

Account

Asymmetric Syntheses Aided by Biocatalysts

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This article summarizes the achievements of the authors' group in the area of biocatalyst-catalyzed organic reactions in recent 10 years. A strain of *Geotrichum* sp. obtained by screening is capable of stereoselectively reducing a number of carbonyl compounds. In many cases, the stereochemistry is complementary with that obtained by baker's yeast. Therefore, this microorganism provides a useful pathway to the preparation of alcohol compounds with specific configurations. On the other hand, a number of plant sources have been screened for oxynitrilases and the hydrocyanation reactions of various arylcarboxaldehydes have been investigated. A "micro-aqueous reaction system" was invented, by which a series of novel optically active cyanohydrins were prepared. On this basis, a high through-put continuous reaction system has been designed. This paper also describes examples of the syntheses of bio-active compounds by using the optically active compounds obtained from the above-mentioned catalytic reactions as precursors.

Keywords asymmetric synthesis, biocatalyst, *Geotrichum* sp., stereoselective reduction, oxynitrilase, enantioselective hydrocyanation

Introduction

Enzyme-catalyzed reactions have emerged during the past 15 years as a valuable addition to the armoury of the synthetic organic chemists.¹ Many synthetic sequences now incorporate a biocatalytic step to provide an optically active building block and the application of biotransformation to asymmetric synthesis shows an increasing tendency. Compared to the traditional chemical transformation, enzymatic method possesses some advantages such as high efficiency, high regio- and stereo-selectivity, mild and ecologically compatible reaction conditions. However, many enzymes are delicate and expensive, their utilization is often difficult for synthetic organic chemists. In contrast, chemical reactions run with whole cell preparation or homogenate of biological materials sometimes give comparable even better results in terms of yield and optical purity of the products with the advantages of relatively simple manipulation, easy availability and low cost. In our endeavors in synthesizing molecules of biological interest, biotransformation has be-

come a powerful tool to provide chiral synthons, which otherwise are difficult to obtain by conventional chemical methods. In this account, we summarize our recent work on biotransformation using intact microorganism cells and by homogenate of plant materials.

Carbonyl reduction mediated by *Geotrichum* sp.

Baker's yeast (*Saccharomyces cerevisiae*) is well known for its ability to convert various classes of compounds, especially carbonyl-containing substrates into the corresponding optically active alcohols usually with (*S*)-configuration. The reduction of carbonyl group takes place to offer the products with the stereogenic outcome following the Prelog rule.² For the synthesis of various kinds of natural products and physiologically active compounds, it is desirable to find that a microorganism which is capable of performing reduction to provide products with the opposite enantioselectivity, a complement to baker's yeast. For this purpose, we carried out a number of screenings on soil samples and have found a strain of fungus, *Geotrichum* sp., which shows the desired properties.

Reduction of aromatic α -diketones

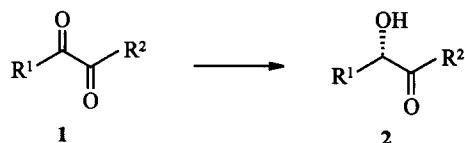
Optically active α -hydroxycarbonyl compounds are important starting materials for the synthesis of complex optically active natural products and are useful stereodirecting groups.³ Routes to these compounds include resolution,⁴ enzymatic reduction⁵ and oxidation of pro-chiral enolates using chiral oxaziridine derivatives.⁶ We have found that *Geotrichum* sp. is effective for converting α -diketones to (*S*)- α -ketols in good yields and enantiomeric excesses as shown in Scheme 1. The features of this reaction are its strict chemoselectivity as in the case of **1a** and only one carbonyl group is reduced. However, (1*R*,2*S*)-diols were obtained when the reaction was conducted with baker's yeast.⁷

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Scheme 1



1a: $R^1 = \text{Me}$, $R^2 = \text{Ph}$, yield 88%, *ee* 86%. **1b–1d:** $R^1 = p\text{-MePh}$, $p\text{-MeOPh}$, $3,4\text{-(MeO)}_2\text{Ph}$; $R^2 = p\text{-MePh}$, $p\text{-MeOPh}$, $3,4\text{-(MeO)}_2\text{Ph}$, yield 73%–88%, *ee* 65%–93%.

Reduction of 1,3-diketones

1,3-Diketones (**3**) can be reduced by *Geotrichum* sp. regioselectively at 3-keto group to give the corresponding alcohols (**4**) with (*R*)-configuration in varying yields and good enantiomeric excesses⁸ (Scheme 2). As a comparison, baker's yeast results in the products with (*S*) stereochemistry from the same starting compounds. These results are very useful because **4** can be afterwards converted into *syn*- or *anti*-1,3-diols, the subunits of many natural products, by conventional methods.

Scheme 2

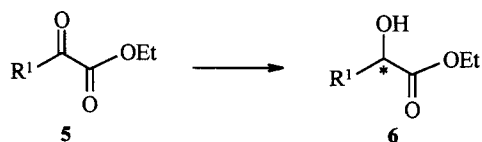


$R = \text{Me}$, Et , $n\text{-Pr}$, $n\text{-Bu}$, Ph ; yield 21%–77%; *ee* 92%–99%.

Reduction of α -oxo esters

Optically active α -hydroxy esters are important intermediates for synthesis of optically active substances having biological interest, such as pheromones⁹ and antibiotics.^{10,11} By using *Geotrichum* sp. at room temperature, α -oxo esters (**5**) can be reduced effectively to the corresponding α -hydroxy esters (**6**) in moderate to excellent optical yields⁸ (Scheme 3). The configuration of the products is determined as (*S*) when $R^1 = \text{alkyl}$ group whereas it is (*R*) when $R^1 = \text{aryl}$ group.

