Chimia 47 (1993) 82–85

ISSN 0009–4293

Biotransformations Leading to Optically Active Synthons for the Preparation of Fine Chemicals

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1. Introduction

Stereospecificity is one of the most salient features of enzyme-catalyzed reactions. Hence, most practical applications of biotransformations aim at the production of optically active molecules. This can be achieved either by enzyme-catalyzed introduction of a new chiral center or by enzymatic resolution of a racemate. Using biotransformation, we have prepared a number of optically active molecules with low molecular weight and multiple functional groups. These molecules have been used as synthons for the preparation of optically active pharmaceuticals and other fine chemicals.

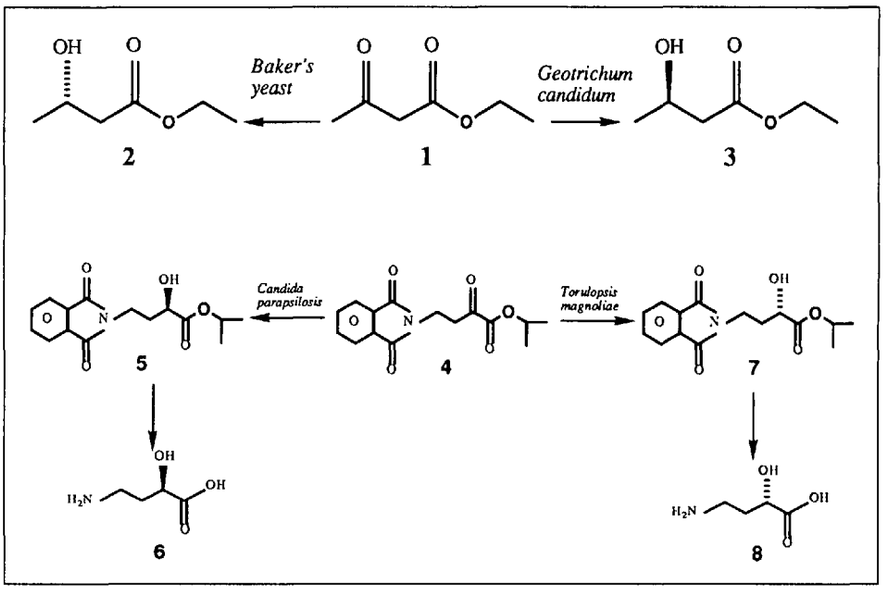
2. Optically Active Synthons Produced by Intact Microorganisms

Several chemical reactions such as the reduction of a ketone, the hydrogenation of a substituted double bond, or the addition of H_2O or NH_3 to a double bond give rise to a new center of asymmetry. If such a reaction is catalyzed by a microorganism containing the appropriate enzyme usually only one of the possible stereoisomers is formed. The use of intact microorganisms has the advantage that cofactors which might be involved in the reaction are regenerated *in vivo*. A detailed overview on the methodology of biotransformations has been given in [1]. The following optically active synthons have been prepared in our laboratories using intact microorganisms.

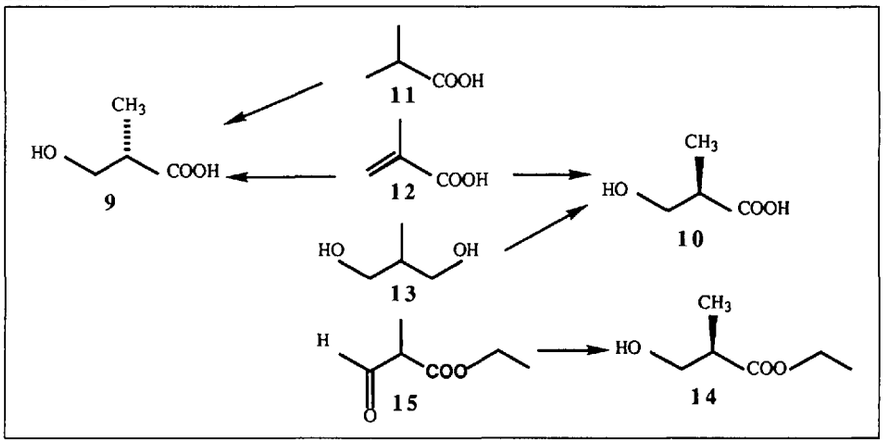
(+)-(S)-Ethyl 3-hydroxybutyrate (**2**, *Scheme 1*) is obtained by stereospecific reduction of the β-keto ester acetoacetate (**1**) with Baker's yeast [2][3]. High chemical (55–60% of purified product) and optical (95–97% ee) yields have been observed by adding the substrate **1** and sucrose continuously to an aerated suspension of Baker's yeast [2]. The (*R*)-configurated product, *(–)-(R)-ethyl 3-hydroxybutyrate* (**3**) is formed (36% yield; 90% ee), if the fungus *Geotrichum candidum* is chosen as the biocatalyst [2]. The moderate enantiomeric excess of this product can be improved almost to 100%, if the fungal mycelium is aged before the biotransformation by incubation in deionized water for 24 h at 27° [4]. The (*R*)-enantiomer **3** is also readily available by depolymerization of polyhydroxybutyrate [5]. Numerous examples for the usefulness of **2** and **3** in the synthesis of a great variety of enantiomerically pure compounds have been compiled by *Seebach et al.* [6].

