

Baker's Yeast Mediated Transformations in Organic Chemistry

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Contents

I. Introduction and General Remarks	50
II. Reductions	50
A. General Remarks	50
B. Reduction of Monocarbonyl Compounds	51
1. Reduction of Cycloalkanones	51
2. Reduction of Bi- and Polycyclic Cycloalkanones	51
3. Reduction of Aliphatic Alkanones	53
4. Reduction of Sulfur-Containing Molecules	54
5. α -Heterocyclic Substituted Ketones	57
6. Nitrocarbonyl Compounds and Masked Amino Ketones	58
C. Reduction of Dicarbonyl Compounds	58
1. Cyclic Diketones	58
2. Acyclic Diketones	62
D. Reduction of α -Keto Esters	64
E. Reduction of α,γ -Diketo Ester and Keto α,γ -Diester	65
F. Reduction of β -Keto Esters	65
1. β -Keto Esters with the Keto Group Being Part of the Ring	65
2. Aliphatic Keto Esters	67
G. Reduction of γ - and δ -Keto Acids and Esters	71
III. C-C Bond-Forming and -Breaking Reactions	72
A. α,β -Unsaturated Systems	72
1. Acyloin-Type Condensations and Reductions of α,β -Unsaturated Compounds	72
2. Decarboxylations	80
B. Miscellaneous C-C Bond-Forming Reactions	80
IV. Reduction of Organometallic Compounds	81
V. Reduction of Fluorine-Containing Compounds	81
A. Ketones	81
B. Keto Esters	83
VI. Oxidations	84
VII. Hydrolyses of Esters	84
A. General Remarks	84
B. Esters of Amino Acids	85
C. Other α -Substituted Carboxylic Esters	86
D. Acyloxy Esters and Lactones	86
E. Alkynol Acetates	88
F. Miscellaneous Hydrolyses	88
VIII. Immobilized Baker's Yeast	89
A. General Remarks	89
B. Examples for the Use of Immobilized Baker's Yeast	89
IX. Miscellaneous Reactions	91
X. References	92

I. Introduction and General Remarks

Microbial transformations, and yeast-mediated transformations in particular, have been widely used since the early days of mankind for the production of bread, dairy products, and alcoholic beverages. All of these early applications used mixed cultures of microorganisms, and all of these biotechnological operations have primarily been directed in the areas of agriculture and human nutrition. It was the merit of Pasteur in 1862¹ to lay a scientific foundation of one of these early applications, namely the oxidation of alcohol to acetic acid by using a pure culture of *Bacterium xylinum*.² Investigations of the oxidation of glucose to gluconic acid³ by *Acetobacter aceti* and of sorbitol to sorbose by *Acetobacter sp.*⁴ followed. The reducing action of fermenting yeast, *Saccharomyces cerevisiae*, was first observed by Dumas in 1874.⁵ He reported that, on addition of finely powdered sulfur to a suspension of fresh yeast in a sugar solution, hydrogen sulfide was liberated. The reduction of furfural to furfuryl alcohol under the anaerobic conditions of fermentation by means of living yeast^{6,7} was the first "phytochemical reduction"⁸ of an organic molecule described in literature. Numerous further enzymatic or microbial biotransformations, bioconversions, biodegradations, and fermentations followed, and as Chaleff⁹ pointed out, in the initial excess of enthusiasm¹⁰ that invariably accompanies the birth of a new field,¹¹ biotransformations were hailed as a panacea that would ultimately displace traditional organic chemistry.^{12,13} But the role is one of support rather than supplantation, of synergy rather than rivalry;⁹ biotransformations should be employed when a given reaction step is not easily accomplished by "ordinary" chemical methods.¹⁴

Contrary to the very early applications, biotransformations are carried out today by pure cultures of microorganisms or plant cells or with purified enzymes, and they should always be considered as a way of performing selective modifications of defined pure compounds into defined final products.¹⁵ The main differences between biotransformations and fermentations have clearly been listed by Yamada.¹⁶