Scheme 3



$R^1 = \text{Me}$, Et , $i\text{-Pr}$, $n\text{-Bu}$, $i\text{-Bu}$, $n\text{-Pen}$, PhCH_2CH_2 ; yield 40%–48%; *ee* (*S*) 83%–98.5%; $R^1 = \text{Ph}$, 1-naphthyl, $p\text{-HOPh}$, $p\text{-AcOPh}$; yield 10%–95%; *ee* (*R*) 39%–100%.

It is interesting to note that phenolic hydroxy group (**5**, $R^1 = p\text{-HOPh}$) is harmful to the microorganism, re-

sulting in slow conversion and low chemical and optical yields (**6**, yield 10% and *ee* 39%). After protecting the hydroxy group by acetyl group (**5**, $R^1 = p\text{-AcOPh}$), the reaction gave satisfactory results (**6**, yield 93% and *ee* 99.5%).

Reduction of β -oxo esters

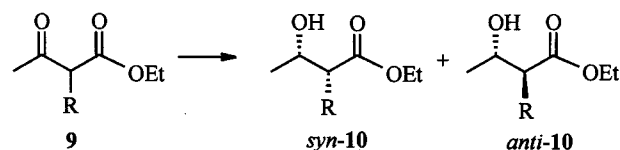
Reduction of β -oxo esters by using baker's yeast is probably one of the most extensively studied topics.² This type of reduction can also be accomplished by using *Geotrichum* sp. resulting in the alcohols with the opposite stereochemistry. For example, reduction of **7** with *Geotrichum* sp. yielded (*R*)-**8** (Scheme 4), whereas baker's yeast gave the antipode (*S*)-form.

Scheme 4



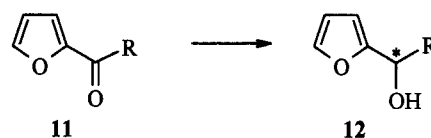
Different from using the pure enzyme, reduction of racemic α -substituted β -keto esters with baker's yeast furnished a mixture of *syn* and *anti* aldol products. *Geotrichum* showed similar enantio- and diastereo-selectivity. For example, reduction of ethyl α -substituted acetoacetate (**9**) with resting cells of *Geotrichum* provided a mixture of *syn* and *anti* **10** (*anti*/*syn* 3:1) in most cases as shown in Scheme 5. If the incubation was performed under anaerobic conditions (nitrogen), the *syn* isomers became the major products. The results are illustrated in Table 1.

Scheme 5

Reduction of α -acylfurans

Fermentation of α -acylfurans with *Geotrichum* sp. produced the corresponding furyl carbinols⁸ (Scheme 6). The latter compounds can be conventionally converted into

Scheme 6

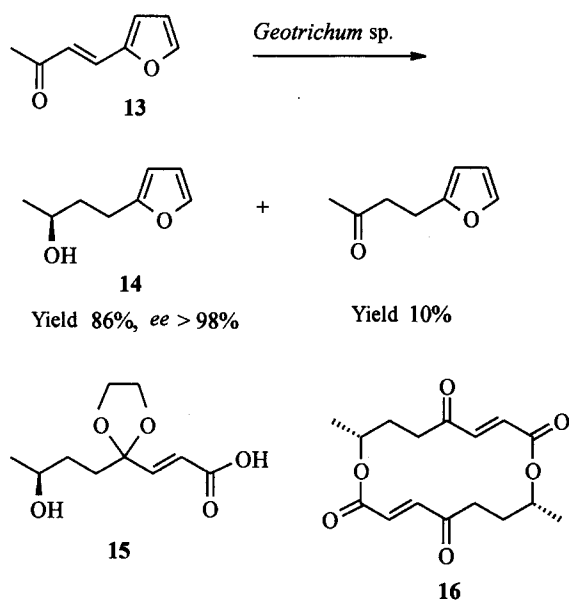


$R = \text{Me}$, Et , Pr , Bu , Pen , Ph ; yield 22%–60%; *ee* 46%–100%.

Table 1 Reduction of α -substituted β -oxo esters by *Geotrichum* sp.

| R | Aerobic condition | | | Anaerobic condition | | |
|-------|-------------------|-----------|--------------------------|---------------------|-----------|--------------------------|
| | Time (h) | Yield (%) | <i>anti</i> / <i>syn</i> | Time (h) | Yield (%) | <i>anti</i> / <i>syn</i> |
| Me | 48 | 78 | 75:25 | 72 | 80 | 40:60 |
| Et | 72 | 88 | 68:32 | 72 | 89 | 32:68 |
| Bu | 48 | 84 | 61:39 | 60 | 87 | 37:63 |
| Allyl | 72 | 91 | 80:20 | 84 | 83 | 25:75 |
| Bn | 60 | 98 | 98:2 | 72 | 99 | 20:80 |

hydropyranones by known methods, which are useful as starting materials for the synthesis of a variety of biologically important molecules. An interesting example is the conversion of α, β -unsaturated ketone (**13**) by *Geotrichum* sp. (Scheme 7). The major product **14** is a precursor of 7-(*S*)-hydroxy-4,4-ethylenedioxy-2-octenoic acid (**15**), which in turn is a building block for the synthesis of (-)-pyrenophorin (**16**), a naturally occurring antifungal macrodiolide.