Reduction of the α-keto ester isopropyl α,1,3-trioxo-2-isoindolinebutyrate (**4**) with *Candida parapsilosis* yields isopropyl (*R*)-α-hydroxy-1,3-dioxo-2-isoindolinebutyrate (**5**) which can easily be converted to *(+)-(R)-4-amino-2-hydroxybutyric acid* (**6**) by removal of the protecting groups [7] (*Scheme 1*). This product is obtained with a biotransformation yield of

Scheme 1



Scheme 2



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90% and an enantiomeric excess (ee) of 99%. It has been used as a precursor of the cerebral insufficiency improver (*R*)-hydroxy-aniracetam [7]. The (*S*)-configurated products **7** or **8**, respectively, are obtained with similar yield and ee-value, if *Torulopsis magnoliae* acts as the biocatalyst. Again, depending on the microorganism used, both enantiomers are accessible in almost optically pure form. The favorite microorganisms for this reaction have been selected in a screening program with more than 100 different microorganisms [7].

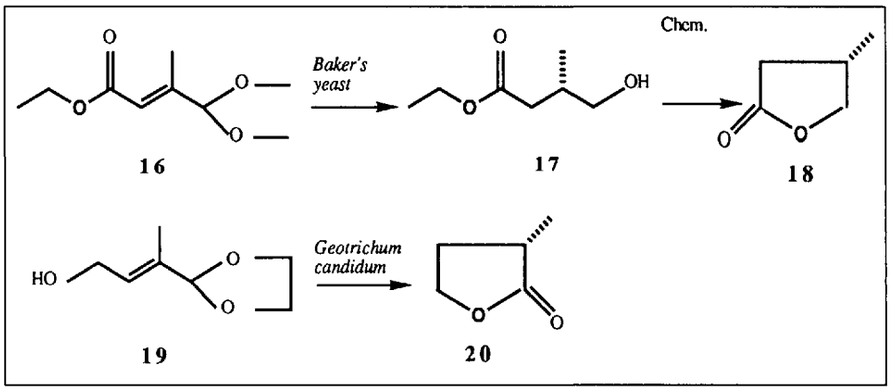
(+)-(S)-Hydroxyisobutyric acid (**9**) and *(−)-(R)-hydroxyisobutyric acid* (**10**, *Scheme 2*) are also accessible by microbial transformations. Hydroxylation of isobutyric acid (**11**) with *Pseudomonas putida* generates the (*S*)-enantiomer **9** [8]. Hydratation of methacrylic acid (**12**) is catalyzed by a couple of microorganisms and yields either enantiomer depending on the microorganism used [9]. The third approach, namely the stereoselective oxidation of prochiral 2-methylpropane-1,3-diol (**13**) is catalyzed by *Gluconobacter roseus* and generates the (*R*)-enantiomer **10** [10]. The same enantiomer is available in the form of its ethyl ester **14** by stereoselective reduction of ethyl α-formylpropionate (**15**) mediated by *Candida humicola* [11]. This last approach is of particular interest since it also works with different substituents in α-position (Et, i-Pr, Ph, or $PhCH_2$ instead of Me), if the appropriate microorganism is selected. It thus makes a whole family of optically active synthons available [11]. Optically active hydroxyisobutyric acid (**9** or **10**) has been used by various authors to synthesize a great variety of enantiomerically pure, pharmacologically active compounds such as monoensin, rifamycin S, lasalocid A, α-tocopherol, and captopril.

Stereospecific hydrogenation of substituted double bonds in the unsaturated substrates **16**, **19**, and **21** by Baker's yeast and *Geotrichum candidum* leads to the chiral products *(−)-(S)-3-methyl-γ-butyrolactone* (**18**, obtained after chemical hydrolysis and cyclization of the intermediate **17**), *(−)-(S)-2-methyl-γ-butyrolactone* (**20**, *Scheme 3*), and *(−)-(6R)-2,2,6-trimethylcyclohexane-1,4-dione* (**22**, *Scheme 4*). All three compounds are obtained with good yield and excellent optical purity [12][13].