The general goals of biotransformations may be considered to be as follows: resolution of racemates, selective conversion of functional groups among several groups of similar reactivities, introduction of a chiral center, and functionalization of a certain nonactivated carbon. Applications in the energy sector or with regard to applications in the areas of environmental pollution problems are of forthcoming interest.¹⁷

Several excellent reviews and monographs have been published on microbial/enzymic transformations. To



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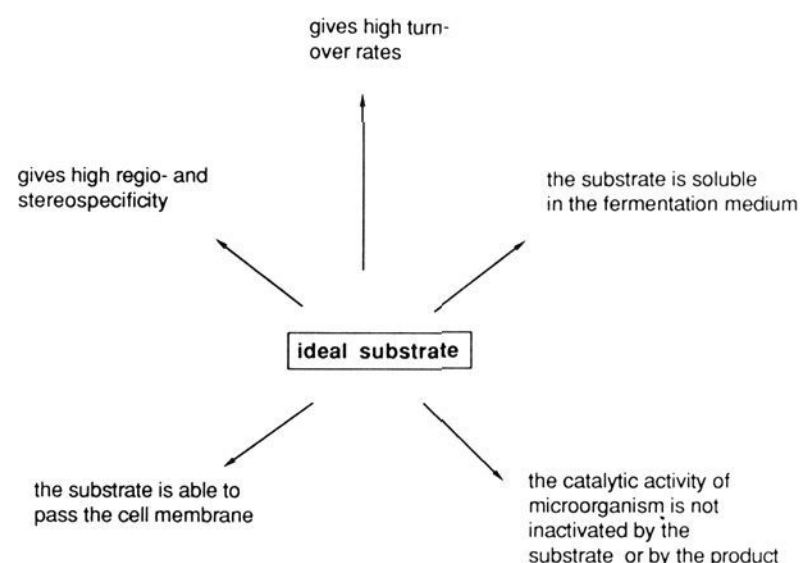


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avoid excessive echoing of these reviews and of other literature in the field, background material will be limited to the minimum commensurate with both the diversity of the readership of this review and the chemical nature of the discussion to follow.

There are two different biotransformation systems, whole cells or isolated enzymes, and both display several advantages.¹⁸ Availability of a certain microorganism is often a deciding factor for an organic chemist turning to the use of biotransformations in synthesis. For ex-

SCHEME 1



ample, baker's yeast (BY), *Saccharomyces cerevisiae*, is a readily available microorganism (world output of BY, 600 000 tons/year¹⁹), but obtaining other microorganisms may require help from a microbiologist and access to fermentation facilities. A further disadvantage of the use of whole cells for laboratory-scale operations is that sterile growing of the cells sometimes is required and the workup is both time-consuming and messy due to separation of the product from the huge amounts of biomass; this process is very often complicated by side reactions that interfere or even dominate the desired transformation. Contrary, enzymes are more often specific for selected reactions and their use may require only small-sized equipment and simpler workup.²⁰ But enzymes are more expensive, and addition of enzyme cofactors or enzyme cofactor recycling might be necessary.^{21,22} The ideal interactions between the substrate and the microorganism (Scheme 1) are scarcely found in praxi; some advice how to deal with basic problems often encountered in such biotransformations is provided in Scheme 2.^{14,16,23,24}

There are some basic ways to perform a reaction with intact baker's yeast: One has to differentiate between using *previously* grown cells, e.g., active cells or spores,²⁵ biotransformations under *fermentative* conditions, or transformations with *immobilized* cells.

