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Microbial hydroxylation of unsaturated, cyclic carboxylic acids protected as benzoxazoles

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Abstract

Microbial hydroxylation of 2-(cyclopent-1-enyl)benzoxazole (**1**) and 2-(cyclohex-1-enyl)benzoxazole (**2**) by *Cunninghamella blakesleeana* DSM 1906 and *Bacillus megaterium* DSM 32, respectively, gave chiral allylic alcohols 3-(benz-1,3-oxazol-2-yl)cyclopent-2-en-1-ol (**3**) and 3-(benz-1,3-oxazol-2-yl)cyclohex-2-en-1-ol (**4**) along with achiral ketones 3-(benz-1,3-oxazol-2-yl)cyclopent-2-en-1-one (**5**) and 3-(benz-1,3-oxazol-2-yl)cyclohex-2-en-1-one (**6**). Both allylic alcohols were produced in enantiomeric excesses higher than 99%. The determination of their absolute configurations (*S* in both cases) is described.

Keywords: Microbial hydroxylation; Benzoxazoles; *Cunninghamella blakesleeana*; *Bacillus megaterium*; Stereochemistry

1. Introduction

Recently, we have shown that hydroxy- and oxocycloalkane carboxylic acids can be prepared from saturated, cyclic carboxylic acids employing a simple protection/hydroxylation/deprotection sequence [1–4]. An example of this "docking/protecting group" concept [5] is shown below (Scheme 1).

Currently, our interest has focused on the conversion of unsaturated substrates prepared from simple alicyclic carboxylic acids for two reasons:

- The products expected from these hydroxylations are more valuable than their saturated analogs for asymmetric organic synthesis [6–8].
- To obtain additional information about the (regio- and stereo-) specificity of the hydroxylation of benzoxazoles by the microorganisms we use (*Bacillus megaterium* DSM 32 or *Cunninghamella blakesleeana* DSM 1906).

2. Results and discussion

2.1. Preparation of starting materials and microbial hydroxylation

The preparation of substrates, 2-(cyclopent-1-enyl)benzoxazole (**1**) and 2-(cyclohex-1-enyl)benzoxazole (**2**), was carried out according to Scheme 2.

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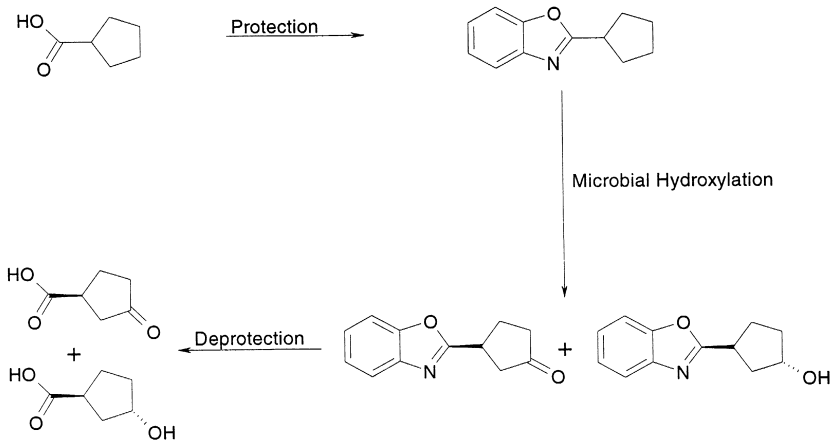
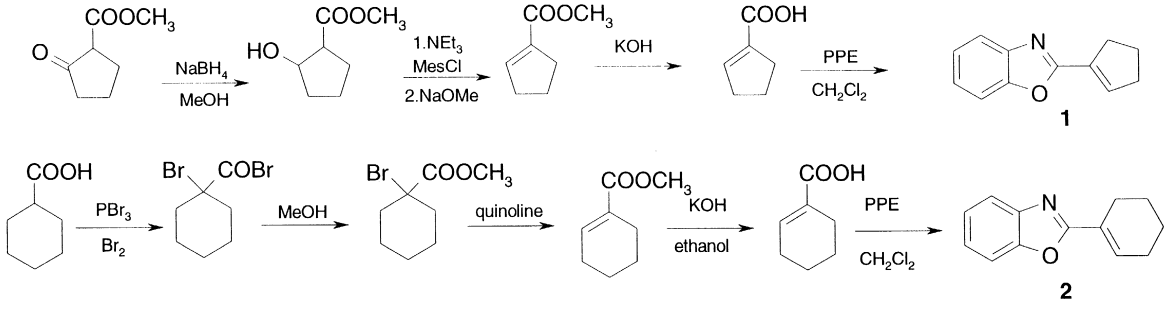


Scheme 1. Example for the hydroxylation of benzoxazoles followed by deprotection.