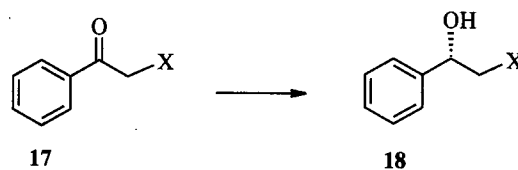
Scheme 7*anti*-Prelog reduction of aryl α -halomethyl or α -hydroxymethyl ketones

Asymmetric reduction of α -halomethyl or α -hydroxymethyl ketones is a useful process to obtain halohydrins or 1,2-diols, which are valuable synthetic intermediates for the preparation of a variety of compounds with biological interest. While chemical reduction by using chiral metal hydrides and organoboranes constitutes a powerful tool, among the most acceptable methods, biotransformations are undoubtedly considered as preferable accesses due in most cases to the high enantio- and chemo-selectivities, milder and environmentally compatible reaction conditions. Baker's yeast¹² and other microorganism or enzymes¹³ were used to reduce some α -haloaryl ketones. However, most of these reductions generally follow the Prelog rule¹⁴ in sense

of the stereochemical outcome. *Geotrichum* sp. **38** has been found to stereoselectively reduce aryl α -halomethyl or α -hydroxymethyl ketones with *anti*-Prelog features.¹⁵

Reduction of α -haloacetophenones

Geotrichum sp. gave the products exclusively having (*S*)-configuration with yields of 80%, 86% and 15%, and *ee* of 75%, 87%, and 98% for F, Cl and Br substitution, respectively (Scheme 8). For comparison, products obtained from baker's yeast possess (*R*)-configuration with yields of 55%, 6% and 0% and *ee* of 35%, 68% and 0%, respectively. Taking into account that the halomethyl moiety is "effectively smaller" than the phenyl moiety,^{14a} we can conclude that *Geotrichum* mediated reduction of α -haloacetophenones affords the *anti*-Prelog halohydrin products.

Scheme 8*Reduction of substituted aryl α -chloromethyl ketones*

A number of para-substituted phenyl α -chloromethyl ketones (**19**) has been treated with *Geotrichum* sp. to give the corresponding (*S*)- α -chloromethyl benzyl alcohol **20** (Scheme 9).

Scheme 9

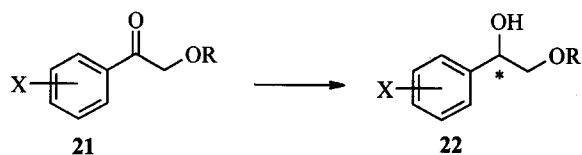
Ar = *p*-HO-C₆H₄, *p*-AcO-C₆H₄, *p*-MeO-C₆H₄, *p*-ClC₆H₄, *p*-MeSO₂-NH-C₆H₄, *p*-O₂N-C₆H₄; yield 15%—90%, *ee* 87%—> 98%.

6-Methoxycarbonylpyridin-2-yl chloromethyl ketone also gave good yield (91%) and *ee* (81%). Surprisingly, (*R*)-benzylic alcohol was obtained from *o*-chlorophenyl α -chloromethyl ketone with low yield (30%) and *ee* (7%).¹⁵

Reduction of α -hydroxy and α -acetoxy ketones

Optically active 1,2-diols are versatile and important synthetic intermediate in organic synthesis. The necessity of preparing such target molecules in an optically pure form has triggered much researcher's interest, leading to the emergence of various synthetic methods based on either conventional chemistry or enzyme-catalyzed reactions. Among these methods, the most important one is the asymmetric dihydroxylation of olefines.¹⁶ Another powerful route to optically active 1,2-diols is the asymmetric reduction of the α -hydroxy ketones.¹⁷ Although a variety of 1,2-diols have been prepared by microbial catalyzed reduction of α -hydroxy ketones,¹⁷ the microorganisms employed are solely baker's yeast. So far, due to the stereopreference of baker's yeast-mediated reduction expressed as the Prelog rule, the low availability of the other configuration enantiomers still remains as an unsolved problem. We found, in addition to the α -halo ketones,¹⁵ the reduction of α -hydroxy and α -acetoxy ketones mediated by *Geotrichum* sp. also gave products with *anti*-Prelog stereochemistry¹⁸ (Scheme 10).

Scheme 10



R = H or Ac; X = H, *o*-Cl, *m*-Cl, *p*-Cl, *m*-NO₂, *p*-NO₂, *p*-MeSO₂NH.

The transformation of 2-acetoxy-1-arylethanones (**21**, R = OAc) gave the diol monoacetates as minor products with yields of 3%–25% and *ee* of 41%–90% in (*S*)-configuration, but the corresponding 1,2-diols as major products with yield of about 60% and *ee* of 55%–92% in (*S*)-configuration. This indicates that, besides oxidoreductase(s), hydrolysase(s) also presents in this microorganism. When 2-hydroxy-1-arylethanones were transformed with *Geotrichum* sp., the corresponding (*S*)-diols were produced in 62%–100% yields and 60%–100% *ee*. It is interesting to point out that *Geotrichum* sp.-mediated transformation of α -hydroxy ketones and α -acetoxy ketones with an *ortho*-substituent on the phenyl ring generally leads to the products of opposite stereochemistry [(*R*)-configuration]. For example, 2-acetoxy-1-*o*-chlorophenyl-ethanone and 2-hydroxy-1-*o*-chlorophenyl-ethanone gave the reduction products of (*R*)-configuration (Scheme 11).

This surprising phenomenon could be explained by that there may be more than one oxidoreductases in the intact cells, which produce alcohols of opposite configuration at different rates.

