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

Hans Georg Leuenberger* and Beat Wirz

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 H₂O or NH₃ 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.

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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]. Hydration 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 methyl 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 PhCH₂) 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.

Sterespecific 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 intermediates 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 ((2R, 4R, 8R, 10R)-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 (+)-(4R, 6R)-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. (3R, 3’R)-zeaxanthin, (3R)-cryptoxanthin, (3S, 3’S)-astaxanthin, etc.) and many naturally occurring degraded carotenoids (e.g. abscisic acid, xanthoxin, loliolide, dehydrodromifoliol, blumenol A and B, theaspirene, picrocrocin) [14].

(S)-Citranal 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 D₃ metabolite (S)-25,26-dihydroxycholcaliferol and the bark-beetle pheromone (15,5R)-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 hydrolysies 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 C₃ and C₄ synthons resulting from the enzymatic asymmetricization of the closely related prochiral precursors 13, 27, and 29, three propane-1,3-diol differently substituted at C(2). The method of choice was the formation of the corresponding chiral monoaclates by asymmetric monoaclylation or by monoaclylolysis of the respective diaclytates: both prochiral approaches give 100% theoretical yield. The two procedures usually lead to monoaclylated 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 C₃ synthon which is useful e.g. for the synthesis of d-
The formation of the (S)-antipode by other investigators [22] in the 2-
resolved L-3-di-0-acetyl-2-0-benzylglycerol in 1%
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 investiga-
tions were extended to the less sterically hindered but
more economically accessible 1,2-aceto-
nide 30b which provided likewise posi-
tive 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 butyl
eresolution of primary alcohol com-
ponents. Fuggatti et al. [32] reported the
enantioselective hydrolysis of the pheno-
acetylester of 30b with immobilized pen-
cillinacilase G generating 31 in 90% ee at
50% conversion (E = 60). Hydrolysis of 2-
me thyl glyc erid e butyrate with porcine pan-
crise lipase by Ladner and Whittenhase
[24] afforded the retained (S)-ester in 51% ee at 60% conversion (E = 3).

Hydrolysis of butyrylated 30b afforded
(S)-2,2,4-trimethyl-1,3-dioxolane-4-
methanol (31) and the retained (S)-bu-
tyrate 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 qual-
ify this enzymatic resolution as extraor-
dinary 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 cyclhex-
anone ketal under similar conditions af-
fected only modest ee values. As reported
by Somer and Antonian [25] the unmeth-
ylated acetone was also hydrolyzed by
several lipases with only moderate enanti-
oselectivity. Hydrolysis was also carried
out effectively in a continuous manner
using purified lipase P covalently immo-
ibilized on Eupergit C [30]. A column
reactor was successfully run for half a year
and was only abandoned for want of sub-
strate. Butyrylated 30b was converted at
a concentration of 7%. Neither deteriora-
tion 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 ra-
cemic alcohol 30b in anhydrous organic

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solvents was also successful providing, as expected, the (R)-configured reaction products [30]: 5 g of 30b dissolved in 50 ml of hexane was acetylated with vinyl acetate (0.55 mol-equiv.) 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.

Some Recent Studies into the Use of Enzymes in Organic Synthesis

Stanley M. Roberts*, Nicholas J. Turner, and Andrew J. Willetts

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 references, is offered.


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