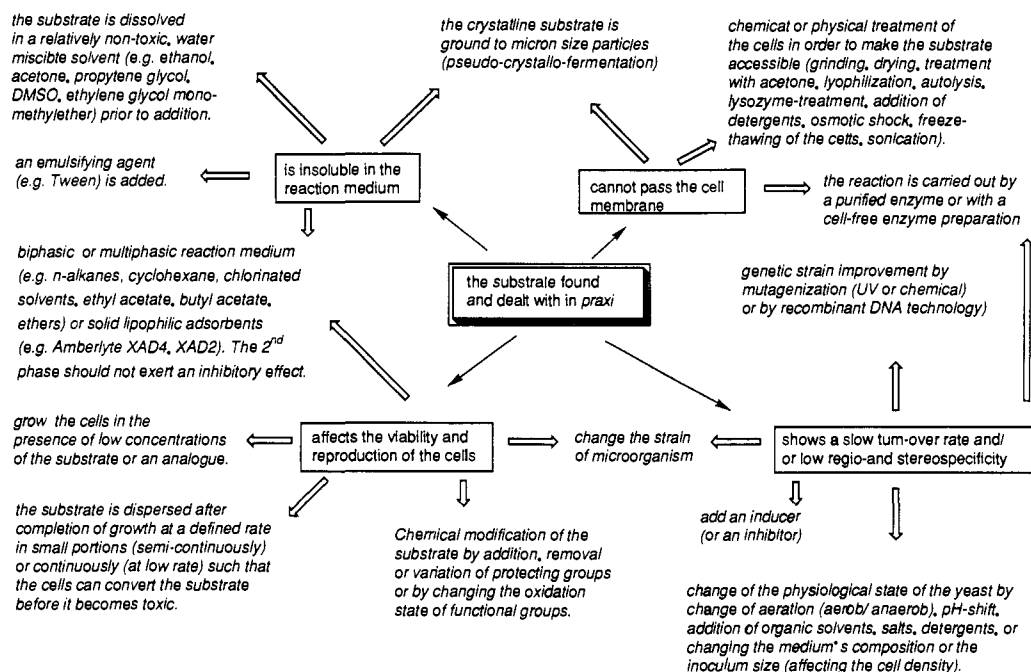
II. Reductions

A. General Remarks

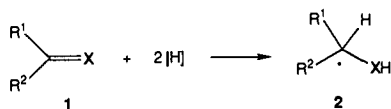
Unsaturated compounds **1** can be reduced by BY (Scheme 3). For **1** the enzyme has to distinguish between the *re* and the *si* face of the π -system to yield chiral **2**.²⁶

The asymmetric reduction of carbonyl-containing compounds by BY constitutes one of the most widely applicable reactions. Originally described in 1898 for the reduction of furfural to furfuryl alcohol,^{6,7} the widespread applications of this reaction are based on systematic investigations by MacLeod²⁷ and Hub.²⁸ Ketones with varying substituents (Me, Et, *n*-Pr, *n*-Bu, Bz) were reduced by BY, and the secondary alcohols obtained were mainly of *S* configuration. Only 3-hydroxyheptanol (from the reduction of 3-heptanone) was predominantly *R*-configured. Sterically hindered ketones (e.g., 4-octanone, *tert*-butyl methyl ketone, isobutyl isopropyl ketone, or *n*-amyl phenyl ketone) were not reduced at all. These results^{27,28} suggested a

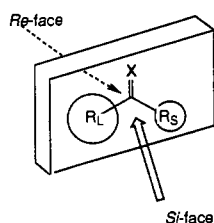
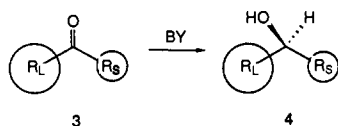
SCHEME 2



SCHEME 3



SCHEME 4



hydrogen transfer to the *re* face of the prochiral ketone 3, with R_L representing a large substituent and R_S a small substituent adjacent to the carbonyl group (Scheme 4) to yield alcohol 4.

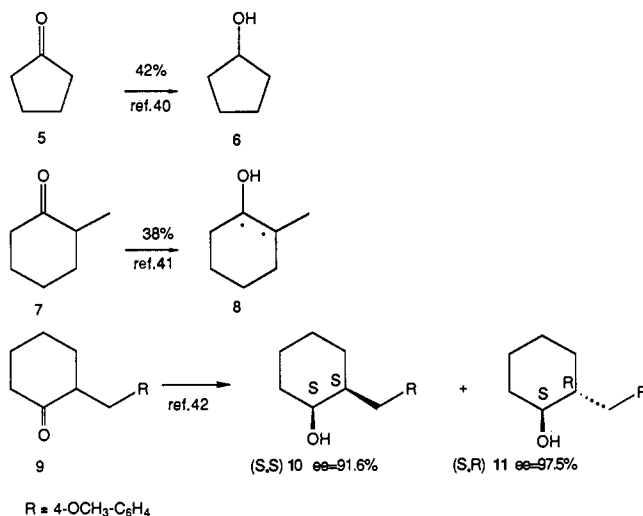
But, as Sih²⁹ pointed out, one should exercise considerable caution when Prelog's rule³⁰ is applied to intact cell systems.

