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Microbial Hydroxylation of 2-Cycloalkylbenzoxazoles. Part III. Determination of Product Enantiomeric Excess and Cleavage of Benzoxazoles

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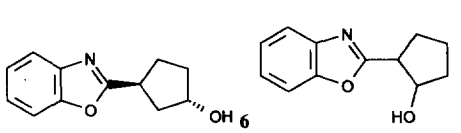
Abstract: An HPLC-system to measure the e.e. of *trans*-3-(benz-1,3-oxazol-2-yl)cyclopentan-1-ol **6**, *cis*-3-(benz-1,3-oxazol-2-yl)cyclopentan-1-ol **38**, 3-(benz-1,3-oxazol-2-yl)cyclopentan-1-one **8** and 2-(benz-1,3-oxazol-2-yl)cyclopentan-1-ol **7** simultaneously in the fermentation mixture of the substrate 2-cyclopentyl-1,3-benzoxazole **5** with *Cunninghamella blakesleeana* DSM 1906 is presented. The scope and limitations of the benzoxazole cleavage reactions is discussed.

INTRODUCTION

In the preceding publications[1,2] we reported the products of the hydroxylations of 2-substituted 1,3-benzoxazoles with *Cunninghamella blakesleeana* DSM 1906 and *Bacillus megaterium* DSM 32. In many cases we were able to obtain enantiomerically enriched chiral alcohols or ketones. Herein we want to report the determination of the enantiomeric excess of these products. An additional requirement for the application of the concept of anchor / protecting groups by the transformation of carbocyclic acids into benzoxazoles is the ease of deprotection after biohydroxylation in good yield to release synthetically useful synthons. The scope and limitations of the cleavage reactions to obtain carboxylic acids or derivatives thereof is discussed.

DETERMINATION OF ENANTIOMERIC EXCESS

The determination of e.e. of hydroxylated products was a crucial part for this work since the e.e. of several compounds varied with the time course of the biotransformation. This was especially the case for 2-cyclopentyl-1,3-benzoxazole **5**[3] which gave two alcohols **6** and **7** and a ketone **8**.



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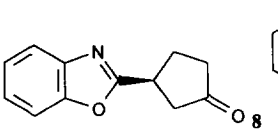

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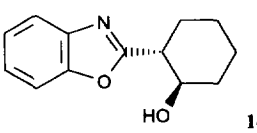

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Formation of ketone **8** during the microbial transformation is apparently enantioselective regarding **6** and *ent*-**6**. **8** is formed in varying but high e.e. (70 - 95 % depending on fermentation conditions), thereby increasing the e.e. of **6** from almost racemic in the beginning to up to 95 % under special conditions and long

fermentation times. Similar effects were also found in other biotransformations of 2-substituted benzoxazoles. Conversion of 2-cyclopentyl-1,3-benzoxazole **5**, however, was the best studied case in our laboratory.

Consequently for the availability of highly enantiomerically enriched products a simple system allowing measurement of all e.e. of interesting products in one measurement was desirable. Experiments to measure e.e. of alcohols **6** and **7** via ^{1}H-NMR with added chiral shift reagent (Eu[hfc]$_3$)[4] failed, because no separation of relevant signals was found in deuteriochloroform as solvent. Measurement was then made possible when the Mosher esters[5] of **6**, **7** and **14** were employed and ^{19}F-NMR spectra were recorded. Interestingly, the separation of the diastereomeric signals for alcohol **6** was only satisfactory when deuteriobenzene was used as solvent. These methods make isolation of the products necessary and involve also a further derivatization step. However, by applying an HPLC system with a chiral column (CHIRALPAK AD; DAICEL) the separation of the enantiomers was possible. For simultaneous measurement of all product e.e. in a fermentation mixture of 2-cyclopentyl-1,3-benzoxazole with *C. blakesleeana* an aliquot of the fermentation broth (typically 5 - 10 mL) was extracted with ethyl acetate / hexanes (1:1) and treated as described in the experimental section. Sample preparation took about 15 minutes and the resulting solution was injected directly. Reproducibility and separation is enhanced by keeping the column and the eluent at 10 °C. Two example chromatograms are shown in Figures 1 and 2. Figure 1 shows a chromatogram of a sample from the fermentation broth 1.5 h after substrate addition. The sample contained unreacted starting material, a small amount of alcohol **7** in 33 % e.e., and racemic alcohol **6**.