Scheme 11



Reduction of sulfur-containing ketones

Sulfides, sulfoxides and sulfones are functionalities which possess great synthetic utility as their possible transformation to a variety of other functional groups. There has been great interest in the bio-reduction of ketones bearing an α -sulfur containing functionality, as this could provide useful synthetic intermediates in asymmetric synthesis of natural products, medicines and other useful materials.¹⁹ However, the bio-reduction of these ketones is critically dependent on both the substituents on the sulfur-containing group and the substituents on the carbonyl group. Most of the bio-reductions afford one isomer of the enantiomeric alcohols following the Prelog's rule.¹⁴ The other isomer is often prepared by tedious chemical transformation. In our studies on the bio-reduction using *Geotrichum* sp., the β -carbonyl phenyl sulfoxides (**23**, **24**) gave the corresponding alcohols (**25**, **26**, **27**, **28**) with good chemical and optical yields, which exhibited *anti*-Prelog's rule stereochemistry, whereas the β -carbonyl sulfides and sulfones produced the corresponding alcohols with low optical purities²⁰ (Scheme 12). After being treated with LAH, **27** and **28** can be converted into (*R*)-alcohol (**29**) (Table 2).

Scheme 12

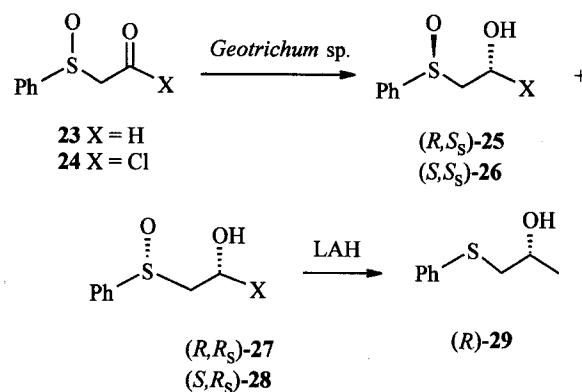


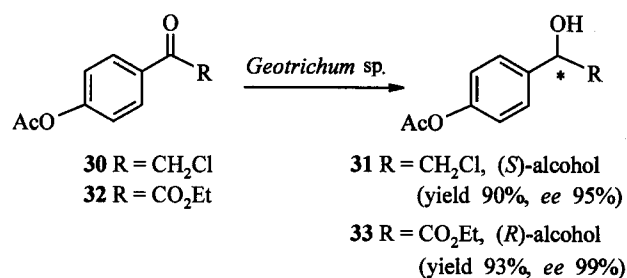
Table 2 Reduction of β -carbonyl phenyl sulfoxides

| X | Time (h) | Yield (%) | <i>ee</i> (%) | Configuration |
|----|----------|----------------------------------|---------------|----------------------------------|
| H | 7 | 76 | 21 :63 | <i>R</i> , <i>S</i> _S |
| | | (21 : 22 = 27:73) | 22 :16 | <i>R</i> , <i>R</i> _S |
| Cl | 20 | 66 | 23 :95 | <i>S</i> , <i>S</i> _S |
| | | (23 : 24 = 81:19) | 24 :88 | <i>S</i> , <i>R</i> _S |

(R)- and *(S)*-Denopamine

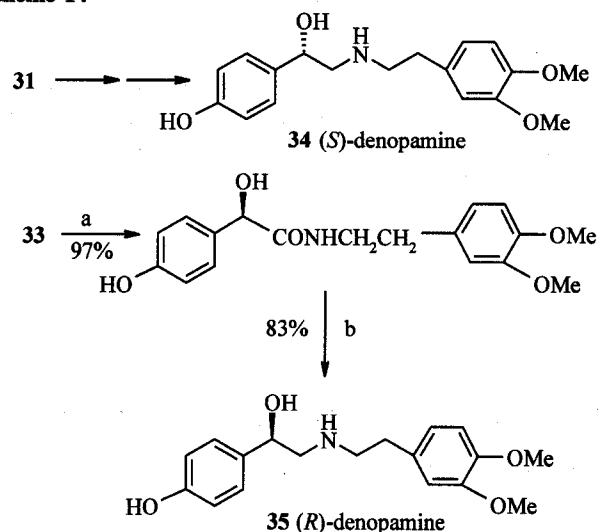
Denopamine, a new selective β_2 -agonist, is important for the treatment of congestive heart failure without promotion of increased myocardial oxygen consumption or heart rate.²¹ But in general, such kind of β -adrenoreceptor active drugs are sold as racemates despite a clear preference for the use of enantiomerically pure drugs, such as *(R)*-(-)-denopamine, which is the active form. One enantioselective synthesis of denopamine has been achieved by using the CBS enantioselective catalytic reduction process.²² By using *Geotrichum* sp., we were able to perform the asymmetric synthesis of denopamine in both *(R)*- and *(S)*-forms.^{8,23} Ketones **30** and **32** were incubated with *Geotrichum* sp. to produce the corresponding alcohols **31** and **33** with *(S)* and *(R)*-configurations, respectively (Scheme 13). Protection of the phenolic hydroxy group is necessary for obtaining high chemical and optical yields. In the case of unprotected ketones, poor *ees* (30% for R = CH₂Cl, 39% for R = CO₂Et) were observed.

Scheme 13



Coupling of **31** with 3,4-dimethoxyphenylethylamine afforded *(S)*-denopamine (**34**) and coupling of **33** with 3,4-dimethoxyphenylethylamine then reduced by diborane gave the *(R)*-enantiomer (**35**) (Scheme 14).

Scheme 14



- (a) 3,4-Dimethoxyphenylethylamine, α -hydroxypyridine, xylene, reflux 5 h;
(b) B₂H₆, THF, reflux 2 h.

