assumed that these strain-specific differences in stereoselectivity could also be found for other hydroxylation reactions, consequently providing a tool for the choice of preferred product stereochemistry.

Acknowledgements

Funding within the framework of the European Community Programme »Human Capital and Mobility«, Project Biooxygenations, Contract No. ERB CHRXCT 930259, as well as by the Christian Doppler Society and Chemie Linz Ges. m.b.H. is gratefully acknowledged. Special thanks to Miss Karin Pongratz for her skilled technical support.

References

1. R. Azerad, *Bull. Soc. Chim. Fr. 132* (1995) 17.
2. H. L. Holland: *Organic Synthesis with Oxidative Enzymes*, VCH Publishers, New York (1992).
3. K. Drauz, H. Waldmann: *Oxidation Reactions. Enzyme Catalysis in Organic Synthesis*, VCH, Weinheim, Germany (1995) pp. 667–791.
4. K. Kieslich: Biotransformations. In: *Biotechnology, Vol. 6a*, H. J. Rehm, G. Reed (Eds.), Verlag Chemie, Weinheim (1984) pp. 1–473.
5. D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Meister, H. M. Leigh, *J. Am Chem. Soc. 74* (1952) 5933.
6. A. Hammoumi, J-P. Girault, R. Azerad, G. Revial, J. Dángelo, *Tetrahedron Asymm. 4* (1993) 1295.
7. J. P. Rosazza, R. V. Smith, *Adv. Appl. Microbiol. 25* (1979) 169.
8. J. B. Sutherland, P. F. Fu, S. K. Yang, L. S. von Tungeln, R. P. Casillas, S. A. Crow, C. E. Cerniglia, *Appl. Environ. Microbiol. 59* (1993) 2145.
9. A. de Raadt, H. Griengl, M. Petsch, P. Plachota, N. Schoo, H. Weber, G. Braunegg, I. Kopper, M. Kreiner, A. Zeiser, *Tetrahedron Asymm. 7* (1996) 467.
10. M. Kreiner, G. Braunegg, A. de Raadt, H. Griengl, I. Kopper, M. Petsch, P. Plachota, N. Schoo, H. Weber, A. Zeiser, *Appl. Environ. Microbiol. 62* (1996) 2603.
11. M. Kreiner: *Screening and Optimisation of Microbial Hydroxylation Systems*, PhD thesis, Technical University of Graz (1996).
12. S. Hotop, J. Möller, J. Niehoff, K. Schügerl, *Process Biochem. 28* (1993) 99.
13. B. Metz, N. W. F. Kossen, *Biotechnol. Bioeng. 19* (1977) 781.
14. A. Whitaker, P. A. Long, *Process Biochem. 11* (1973) 27.
15. W. H. Hanisch, P. Dunnill, M. D. Lilly, *Biotechnol. Bioeng. 22* (1980) 555.
16. H. Steinbrenner, A. Lehmann, M. Petsch, H. Griengl, A. Asperger, *Third International Symposium on Cytochrome P450 Biodiversity* (1995).

Razlike u regioselektivnosti i stereoselektivnosti hidroksilacije derivata ciklopentankarboksilne kiseline koristeći različite vrste Mucorales

Sažetak

Utvrđeno je da su vrste **Mucorales**, *i to* **Absidia glauca, Mortierella alpina, Mucor plumbeus, Phycomyces blakesleeanus, Rhizopus oryzae** *i* **Syncephalastrum racemosum**, *djelotvorni biokatalizatori za biotransformaciju derivata ciklopentankarboksilne kiseline u hidroksi i oksospojeve. Autori su ciklopentankarboksilnu kiselinu kemijskom transformacijom u 2-ciklopentilbenzoksazol* **1** *zaštitili od mikrobne razgradnje. Biotransformacijom je kao glavni produkt nastao 3-(benzoksazol-2-il) ciklopentan-1-ol* **3**. *Kao nusprodukti dobiveni su 2-(benzoksazol-2-il) ciklopentan-1-ol* **2** *i 3-(benzoksazol-2-il) ciklopentan-1-on* **4**.

Enantiomerski sastav i optička čistoća glavnih proizvoda utvrđeni su kako bi se usporedile međusobne specifične razlike u regioselektivnosti i stereoselektivnosti fungalnih vrsta.

Glavni proizvod **3**, *dobiven s pomoću* **M. plumbeus, R. oryzae** *i* **S. racemosum**, *bio je pretežno (*1S,3S*)-enantiomer dok je s pomoću* **A. glauca, M. alpina** *i* **P. blakesleeanus** *nastao uglavnom (*1R,3R*)-enantiomer.*

Dobiveni rezultati upućuju na to da, iako srodne gljive mogu proizvesti sličan niz spojeva, postoje razlike u regioselektivnosti i stereoselektivnosti karakteristične za pojedinu vrstu.