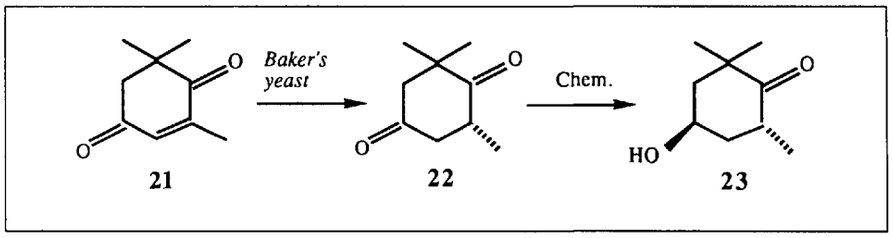
The lactones **18** and **20** served as optically active building blocks for the synthesis of the side chain of natural vitamin E ((2*R*, 4'*R*, 8'*R*)-α-tocopherol).

The optically active cyclohexane derivative **22** (*Scheme 4*) can readily be reduced at the less hindered keto group by chemical methods and yields with high excess the *trans*-diastereomer (−)-(4*R*,6*R*)-4-hydroxy-2,2,6-trimethylcyclohexanone (**23**). The latter compound is an ideal chiral precursor for the synthesis of optically active 3-hydroxycarotenoids (*e.g.* (3*R*, 3'*R*)-zeaxanthin, (3*R*)-cryptoxanthin, (3*S*, 3'*S*)-astaxanthin, *etc.*) and many naturally occurring degraded carotenoids (*e.g.* abscisic acid, xanthoxin, loliolide, dehydrovomifoliol, blumenols A and B, theaspirone, picrocrocin) [14].

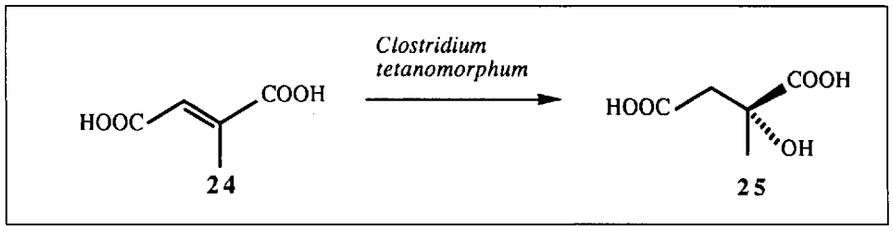
Scheme 3



Scheme 4



Scheme 5

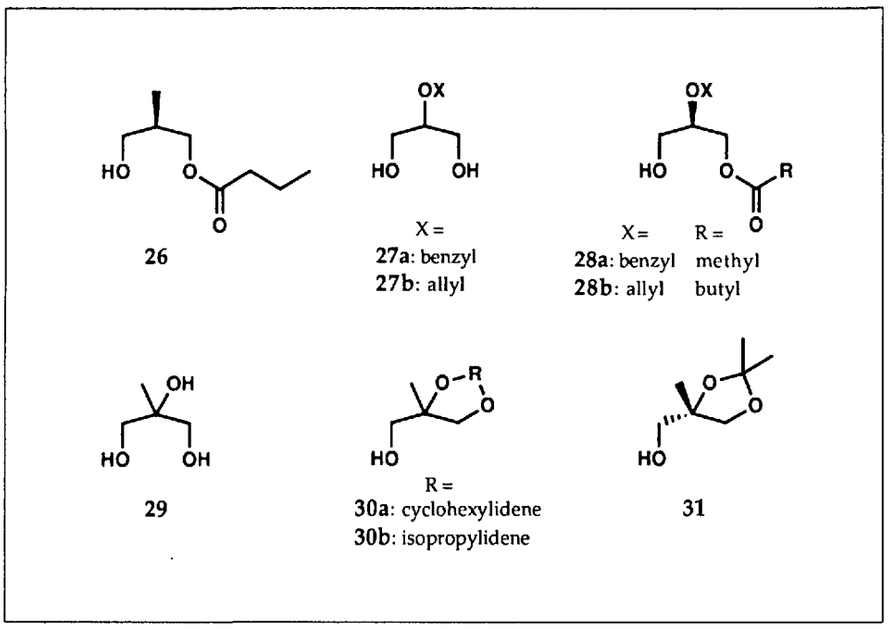


(S)-Citramalic acid (**25**, *Scheme 5*) can be prepared by asymmetric hydration of the double bond of mesaconic acid (**24**) with *Clostridium tetanomorphum*. This compound has been used to synthesize the optically active chroman moiety of natural α-tocopherol [15] and other optically active compounds such as (*R*)-linalol, the vitamin D_3 metabolite (*S*)-25,26-dihydroxycholecaliferol and the bark-beetle pheromone (1*S*,5*R*)-frontalin [16].