Synthesis of *(R)*- and *(S)*-fluoxetine

The serotonin-uptake inhibitor fluoxetine is the most exciting new therapeutic agent. Recent study shows that fluoxetine is not only active as an antidepressant but also used for treatment of anxiety, alcoholism, chronic pain and eating disorder such as obesity and bulimia.²⁴ The enantiomers of fluoxetine have been found to exhibit different activities and metabolism rates.²⁵ Chemical enantiomeric routes to fluoxetine have been described by Sharpless²⁶ using selective reduction of 2,3-epoxycinnamoyl alcohol with Red-Al, and by Corey²⁷ comprising the CBS enantiomeric catalytic reduction process, respectively. With the aid of microorganisms, we have accomplished the synthesis of both enantiomers of *(R)*- and *(S)*-fluoxetine in good yields with high optical purities.^{8,28}

Ethyl benzoyl acetate (**36**) was fermented with baker's yeast and *Geotrichum* sp. to give *(S)*- and *(R)*-alcohol, respectively, which provided the target compounds through 4 steps transformations as shown in Scheme 15.

Synthesis of sitophilate

Sitophilate (**40**), the aggregation pheromone of a grainary weevil *Sitophilus granarius*, has been synthesized from diketone **39** by us using biocatalytic reduction protocol as shown in Scheme 16.⁸

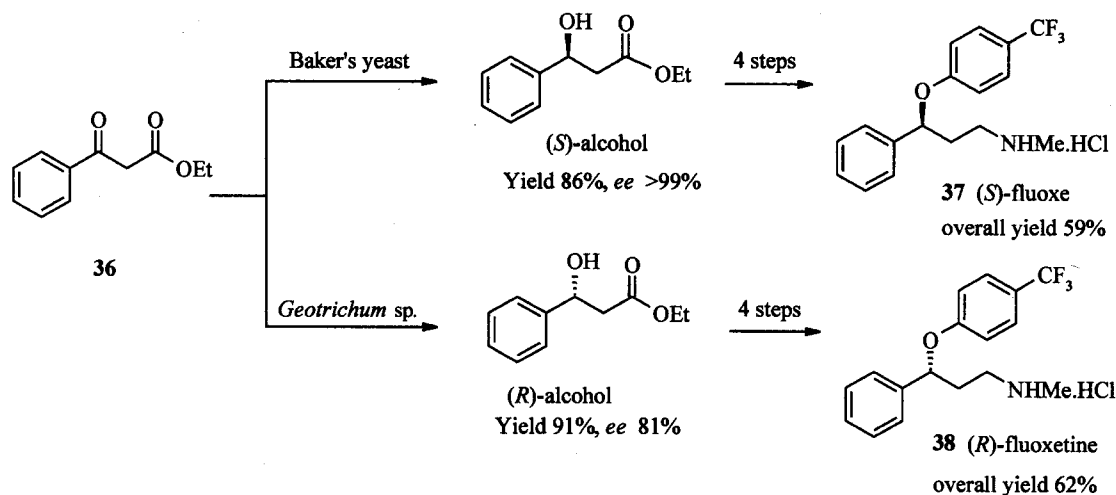
Synthesis of Ro 25-8210 and Ro 25-6630

Ro 25-8210 (**41**) and Ro 25-6630 (**42**) have been used to prepare inhibitors of the matrix metalloproteinase stromelysin-1.²⁹ Only one of the enantiomers was synthesized by Hull³⁰ via an enantioselective oxazaborolidine-catalyzed borane reduction of bromomethyl ketone **43**. However, compounds containing a pyridine ring are poor substrates for the conventional asymmetric reduction, as the *ee*'s of the products are usually not high. Therefore, it is valuable to develop new methods of asymmetric synthesis of **41** and **42**. By using *Geotrichum* sp. and baker's yeast, we have been able to accomplish the chemoenzymatic synthesis of these two compounds starting from pyridinyl α -chloromethyl ketone (**44**), as shown in Scheme 17.³¹

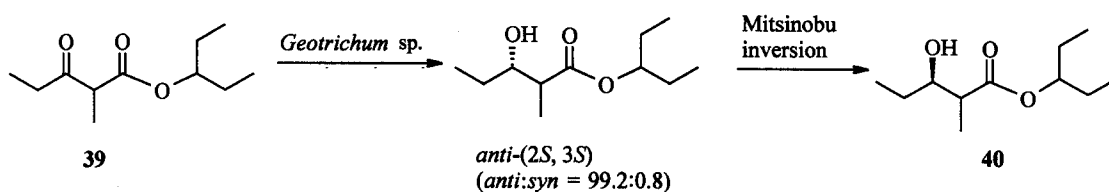
Synthesis of (7*aR*)-5,6-dihydro-7*a*-methyl-1*H*-indene-2,7(4*H*,7*aH*)-dione

Enantiomerically pure bicyclic enones **46**–**49** (Scheme 18) may constitute useful building blocks for the establishment of synthetic strategies in the field of steroids, terpenoids and related molecules. The enantiomerically enriched forms of Wieland-Miescher ketone (**46**) and Hajos-Parrish ketone (**47**) could be efficiently prepared by asymmetric cyclization of their prochiral precursors by the use of proline-enamine³² or catalytic antibody.³³ However, ring