B. Reduction of Monocarbonyl Compounds

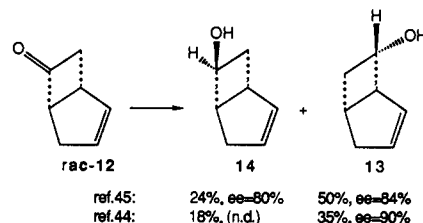
1. Reduction of Cycloalkanones

Only a few examples for the reduction of cycloalkanones³¹ bearing no further functionalities (or obviously not participating remote functional groups) have been described so far,³² however, there are numerous reports on the reduction of steroids,³³⁻³⁸ but some of these reductions claimed for the action of BY are attributable to the action of bacteria having contaminated the yeast.³⁹ Cyclopentanone (5) (Scheme 5) was re-

SCHEME 5



SCHEME 6

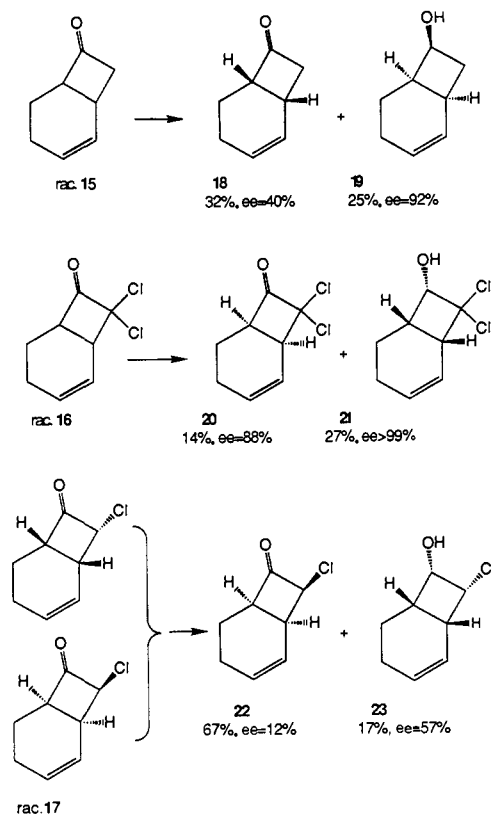


duced into cyclopentanol (6),⁴⁰ and racemic 2-methylcyclohexanone (7) was found to yield dextrarotatory 2-methylcyclohexanol (8).⁴¹ Similarly, 9 gave a 1:1 mixture of *cis*-(1*S*,2*S*)-2-(4-methoxybenzyl)-1-cyclohexanol (10) (91.6% ee) and of *trans*-11 (97.5% ee).⁴² Reduction of racemic *cis*-2,4-dimethyl-1-cyclohexanone provided all possible stereoisomers in 4% overall yield.⁴³

2. Reduction of Bi- and Polycyclic Cycloalkanones

The first reported reduction of a cyclobutanone is represented by the BY-mediated transformation of

