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Biocatalytic synthesis of cyclopropanol from cyclopropyl methyl ketone using whole cells of *Rhodococcus erythropolis*

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Abstract

Whole cells of *Rhodococcus erythropolis* DSM 1069 are found to be effective catalysts for the synthesis of cyclopropanol from cyclopropyl methyl ketone. The reaction most probably comprises a Baeyer-Villiger type oxidation followed by hydrolysis of intermediate cyclopropyl acetate.

Keywords: Cyclopropanol; Cyclopropyl methyl ketone; Microbial Baeyer-Villiger oxidation; *Rhodococcus erythropolis*

1. Introduction

Cyclopropanol **3** (Scheme 1) is the smallest member of the cyclic monohydric alcohols. Its chemical and physical properties have been well documented [1,2]. Reports on its use as a synthon in organic chemistry are limited, however, probably due to its relative inaccessibility. Although, several chemical methods for the synthesis of cyclopropanol have been described [3–5], none of these have led to a commercially viable production process. Previously we showed that cyclopropanol can be obtained by a chemo-enzymatic route [6]. In the first step cyclopropyl acetate **2** was synthesized in a Baeyer-Villiger oxidation of commercially available cyclopropyl methyl ketone **1** using trifluoroperoxyacetic acid [7,8]. Subsequently, hydrolysis of **2** using esterase from porcine liver afforded cyclopropanol essentially free of contaminating alcohols. For a large-scale production of cyclopropanol this method is inconvenient due to the requirements of the first step (huge amounts of anhydrous disodium hydrogen phosphate, trifluoroperoxyacetic acid). In order to overcome these limitations, we investigated the possibilities of a fully biocatalytic procedure.

2. Experimental procedures

Baeyer-Villiger mono-oxygenase from *Acinetobacter calcoaceticus* NCIMB 9871 was kindly donated by G. Ottolina; cyclohexanone mono-oxygenase from *Xanthobacter* sp. was from Fluka, Buchs. Cyclopropyl methyl ketone (96% purity) was from Fluka. All other chemicals were of analytical grade, purchased from commercial suppliers, and used as provided. *Rhodococcus erythropolis* DSM 1069 was cultivated essentially as described [9]. Precultivation was carried out in a medium consisting of 2% (w/v) D-glucose, 1% (w/v) bactopeptone, 0.5% (w/v) NaCl, and 0.05% (w/v) yeast extract. Cells were grown under aerobic conditions at 30 °C for 48 h by shaking at 200 rpm in

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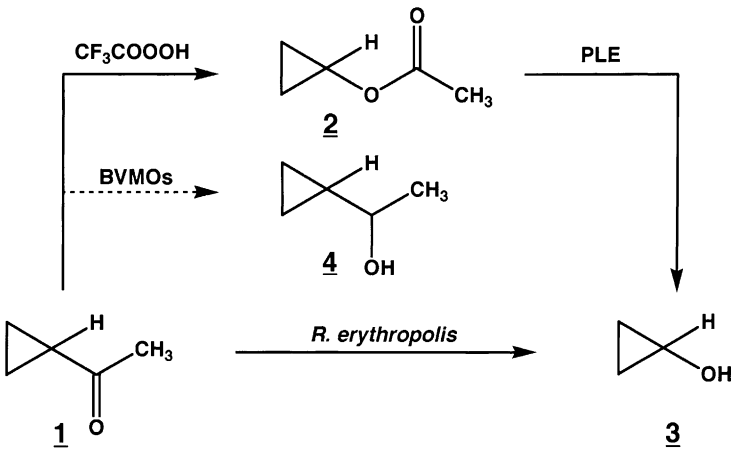

Scheme 1. Synthesis of cyclopropanol from cyclopropyl methyl ketone.

a rotary shaker incubator. Preculture (25 ml) was used to inoculate 2 l Erlenmeyer flasks containing 500 ml mineral salt medium [9], 0.05% (w/v) yeast extract, and 5% (w/v) cyclohexanol. Flasks were shaken aerobically at 30 °C and 200 rpm. Cells were harvested in the late exponential growth phase (ca. 48 h) by centrifugation at 5000 × *g* and 4 °C. To an aerated suspension of washed cells (4.4 g, wet weight) in aqueous phosphate buffer (50 mm, pH 7.5, 100 ml) cyclopropyl methyl ketone **1** was added (500 μl, 5.05 mmol). After 48 h at 25 °C 75% of the alcohol was formed as determined by GC. Extraction of the reaction mixture [6] yielded 123 mg (2.1 mmol, 42%) of pure cyclopropanol as judged by GC (HP-FFAP, 40 °C), NMR, and MS.

3. Results and Discussion

For the bioconversion of **1** to **2** (Scheme 1) we investigated the use of two Baeyer-Villiger type mono-oxygenases (BVMOs): crude enzyme from *Acinetobacter calcoaceticus* NCIMB 9871 [10], and commercially available cyclohexanone mono-oxygenase from *Xanthobacter* sp. Since both BVMOs require NADPH as a coreductant, the glucose-6-phosphate/glucose-6-phosphate dehydrogenase recycling protocol was used to regenerate NADPH during the reaction. Whereas some reduction of **1** to the corresponding alcohol **4** was observed, no detectable formation of **2** could be measured with these enzymes after 24 h of incubation. We concluded that **1** might not be a good substrate for these enzymes. This conclusion is in line with the observation that most reports of enzymatic Baeyer-Villiger reactions describe the production of (i.e. enantiomerically pure) [11] lactones from cyclic ketones, while only limited information is available on the enzymatic Baeyer-Villiger oxidation of ketones that do not carry the carbonyl function as part of an aliphatic ring system.

Bacteria belonging to the genus *Rhodococcus* are versatile in the metabolization of natural and synthetic compounds [12–14]. The capability of *Rhodococcus* sp. to catalyze Baeyer-Villiger type reactions when grown on cyclohexanone as a carbon and energy source has been described [15]. We investigated the use of cyclohexanol-induced whole cells of *Rhodococcus erythrnpolis* DSM 1069. Recycling of NADPH is expected to take place as part of the overall cellular metabolism, thus eliminating the need for an external recycling protocol. Using GC (HP-FFAP, 40 °C) we were able to monitor the levels of the ketone **1**, the ester **2**, and the alcohol **3**. During the course of the reaction the levels of the expected intermediate, cyclopropyl acetate **2**, remained below the detection limit (estimated at 2% w/v). Since an alternative route from **1** to **3** appears rather unlikely, we concluded that the intermediate ester **2** is immediately converted into the alcohol **3** by hydrolases

present in the microorganism. Indeed, upon incubation of **2**, prepared by independent synthesis [6], with freshly cultivated cells of *R. erythropolis*, rapid hydrolysis was observed under the conditions employed (results not shown). As the cells are not supplied with a nitrogen source, it appears reasonable to attribute the observed losses to the formation of compounds other than biomass. Formation of the alcohol **4**, derived from the reduction of the ketone **1**, as observed during the reaction of **1** catalyzed by BVMOs, was not detected.

We conclude that the biocatalytic one-pot conversion of cyclopropyl methyl ketone into cyclopropanol by cyclohexanol-induced whole cells of *R. erythropolis* DSM 1069 described here, constitutes a convenient and straightforward synthetic method. Yields (75% in the reaction mixture, 42% after work-up) can probably be increased by optimization of the downstream processing, i.e. by continuous extraction. The fully biocatalytic approach then offers a clear promise for the commercial production of this simplest member of the cyclic monohydric alcohols, turning it into a commodity chemical.

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