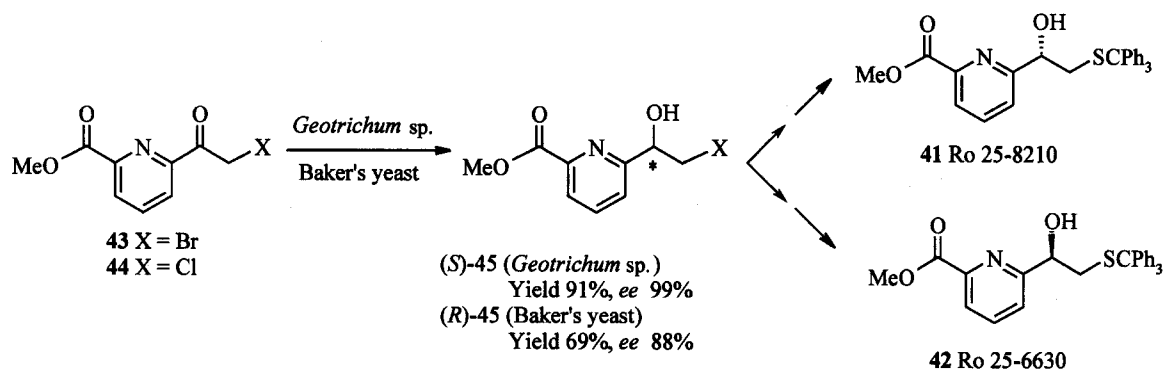
Scheme 15



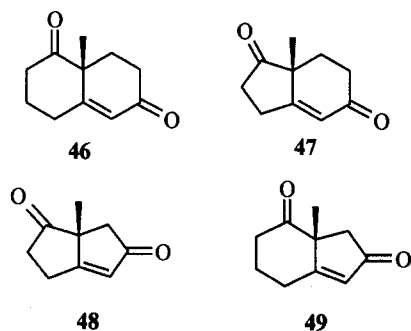
Scheme 16



Scheme 17



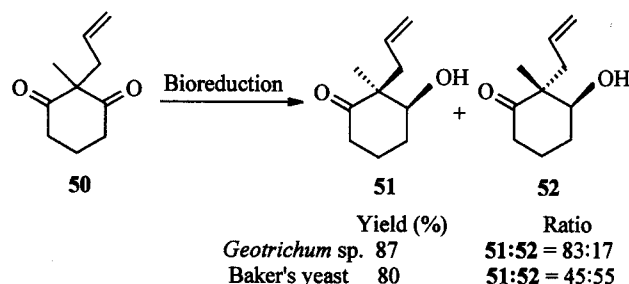
Scheme 18



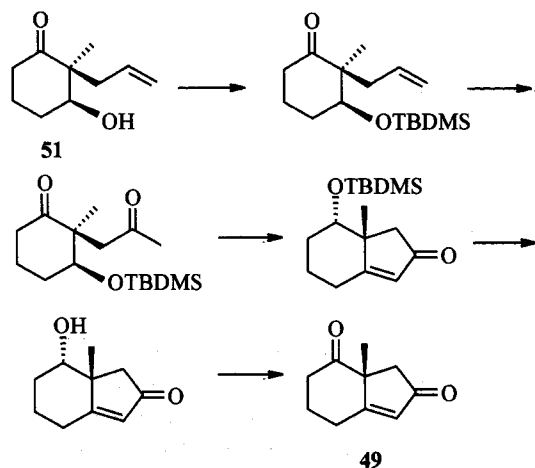
construction of the fused cyclopentenones **48** and **49** using the standard aldol condition is quite difficult.³⁴ Though optically active **48** has been asymmetrically synthesized by Trost³⁵ and Brooks,³⁶ the enantioselective synthesis of **49**

had not been reported until we discovered the chemoenzymatic transformation by using *Geotrichum* sp. starting from prochiral diketone **50** (Scheme 19).³⁷ Followed by 3 steps chemical transformation, compound **51** was converted to **49** (Scheme 20).

Scheme 19



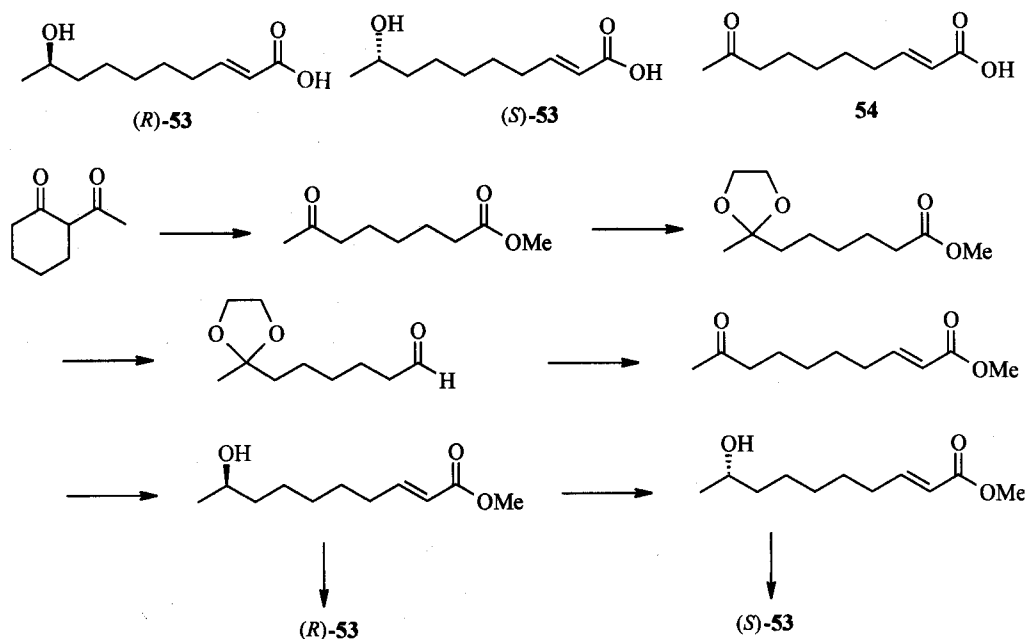
Scheme 20



Synthesis of (*R*)- and (*S*)-9-hydroxy-(*E*)-2-decenoic acid (9-HDA)