SCHEME 7

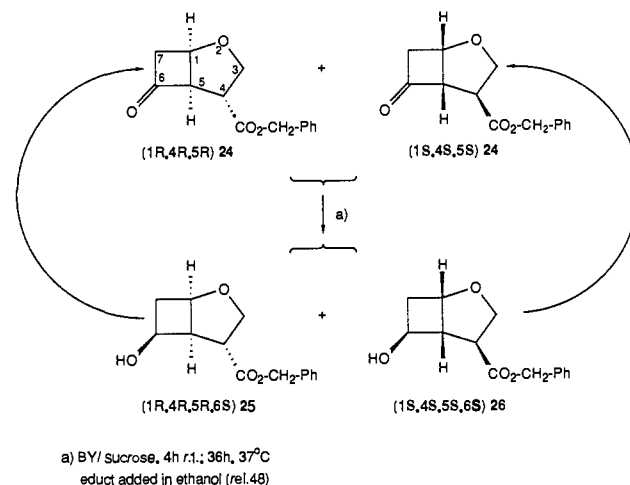


(±)-bicyclo[3.2.0]hept-2-en-6-one (**12**) (Scheme 6). **13** was obtained in about 90% optical purity; the optical purity of the byproduct **14** (obtained in ca. 18% yield) was not determined. It is of interest to note that additional riboflavin and commercial yeast nutrient were added to the reaction mixture.⁴⁴

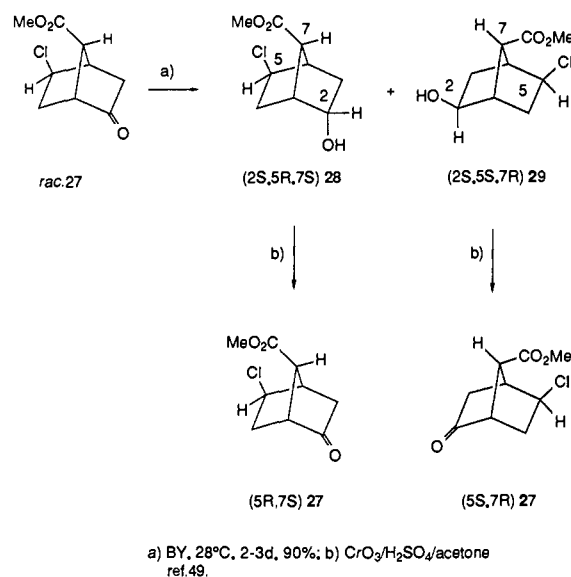
Since these bicycloheptenones represent important building blocks for the synthesis of prostaglandins PGE₂, PGFA_{2α}, and PGA₂, the reduction of **12** was recently reinvestigated in more detail.⁴⁵ In order to improve the low substrate enantioselectivity achieved by using commercially available BY, other different yeast strains were screened and marked differences established (the ratio of **13** to **14** for different strains of *Saccharomyces cerevisiae* was found to be 1:1 to 7:1). In addition, the endo to exo ratio (**13** to **14**) changed on prolonged incubation (7:3 in 24 h to 3:2) but could kept constant (5:2), maintaining a glucose concentration of 350 g/L. The yeast reduction is inhibited by 50% at a concentration of 15 g/L of ketone **12**.⁴⁵ The best results for this reduction, however, were obtained with *Mortierella ramanniana* (Glaxo C2506), giving rise to an endo to exo ratio of >30:1; no increase in the conversion rate by increasing the oxygen-transfer rate but definitive requirement for oxygen were demonstrated.

Reduction of the bicyclo[4.2.0]octenones **15**–**17** (Scheme 7) was found to be completely diastereoselective for reduction from the ketone's exo face in addition to being highly enantioselective. Thus, reduction of racemic **15** afforded 32% of **18** (40% ee) and 25% of **19**; **16** gave within 45 min 14% of **20** (88% ee) and 27% of **21** (ee >99%), whereas **17** yielded after 6 h 67% of **22** (12% ee) and 17% of **23** (57% ee).⁴⁶ An enzymic reduction of chlorinated bicyclo[3.2.0]hept-2-en-6-ones of similar substrate and product enantioselectivity has been reported.⁴⁷