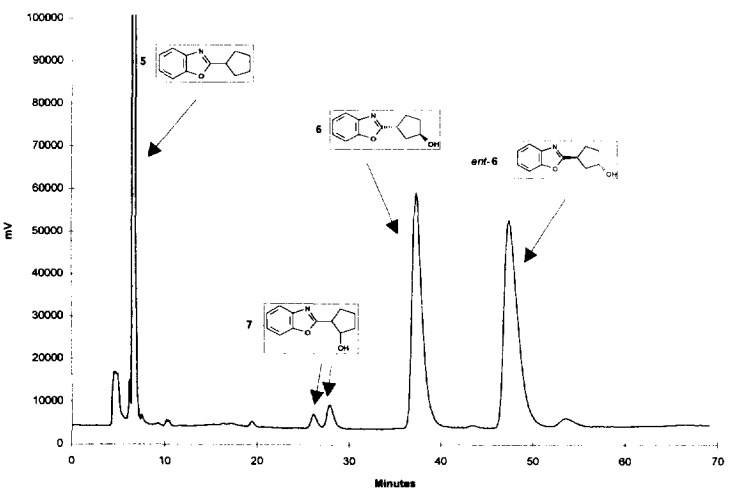


Figure 1: Conversion of 2-Cyclopentyl-1,3-benzoxazole **5** with *C. blakesleeana*; the sample was taken 1.5 h after substrate addition. Column: CHIRALPAK AD; eluent: heptane / 2-propanol 95:5; flow rate: 0.50 mL/min; detection: UV 230nm, temperature: 10°C

After 48 h (Figure 2) all products of the biotransformation were already formed. The e.e. of ketone **8** was 70 % and the e.e. of alcohol **6** 60 %. A small amount of *cis*-alcohol **38** had been formed and the e.e. of alcohol **7** was virtually unchanged. The compounds were identified by the addition of pure authentic samples.

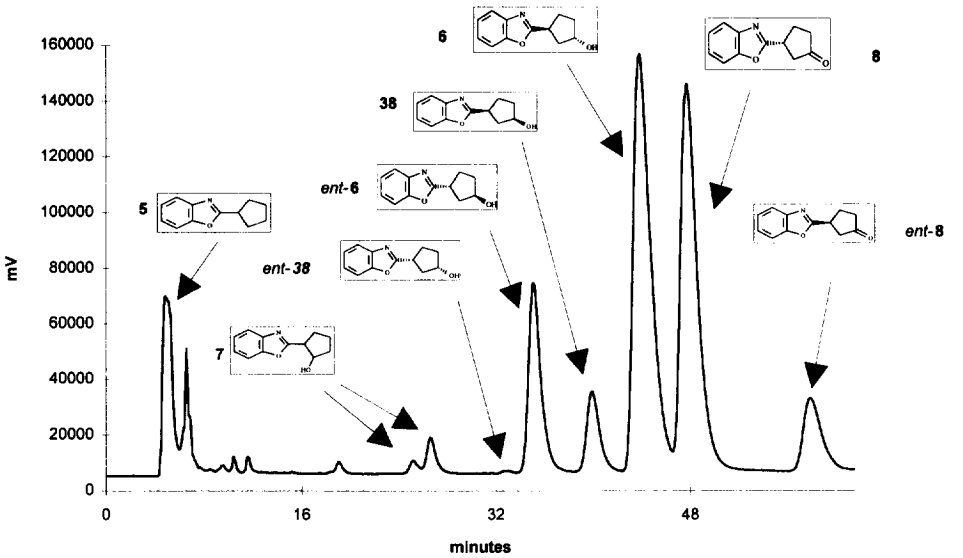


Figure 2: Conversion of 2-Cyclopentyl-1,3-benzoxazole **5** with *C. blakesleeana*; the sample was taken 48 h after substrate addition. Column: CHIRALPAK AD; eluent: heptane / 2-propanol 95:5; flow rate: 0.50 mL/min; detection: UV 230nm, temperature: 10°C

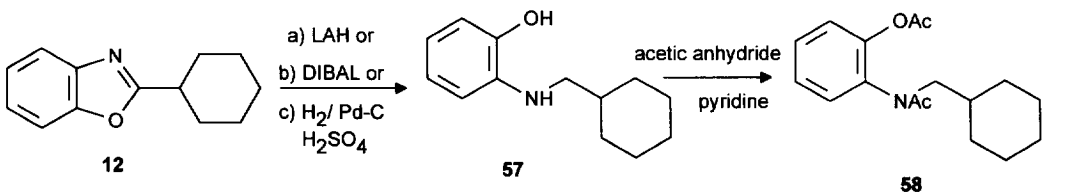
The effects of fermentation conditions (media and additives) upon the time course of the enantiomeric excesses will be described elsewhere.[6]