9-HDA is a mandibular pheromone secreted by the queen honeybee. Biological tests of both the enantiomers show that the (*R*)-isomer is 10 times more effective in swarm settling.³⁸ Although a number of syntheses of **53** involving the 9-keto intermediate (**54**) have been reported,³⁹ the lack of an effective chiral reduction reagent has resulted in racemic products. Only one enantiomeric synthesis of 9-HDA has been reported where the desired stereogenic center of unsaturated acid **53** was achieved by using organocuprate-catalyzed ring opening of homochiral methyl oxirane.⁴⁰ By using *Geotrichum* sp., we have succeeded the stereoselective synthesis of the two enantiomers of 9-HDA⁴¹ as shown in Scheme 21.

Scheme 21



Asymmetric hydrocyanation mediated by almond meal (*Prunus amygdalus*)

Cyanohydrins are useful intermediate in synthesis because they bear one chiral carbon atom with two functional groups, which can be readily converted into other functional groups. Asymmetric hydrocyanation has been accomplished by either chemical processes or enzymatic ones.³⁸ Hydronitrile lyases (HNLs) (or oxynitrilases) are widely spread in plants as well as in some kinds of microorganisms and insects. They catalyze the cleavage of cyanohydrins in a reversible way. Therefore, the enzyme can be used to catalyze the asymmetric formation of cyanohydrins by the addition of HCN to aldehydes or ketones. Several HNLs have been isolated from the enzyme sources and some of the recombinant enzymes are also available. Among all HNLs, the one from almond, which exhibits (*R*)-enantioselectivity, has been extensively studied and exploited.⁴²

Due to the competitive racemization reaction, the enzyme-catalyzed hydrocyanation in aqueous media often gives unsatisfactory optical yield. To overcome this problem, efforts have been made using the immobilization technique or carrying out the reaction in aqueous-organic biphasic systems at lower temperature (such as 4 °C).

"Micro-aqueous condition" for asymmetric hydrocyanation

The oxynitrilase-catalyzed reactions were carried out by using the crude enzyme preparation, isolated (or recombinant) or immobilized enzymes in aqueous or aqueous/organic biphasic media.^{42a,43} Application of these procedures in a large scale is hampered by limitations such as

enzyme activity and thermo-stability, through-put and the cost. Immobilization of enzyme usually results in appreciable loss of enzyme activity and stability during the immobilization.

A unique "micro-aqueous condition" process has been developed by us for asymmetric hydrocyanation of aliphatic aldehydes and ketones, aromatic aldehydes and heteroaromatic aldehydes.⁴³ The corresponding cyanohydrins were obtained in high yields and enantiomeric excess values comparable to, in some cases even better than, those obtained using the purified or recombinant enzymes. This process involves (1) preparation of meal of a material containing the enzyme such as almond kernels by soaking the material in water, pulverizing the swollen material in a homogenizer and then defatting the obtained meal and (2) carrying out the reaction in an organic solvent such as isopropyl ether, ethyl acetate. This system keeps the enzyme remaining in its *in vivo* nature and behaves like an immobilized enzyme. In addition, this method is inexpensive and convenient to synthetic organic chemists and has been successfully used by our group in the synthesis of a variety of cyanohydrins.

Synthesis of fluorinated mandelonitriles

Optically active fluorine-containing cyanohydrins are important starting materials for the synthesis of fluorine-containing molecules of special biological interest. Surprisingly, syntheses of fluorine-containing cyanohydrins have not been reported until recently⁴⁴ (Scheme 22). As shown in Table 3, monofluorobenzaldehydes produce the corresponding monofluoromandelonitriles with good chemical and optical yields, while the results of difluorobenzaldehydes vary with the substitution pattern. Trifluoro- and pentafluoro-benzaldehydes, however, gave racemic products.

Scheme 22

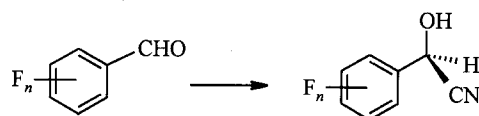


Table 3 Conversion of fluoro-benzaldehydes to fluoro-mandelonitriles

| Benzaldehyde | Cynaohydrin | Yield (%) | ee (%) | Configuration |
|------------------|------------------|-----------|--------|---------------|
| 4-Fluoro- | 4-Fluoro- | 90 | 94 | R |
| 2-Fluoro- | 2-Fluoro- | 96 | 84 | R |
| 3,4-Difluoro- | 3,4-Difluoro- | 71 | 84 | R |
| 2,3-Difluoro- | 2,3-Difluoro- | 92 | 46 | R |
| 2,6-Difluoro- | 2,6-Difluoro- | 70 | 41 | R |
| 3,4,5-Trifluoro- | 3,4,5-Trifluoro- | 37 | 0 | — |
| Pentafluoro- | Pentafluoro- | 90 | 0 | — |

Synthesis of heteroaryl cyanohydrins

Scheme 23



R = substituted furanyl, thienyl, pyrrolyl, indolyl, pyridinyl, imidazolyl, thiazolyl

Heteroaryl cyanohydrins and their derivatives are important skeletons in molecules of biological activity.⁴⁵ Asymmetric synthesis of heteroaryl cyanohydrins has been a topic of interest. Regarding the bio-catalyzed synthesis, however, only few successful examples have been reported. In addition to the known heteroaryl carboxaldehydes, we examined a number of new substrates. Among them, some gave promising results with yields and *ee* of synthetic significance. Pyrrolyl carboxaldehydes gave no product when treated with the pure oxynitrilases.^{42a} However, after the hydrogen atom of the amino group in the pyrrolyl ring was substituted with methyl group or methoxymethyl group, the hydrocyanation of pyrrolyl carboxaldehydes gave the corresponding cyanohydrins with moderate yields and *ee*. Bulky protection groups such as Ts and Boc were found to be of little help to the reaction.⁴⁶ Some examples are illustrated in Scheme 24.