SCHEME 8



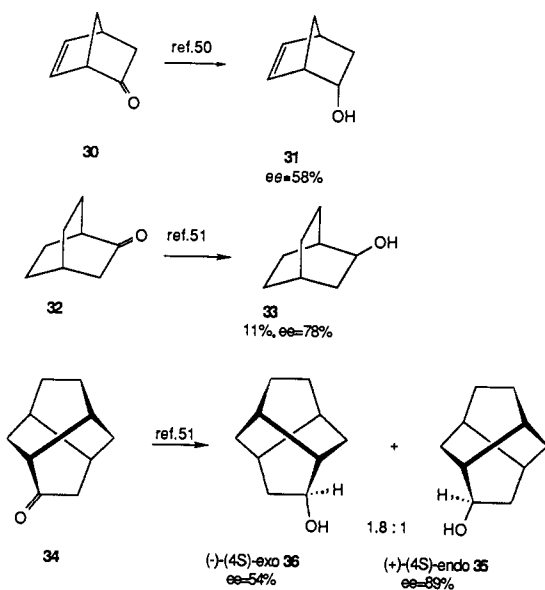
SCHEME 9



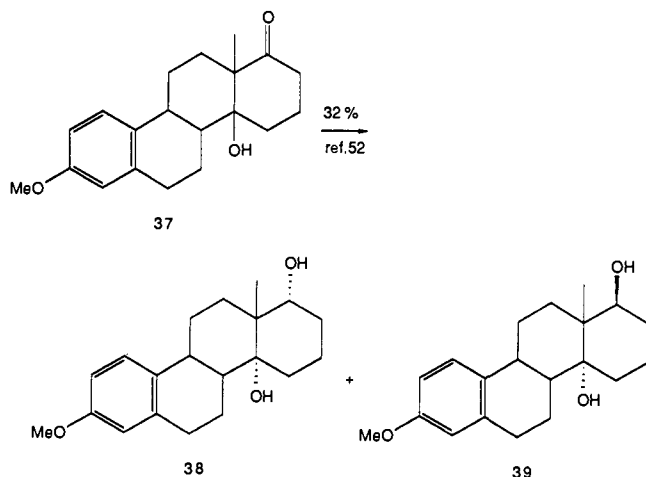
Similarly, reduction of racemic (1 α ,4 α ,5 α)-4-(benzyloxycarbonyl)-2-oxabicyclo[3.2.0]heptan-6-one (**24**) (Scheme 8) by BY gave 59% of a separable mixture of the corresponding diastereomeric 6-hydroxybicycloheptanols **25** and **26** in 10% and 2% isolated yields, respectively. **25** and **26** afforded upon separate reoxidation with pyridinium chlorochromate the resolved enantiomers (1R,4R,5R)-**24** and (1S,4S,5S)-**24**. Interestingly, 2-(4-hydroxyphenyl)ethanol was obtained as a byproduct of this BY-mediated reduction.⁴⁸

An analogous sequence of reduction and reoxidation for obtaining the pure enantiomers was used for preparation of enantiomerically pure methyl 5-chloro-2-oxobicyclo[2.2.1]heptane-7-carboxylates (**27**) from the corresponding racemate (Scheme 9). Racemic **27** afforded upon treatment with BY 90% of a mixture of methyl (2S,5R,7S)-5-chloro-2-hydroxybicyclo[2.2.1]heptane-7-carboxylate (**28**) and methyl (2S,5S,7R)-5-chloro-2-hydroxybicyclo[2.2.1]heptane-7-carboxylate (**29**), which were each reoxidized by CrO₃/H₂SO₄/acetone to yield both enantiomers of **27**. A drawback of these reductions is the laborious workup, which could be improved by performing the reduction with *Candida utilis* and stopping the reduction at 52–53% conversion of racemic **27**.⁴⁹

SCHEME 10



SCHEME 11



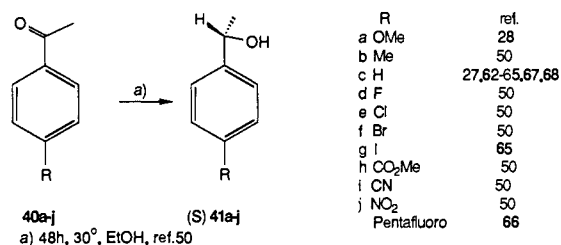
Anaerobic reduction of norbornenone (30) (Scheme 10) afforded only *endo*-norborneol (31) but with moderate enantioselectivity. As shown by differential scanning calorimetry, 31 crystallizes as a pseudoracemate and it is therefore not possible to improve the low ee (58%) by repeated recrystallization.⁵⁰ Bicyclo[2.2.2]octan-2-one (32) afforded only 11% of 33 (78% ee). Racemic 4-twistanone (tricyclo[4.4.0.0^{3,8}]decan-4-one, 34) gave a 1:1.8 mixture of *endo* and *exo* alcohols 35 and 36 of 89% and 54% ee, respectively.⁵¹ Better yields and higher ee values were obtained with *Rhodotorula rubra*.⁵¹