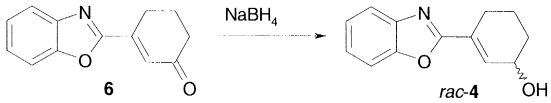
Scheme 2. Preparation of substrates (PPE = polyphosphoric acid ethyl ester).

The other possible regio-isomers (double bond in either position 2 or 3) are still under investigation and will be published elsewhere. **1** was obtained from methyl 2-oxocyclopentanecarboxylate in 50% overall yield and **2** from cyclohexanecarboxylic acid in 34% overall yield [9,10].

Conversion of **1** and **2** yielded allylic alcohols, 3-(benz-1,3-oxazol-2-yl)cyclopent-2-en-1-ol (**3**) and

Table 1
Conversion of substrates by *C. blakesleeana* DSM 1906 and *B. megaterium* DSM 32

1 with *C. blakesleeana*	**3**	Yield: 40 % Abs. Conf.: 3*S* e.e. > 99 % $[\alpha]_D^{20}$=-22.6 (c 0.3; CH_2Cl_2) mp=165-6°C	**5** Yield: 27 % mp=182-3°C
2 with *B. megaterium*	**4**	Yield: 43 % Abs. Conf.: 3*S* e.e. > 99 % $[\alpha]_D^{20}$=+21.8 (c 1.0; CH_2Cl_2) mp=114-5°C	**6** Yield: 28 % mp=102-3°C



Scheme 3. Nonselective reduction of ketone **6** to produce racemic **4** used as a reference material for chiral HPLC analysis.

3-(benz-1,3-oxazol-2-yl)cyclohex-2-en-1-ol (**4**), as main products. In both cases, further oxidation by the respective microorganism occurred to produce ketones 3-(benz-1,3-oxazol-2-yl)cyclopent-2-en-1-one (**5**) and 3-(benz-1,3-oxazol-2-yl)cyclohex-2-en-1-one (**6**). The hydroxylation reaction was more specific in these cases compared with the saturated compounds described previously. In the case of 2-cyclopentylbenzoxazole, two alcohols were formed (hydroxylation in positions 2 and 3) and for 2-cyclohexylbenzoxazole, two alcohols (hydroxylation in positions 2 and 4) were also afforded [2]. However, the unsaturated substrates **1** and **2** only gave one allylic alcohol **3** and **4**, respectively, without additional hydroxylation products. We were also pleased that the enantiomeric excesses (e.e.) of these alcohols were much higher than those found for the saturated analogs, although fermentation optimisation had not been carried out. This excellent outcome rendered any additional effort to raise product e.e. as unnecessary (Table 1).

2.2. Determination of enantiomeric excesses

The e.e. of **4** was determined by HPLC using a chiral column (CHIRALPAK AD from Daicel) with heptane/2-propanol 95:5 as eluent at 10°C. Since the e.e. measured was very high, we proved that separation of both enantiomers could be achieved by the preparation of a racemic mixture of **4** through unselective reduction with sodium borohydride from ketone **6**. The racemate showed two well-separated peaks. Injection of the product after fermentation showed only one peak as can be seen in the chromatogram (Scheme 3, Plate 1).

Unfortunately, this chiral HPLC column did not separate the enantiomers of *rac*-**3** prepared by $NaBH_4$ reduction of **5**. However, the racemic acetate prepared from **3** was separable on a GC equipped with a chiral Lipodex E column. Injection of acetylated **3** prepared from the fermentation product gave an e.e. $> 99\%$.