3. Optically Active Synthons Produced with Purified Enzymes (26–31)

For the preparation of chiral building blocks, the use of (partially) purified enzymes is also a well established tool in organic synthesis. In most cases, commercially available hydrolases, which do not need any cofactors, are used. Their ability to catalyze stereoselective hydrolyses as well as the inverse condensation reactions is applied to the introduction of chirality in prochiral compounds or the resolution of racemates, respectively. In the following part, we describe the preparation of some valuable C_3 and C_4 synthons resulting from the enzymatic asymmetrization of the closely related prochiral precursors **13**, **27**, and **29**, three propane-1,3-diols differently substituted at C(2). The method of choice was the formation of the corresponding chiral monoacylates by asymmetric monoacylation or by monohydrolysis of the respective diacylates; both *prochiral approaches* give 100% theoretical yield. The two procedures usually lead to monoacylated products of opposite configuration. In many cases, the configuration of the chiral product can be altered as well by appropriate protecting group management.

(−)-(R)-3-Hydroxy-2-methylpropyl butyrate (**26**) is a bifunctional C_4 synthon which is useful *e.g.* for the synthesis of *d*-



α-tocopherol [17]. In an enzyme screening for the asymmetric hydrolysis of diacylated **13**, lipase P (Amano) showed best results. Among several diacylates tested, the dibutyrate turned out to be superior with respect to stereoselectivity *and* hydrolysis rate [17]. Various reaction parameters were optimized: low temperatures (0°) combined with the application of salting-in salts or (polyhydric) alcohols turned out to be the most suitable systems providing **26** in 96% ee and 92% yield. The formation of the (*S*)-antipode by acetylation of diol **13** in organic solvents has been reported by *Xie et al.* [18] (>99% ee; 33% yield) and *Santaniello et al.* [19] (>98% ee; 40% yield).

Optically active glycerol synthons play an important role in the syntheses of natural and unnatural glycerides or glyco-, phospho-, and ether lipids and are also key intermediates in the synthesis of β-blockers and natural compounds [20]. The asymmetric enzymatic acylation [21] of 2-*O*-benzylglycerol (**27a**) and the hydrolysis [22] of the corresponding 1,3-diacylates has been demonstrated by several authors. We also enzymatically hydrolyzed a 2-*O*-benzyl protected diglyceride [23] using an optimized low-temperature system (0°): 1,3-di-*O*-acetyl-2-*O*-benzylglycerol in 1% concentration was treated with lipase P providing the *(R)-1-O-acetyl-2-O-benzylglycerol* (**28a**) in 95% ee at 50% conversion (ester eq.). At a higher substrate concentration (3.7%), **28a** was obtained in 93% ee and 87% yield. With respect to enantiomeric purity *and* chemical yield, this compares favorably to the results obtained by other investigators [22] in the 2-*O*-benzyl-protected series.

Holding some potential synthetical advantages, allyl protected *(R)-2-O-allyl-1-O-valerylglycerol* (**28b**) was also prepared by asymmetric hydrolysis [23]. Hydrolysis of the divalerate using lipase M-AP (Amano) showed best results: by combination of several favorable parameters (4°, 0.1 M $CaCl_2$) **28b** was produced in 95% ee (50% conversion) at 3.7% substrate concentration.

In the search for chiral glycerol building blocks, the enzymatic *racemic resolution* of, among others, glycidyl esters [24] and glycerol acetonide esters [25] have also been described.

Chiral 2-methylglycerol derivatives can be used instead of citramalic acid (**25**) in the construction of chiral quarternary C-atoms encountered, for example, in *d*-α-tocopherol [12][15][26], pheromones [16][27], or natural vitamin D_3 metabolites [28]. A prochiral approach again suggested itself for the enzymatic generation of optically active 2-methylglycerol compounds. However, unlike the 2-monosubstituted substrates **13** and **27**, no monoacylates could be obtained for 2-disubstituted substrate **29** with satisfactory stereoselection after screening several diacylates with a series of commercial hydrolases. Since discrimination between enantiomers or enantiotopic groups is frequently better, when the difference in size of the substituents at the chiral center is large [29], the tertiary OH group was benzylated. A screening with 2-*O*-benzyl-2-methyldibutyrin afforded the monobutyrate in maximum enantiomeric purity of 79% (lipase from *C. cylindraceae*), however, at the cost of the chemical yield (formation of the diol). Esterification experiments were likewise unsuccessful.