CLEAVAGE OF BENZOXAZOLES

One important feature of a good protecting group is its ease to be removed after having served its purpose.[7] In some cases benzoxazoles do fulfill this requirement but only partially because they are remarkably stable against a variety of reagents. These compounds normally do not react with Grignard reagents[8] (except allylic Grignard reagents)[9] and are stable against hydrogenation with many hydrogenation catalysts.

Reductive Cleavage

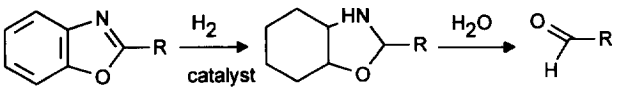
Reductive reactions normally lead to ring opening of the oxazole but the aminophenol is not eliminated during the reaction giving an amine as product.[10] This reaction can carried out by $LiAlH_4$, DIBAL, $NaBH_4$/acetic acid[10] or hydrogenation of a highly acidic solution of the benzoxazole with Pd on carbon as catalyst. As an example the reduction of 2-cyclohexyl-1,3-benzoxazole **12** to the secondary amine **57** is shown.



The amine **57** was then usually protected as diacetate to inhibit decomposition as solutions of these amines normally tend to darken upon standing at room temperature. Interestingly, the polarity of amines like **57** is

remarkably low compared with other amino alcohols leading to a high R_f-value on TLC. An intramolecular hydrogen bridge between the NH- and the OH-group on the benzene ring might be responsible for this behavior. Further evidence for this was that the polarity increased once **57** was diacetylated to **58**, where no hydrogen bridge exists.

Numerous attempts to change the outcome of the hydrogenation so that aldehydes, carboxylic acids or derivatives thereof would be obtained, were undertaken. A desirable reaction is shown below:



Hydrogenation of the aromatic system would give an intermediate oxazolidine, which presumably is hydrolytically unstable and would break apart to give rise to an aldehyde. Attempts to hydrogenate benzoxazoles with catalysts such as Rh/Al_2O_3 or PtO_2 at normal pressure gave no conversion.[11]

Alcoholytic Cleavage

Eventually we resorted to a second more valuable reaction, which was the alcoholytic cleavage of benzoxazoles with hydrochloric acid and zinc chloride.[12,13] When the published procedure[13] was used, the main product of the reaction was a hydroxy carboxylic acid, which could only be isolated in relatively low yields due to its polarity. We found that the addition of higher amounts of alcohol (methanol or ethanol) mainly produced the ester, which enhanced the yields and gave the esters in higher purities (Figure 3).

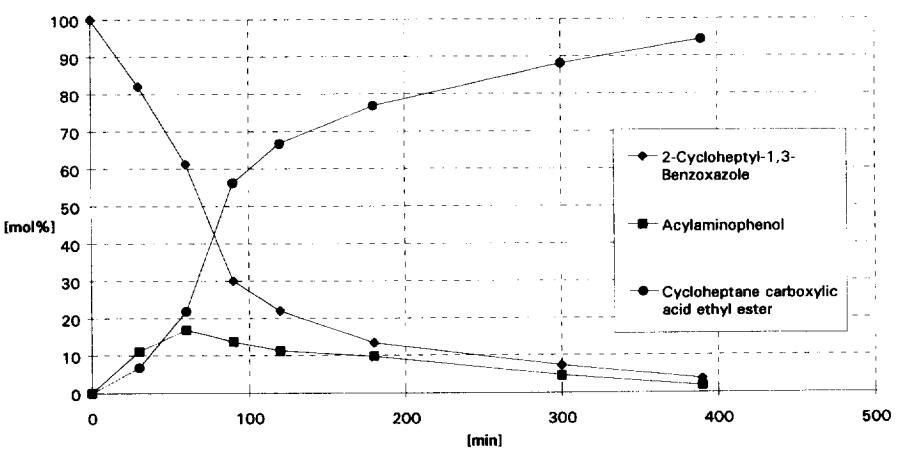
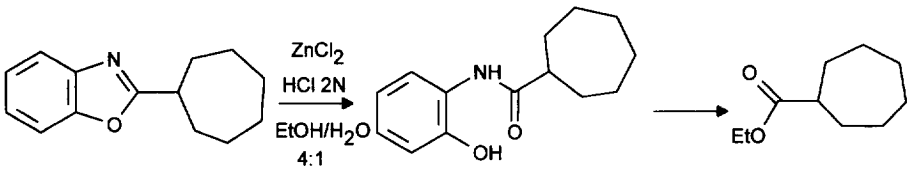