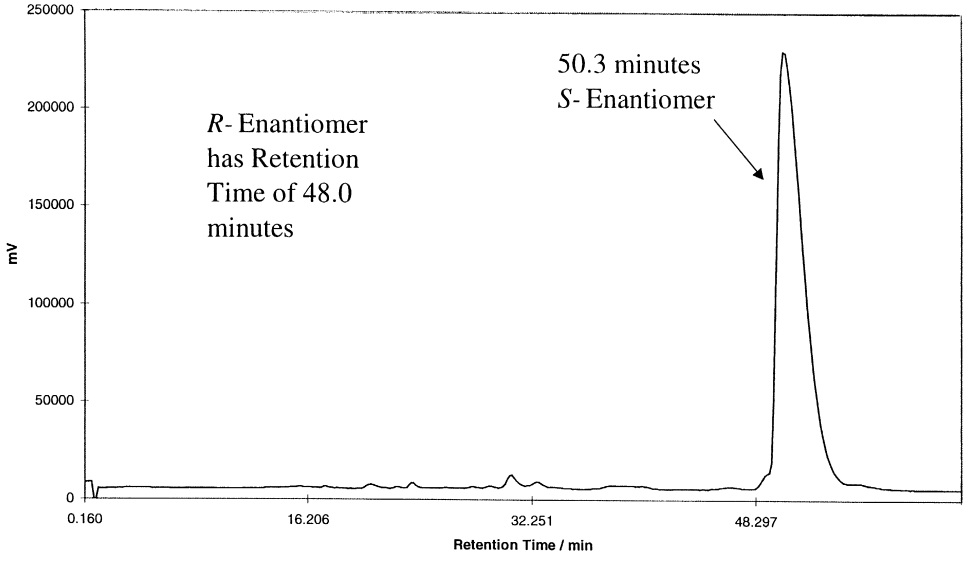
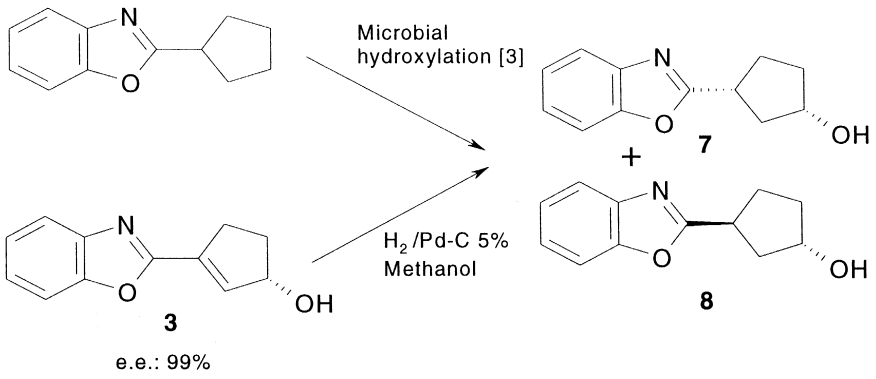


Plate 1. HPLC chromatogram of enantiomerically pure alcohol **4** injected on CHIRALPAK AD (heptane/isopropanol 95:5, 10°C).



Scheme 4. Determination of the absolute configuration of **3**.

2.3. Determination of the absolute configuration

The absolute configuration of alcohol **3** was determined by hydrogenation of **3** with Pd–Carbon 5% as catalyst to produce a mixture of the diastereomeric saturated alcohols **7** and **8** (Scheme 4).

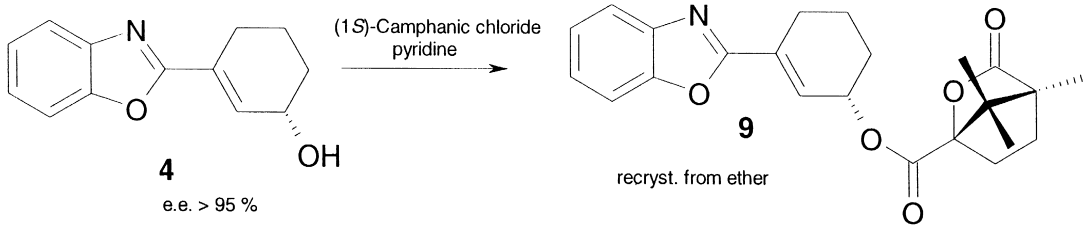
The *cis*-isomer **7** predominated by 5:1 and assignment was possible by comparison of the HPLC chromatograms of this mixture with a known sample prepared previously from reduction of 3-(2-benzoxazol-1, 3-yl)cyclopentanone [3]. This confirmed that the 3*S*-isomer had been formed, which is in accordance to the stereochemistry found for the saturated series [1,2].

For the determination of the absolute configuration of alcohol **4**, we resorted to the preparation of a derivative with 1*S*-camphanoic acid that would give crystalline samples for the investigation by X-ray analysis (Scheme 5, Fig. 1).

Inspection of the X-ray structure showed that the 3*S*-enantiomer had been produced in the fermentation.

The time dependence of formation of products from substrate **2** is shown in the following diagram. As previously reported, it becomes clear that ketone **6** is probably produced from **4** by an alcohol dehydrogenase [1–4]. However, it was found that the e.e. of **3** and **4** was very high immediately after the start of the hydroxylation suggesting a high selectivity for the hydroxylation step (Plate 2).