The prochiral approach was finally abandoned in favor of a kinetic *racemic resolution*. The racemic 1,2-ketals **30a** and **b** were chosen as synthetically readily accessible substrates which at the same time promised simple racemization of the unwanted enantiomer (for both alcohol and acylate). After successful resolution of spiroketal **30a**, the investigations were extended to the less sterically hindered but more economically accessible 1,2-acetonide **30b** which provided likewise positive results [30]: the enzymatic reaction proceeded with high enantioselectivity showing a maximum enantiomeric ratio [31] of $E > 150$ for the *acylation* of **30b** and $E > 1000$ for the *hydrolysis* of its butyryl ester. These are unusually high values for the resolution of *primary* alcohol compounds. *Fuganti et al.* [32] reported the enantioselective hydrolysis of the *phenylacetyl* ester of **30b** with immobilized penicillinacylase G generating **31** in 90% ee at 50% conversion ($E \approx 60$). Hydrolysis of 2-methylglycidyl butyrate with porcine pancreas lipase by *Ladner* and *Whitesides* [24] afforded the retained (*S*)-ester in *ca.* 51% ee at 60% conversion ($E \approx 3$).

Hydrolysis of butyrylated **30b** afforded *(S)-2,2,4-trimethyl-1,3-dioxolane-4-methanol* (**31**) and the retained (*S*)-butyrate in >99% ee near 50% conversion. Lipase P again turned out to be the most suitable enzyme retaining its excellent enantioselectivity and activity also at a high substrate concentration (16%, $E >$ 700). The high enantiomeric ratios ($E >$ 200) obtained with a comparatively large number (six) of commercial enzymes qualify this enzymatic resolution as extraordinary for a *primary* alcohol ester. One is inclined to associate this performance with the comparatively rigid dioxolane ring. However, the tertiary Me group also seems to play an important role, since enzyme screening with the unmethylated cyclohexanone ketal under similar conditions afforded only modest ee values. As reported by *Sonnet* and *Antonian* [25] the unmethylated acetonide was also hydrolyzed by several lipases with only moderate enantioselectivity. Hydrolysis was also carried out effectively in a *continuous* manner using purified lipase P covalently immobilized on Eupergit C [30]. A column reactor was successfully run for half a year and was only abandoned for want of substrate. Butyrylated **30b** was converted at a concentration of 7%. Neither deterioration of the enantioselectivity nor enzyme bleeding was observed in the course of this long-term experiment. *Racemization* of alcohol **31** as well as of its acylate was achieved very simply by incubating them in acetone in the presence of TsOH and, in case of the acylate, of racemic alcohol **30b**.

Enantioselective *esterification* of racemic alcohol **30b** in anhydrous organic

solvents was also successful providing, as expected, the (*R*)-configurated reaction products [30]: 5 g of **30b** dissolved in 50 ml of hexane was acetylated with vinyl acetate (0.55 mol-eqiv.) within 2 h (45% conversion) using 0.24 g of lipase P adsorbed on porous glass beads ($E > 150$). Repeated batchwise use of the catalyst (seven runs) did not reveal any noticeable inactivation.

All the favorable features mentioned suggest technical potential for the present enzymatic procedure.

The enzyme transformations outlined above demonstrate that minor changes in substrate structure may entail consequences ranging from having a new optimum enzyme or acyl moiety to the necessity of a completely new synthetic route for the target compound.

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Chimia 47 (1993) 85–92

ISSN 0009-4293

Some Recent Studies into the Use of Enzymes in Organic Synthesis

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Fifteen years ago only a small number of academic groups and a handful of industrial Companies were genuinely interested in the employment of enzymes in organic synthesis. Nowadays the situation is different. Most organic chemists acknowledge that enzyme-catalysed reactions (biotransformations) may be of potential utility in their work. Thus, it is widely appreciated that enzymes may allow chiral synthetic intermediates (synthons) to be prepared in optically active form. It is also understood that enzymes can catalyse reactions that are difficult or, at the present time, impossible to emulate using other techniques of organic chemistry.

There are several factors which have helped to strengthen the impact of enzyme-catalysed reactions in organic chemistry. First, a wide-range of enzymes are available from commercial suppliers. Secondly, there is a much better understanding of the chemo-, regio-, and stereoselectivity of the reactions catalysed by various enzymes and several textbooks are available [1] to help newcomers to become conversant with these data. In addition a compendium of validated procedures, with

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