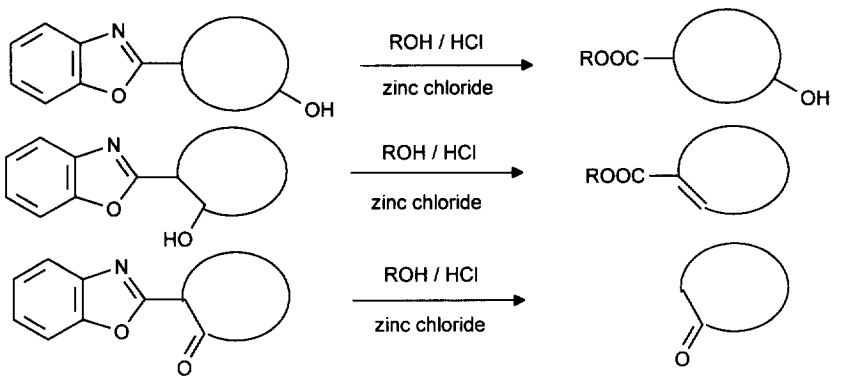
Figure 3: Time course of the alcoholytic cleavage of 2-cycloheptyl-1,3-benzoxazole **19** as followed by GC-Analysis. Conditions: 3 equ. zinc chloride, 2N HCl, ethanol/water 4:1

A rapid decrease of the starting benzoxazole **19** can be found leading to the formation of the oxazole-ring opened amide which is further hydrolyzed to the respective carboxylic acid ester.



19 acylamidophenol

This procedure worked also well for hydroxylated benzoxazoles, which were not substituted in position 2 of the cycloalkyl ring. 2-Hydroxycycloalkyl-1,3-benzoxazoles mainly underwent elimination to give an olefin, and 2-oxocycloalkyl-1,3-benzoxazoles gave the intermediate 2-oxocycloalkylcarboxylic acid, which was prone to undergo decarboxylation.[14]



EXPERIMENTAL

Analytical Methods: ^{1}H, ^{13}C *NMR*: Gemini 200 (Varian), MSL 300 (Bruker) solvent as internal standard. ^{19}F-*NMR*: MSL 300 (Bruker), CCl_3F as external standard. *HPLC:* JASCO system containing pump 880-PU, UV-detector 875-UV and AXXIOM Model 727 chromatography software; chiral column: CHIRALPAK AD from DAICEL with the eluent heptane / 2-propanol 95:5. For better separation the column was cooled to 10° C. Detection of benzoxazoles was at 230 nm. *LC*: Silica gel 60, 70-230 mesh (Merck) *GC*: Hewlett Packard HP 5890 Series II with autosampler HP 7673 and integrator HP 3396 A, quartz capillary column: 1 m x 0.32 mm, 0.52 µm HP1 and 25 m x 0.32 mm, 0.52 µm HP5, carrier gas He, FID. *TLC*: Silica gel 60 F_{254} aluminum plates (Merck), Detection: a. UV (254 nm), b. spraying reagent A (5% vanillin in concentrated H_2SO_4) or spraying reagent B (10% H_2SO_4, 10% $(NH_4)_6Mo_7O_{24}$ x 4 H_2O and 0.8% $Ce(SO_4)_2$ x 4 H_2O in water) and developing on a heat plate (600 °C).

General Method for the Preparation of Mosher Esters[5]

A solution of alcohol (1.0 equiv.), Mosher chloride (1.1 equiv.), pyridine (1.1 equiv.) and 4-dimethylaminopyridine (catalytic amount) in dichloromethane is allowed to stand at r.t. overnight. The reaction mixture is then washed with 1 N HCl, saturated $NaHCO_3$ solution and brine. Drying (Na_2SO_4) and evaporation gives the product, which is dissolved in $CDCl_3$ and measured directly.