This can be seen by inspection of Scheme 6. The predominantly substituted hydrogen atoms are encircled. Obviously, the hydroxylation occurs *trans* to the docking benzoxazole group since only very small amounts of *cis*-isomers could be found. With the saturated substrate, however, the enzyme does not distinguish between the two hydrogens below the ring in position 3 leading to relatively low e.e. of



Scheme 5. Preparation of 1*S*-camphanoate **9** for X-ray analysis.

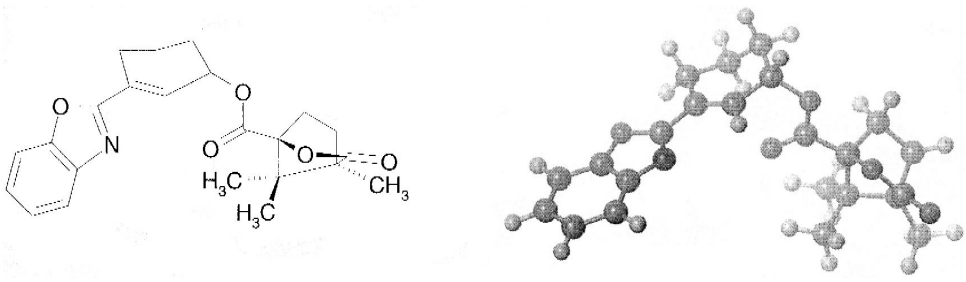


Fig. 1. X-ray structure of **9** drawn in SYBYL.

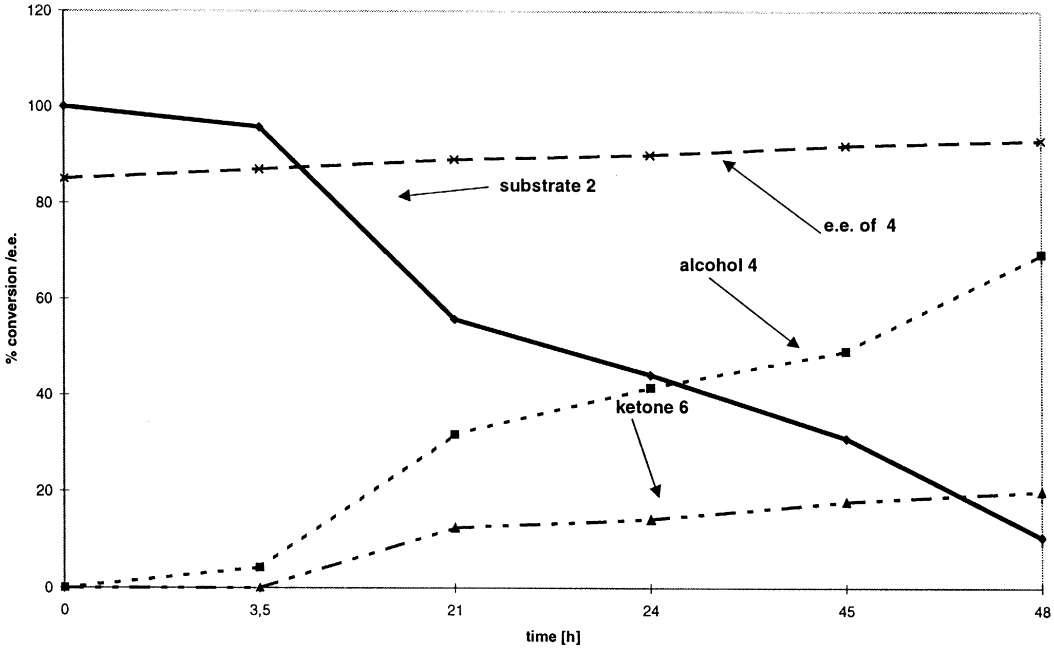
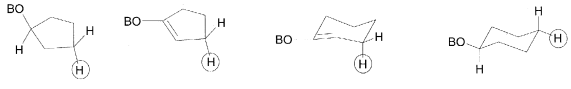


Plate 2. Time dependence of the formation of alcohol **4** and ketone **6** from **2** in a buffered medium.

30%, which can only be enhanced by selective oxidation. With the unsaturated substrates however, the *trans*-selectivity combined with the higher reactivity of the allylic position lead to a very high e.e. of the product immediately after the hydroxylation step.



Scheme 6. Comparison of substrates investigated earlier with those presented in this report. The circle indicates the hydrogen atom that is replaced by a hydroxyl group and "BO" is the benzoxazole moiety.

3. Summary

Both allylic alcohols **3** and **4** could be directly obtained from the fermentation in high optical purity. There was no need for further purification and after deprotection, the afforded acids are useful precursors for the synthesis of biologically active molecules.

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