Efforts in searching for other HNL sources

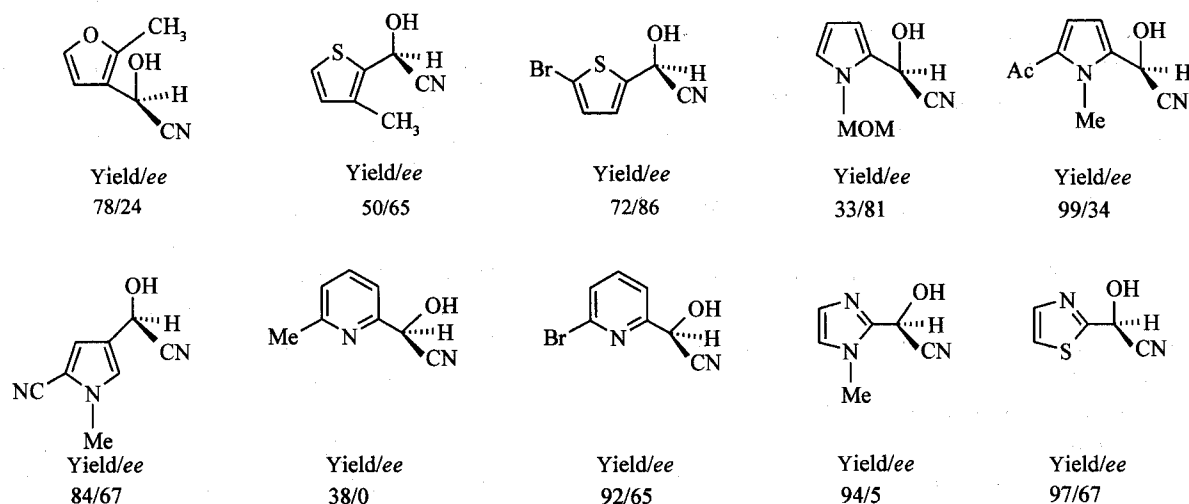
Searching for new sources of oxynitrilases is a continuous task in our group. For synthetic purpose, it is important to find new sources of oxynitrilases, which can catalyze various kinds of substrates (especially artificial substrates) giving high conversion and optical outcome with either (*R*)- or (*S*)-preference and can work well under our micro-aqueous condition.

According to the records in the traditional Chinese medicines, we screened several plant materials (peach, loquat, *Nandina domestica* Thumb, ginkgo and *Ilcx chinensis* Sims.) for their oxynitrilase activity and found that peach exhibited the enzyme activity similar to that of almond under the micro-aqueous condition, whereas loquat exhibited low enzyme activity.⁴⁷

Hydrocyanation by continuous process

The unswollen property of almond meal in the micro-aqueous media allows us to build up a column reactor for continuous process of hydrocyanation to produce optically pure cyanohydrins from aryl carboxaldehydes and heteroaryl carboxaldehydes in a high throughput manner.⁴⁸ Both chemical and optical yields are dependent upon the nature of the substrate, flow rate, reaction temperature,

Scheme 24



etc. Generally, higher flow rate results in higher *ee* values but lower chemical yields, and *vice versa*. Optimization can be made by tuning these factors to achieve a balance. For example, mandelonitrile can be obtained from benzaldehyde in 95% yield and >99% *ee* with a throughput of 3630 g/(L·d) and a ratio of substrate to catalyst (S/C) of 66.7 (mol/g) under the conditions of flow rate at 3 mL/min and *T* at 15 °C; furanyl 2-cyanohydrin from furanyl carboxaldehyde in 100% yield and >99% *ee* with a throughput of 1176 g/(L·d) and S/C of 40 under the conditions of flow rate at 1 mL/min and *T* at 15 °C. The catalyst remains its activity for many reaction recycles, for example, more than 200 recycles in the case of benzaldehyde. No purification is needed for the resultant cyanohydrins. This process is of the advantages of high efficiency, easy manipulation and low cost.

Summary

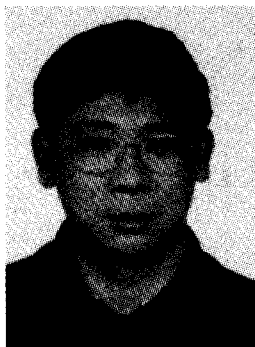
In this account, we summarized our study on biotransformations using *Geotrichum* sp. and almond meal preparation. Syntheses of some biologically interesting molecules starting from the bio-transformed intermediates are also presented.

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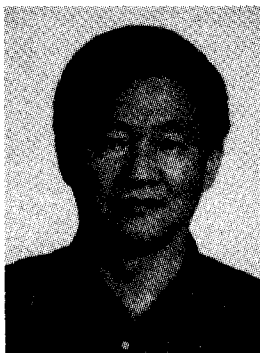
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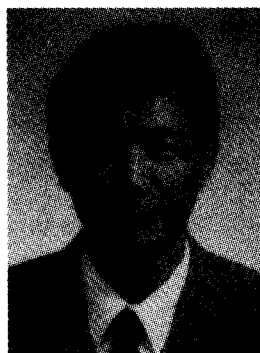
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