Preparation of Samples for HPLC from Fermentation Mixtures

5 - 10 mL of fermentation mixture was centrifuged and the upper phase was extracted with 30 mL ethyl acetate / hexanes 1:1. The extract was then filtered over a small column containing 5 g of silica gel. Afterwards the filtrate was evaporated to dryness and then dissolved in heptane/2-propanol 95:5 (150 µL). A further filtration to remove traces of solid impurities gave the filtrate which was ready for HPLC injection.

2-Cyclohexylmethylaminophenol 57

Hydrogenation: A suspension of 2-cyclohexyl-1,3-benzoxazole **12** (260 mg; 1.29 mmole) and Palladium on carbon (50 mg; 5 %) in sulfuric acid (40 %; 20 mL) is hydrogenated under stirring at 60 °C at ambient pressure for two days. The catalyst is removed by filtration, the filtrate neutralized by addition of solid sodium bicarbonate and the water phase extracted with ethyl acetate. Drying (Na_2SO_4) and evaporation gives a yellow oil which is purified by flash chromatography (ethyl acetate / hexanes 1:8) to yield the amine (110 mg; 42 %).

Lithiumaluminiumhydride Reduction: To a suspension of lithium aluminium hydride (29 mg; 0.753 mmole) in THF (3mL) 2-cyclohexyl-1,3-benzoxazole **12** (100 mg; 0.502 mmole) in THF (2 mL) is added and the reaction mixture is heated to reflux for 3 h. Then the suspension is cooled with ice and ice water (1 mL) is added slowly. The precipitated solid is removed by filtration, washed with ether, dried (Na_2SO_4) and evaporated. Flash chromatography (ethyl acetate/hexanes 1:8) gives an oil (89 mg; 85 %).

DIBAL-Reduction: A solution of 2-cyclohexyl-1,3-benzoxazole **12** (106 mg; 0.53 mmole) in dry THF (5 mL) is cooled to -78 °C under argon and DIBAL-H (140 mg; 0.99 mmole; 1 mL of a 1M solution in hexane) is added dropwisely. The solution is allowed to warm to r.t. and stirred overnight. Then the reaction mixture is filtered over celite, the filtrate is dried (Na_2SO_4) and evaporated. This yields an oil (100 mg; 91 %).

^{13}C-NMR ($CDCl_3$): 23.1 (C-4); 26.3 (C-3, C-5); 31.6 (C-2, C-6); 37.9 (C-1); 51.1 (CNH); 111.7 (C-3′); 114.4 (C-6′); 117.0 (C-5′); 121.1 (C-4′); 138.1 (C-2′); 144.3 (C-1′).

2-(N-Acetyl-N-cyclohexylamino)phenylacetate 58

A solution of **57** (1 mmole), acetic anhydride (2.1 mmole), pyridine (2.1 mmole) and a catalytic amount of 4-dimethylaminopyridine in dichloromethane (25 mL) is allowed to stand at r.t. overnight. The reaction mixture is then washed with 1 N HCl, saturated $NaHCO_3$ solution and brine. Drying (Na_2SO_4) and evaporation gives a crude product, which is purified by flash chromatography.

Yield: 85 %; ^{1}H-NMR ($CDCl_3$): 0.90-1.38 (m, 11H); 1.80 (s, 3H); 2.25 (s, 3H); 3.12 (ddd, 1H); 3.83 (ddd, 1H); 7.17-7.41 (m, 4H). ^{13}C-NMR ($CDCl_3$): 20.9 (Ac); 22.5 (Ac); 26.0 (C-4); 26.6 (C-3, C-5) 31.0 (C-2, C-6) + 31.3 (rotamers); 36.7 (C-1); 54.3 (C-NH); 124.4 (C-3′); 127.0 (C-6′); 129.2 (C-5′); 130.4 (C-4′); 136.0 (C-2′); 147.4 (C-1′); 168.9 (COAc); 171.3 (COAc).

General Procedure for Alcoholytic Benzoxazole Cleavage

The benzoxazole (2 mmole) is dissolved in the alcohol (25 mL), aqueous HCl (4 N, 25 mL) and zinc chloride (5 mmole) are added. The resulting solution is refluxed for several hours. Then water (50 mL) is added and the reaction mixture is extracted with dichloromethane (5 x 30 mL). The dichloromethane phase is dried (Na_2SO_4) and evaporated. Flash chromatography yields the pure ester. Yields are given in part II[2] of this series.

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