Reduction of the racemic estradien derivative 37 (Scheme 11) with BY (*Saccharomyces cerevisiae* Heyen ex. Hansen) afforded in 32% yield the corresponding alcohols 38 and 39 in optically pure form whereas reduction of 37 using lithium aluminum hydride afforded the corresponding racemic materials. Better yields, however, were obtained by using *Rhizopus nigricans*.⁵²

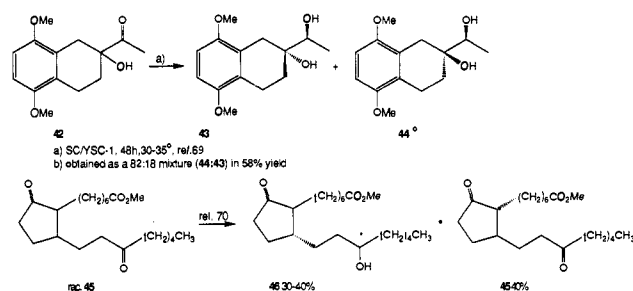
3. Reduction of Aliphatic Alkanones

The reduction of aldehydes and ketones by means of fermenting BY is long known, and this subject has been covered in several reviews.^{26,40,53-56} The stereochemical course of these reductions has been investigated by

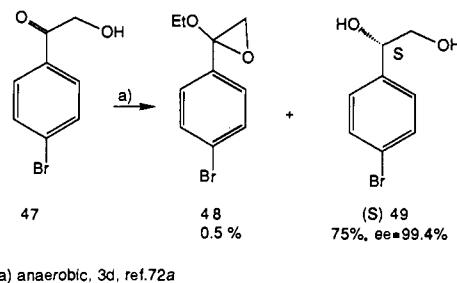
SCHEME 12



SCHEME 13



SCHEME 14



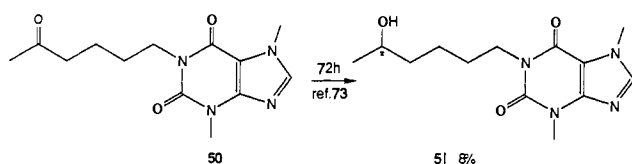
several deuteration studies.⁵⁷⁻⁵⁹ Hence, the number of examples for these reductions of carbonyl groups with the carbonyl moiety being part of an acyclic chain will be limited. A variation of these reductions has been established by Simon et al.,^{60,61} namely the electromicrobial reduction that brought about several advantages. This topic has been reviewed recently.²⁶

Fermentative reduction of substituted acetophenones 40a-j (Scheme 12) afforded (*S*)-1-arylethanol 41a-j in low to moderate yields and ee values between 82% and 96%.^{27,28,50,62-68} No influence on the steric course of the reduction was observed when the substituents were changed, but the velocity of the reaction was decreased by electron-donating substituents.^{50,62}

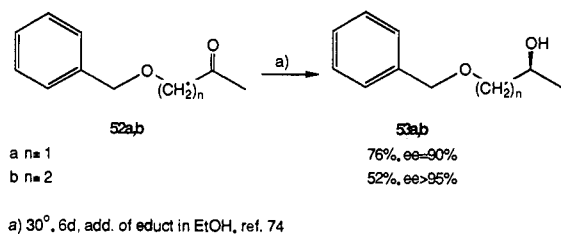
The advantage of yeast-mediated reductions of ketones is their high *re* selectivity, often resulting in high optical yields of products. In addition, when the ketone carries a substituent capable of coordination through hydrogen bonding or lone-pair interaction, the products are predominantly of erythro configuration.

Thus, treatment of racemic 42 (Scheme 13) (cf. reduction of racemic 211) with *Saccharomyces cerevisiae* (YSC-1/Sigma) gave 91% of the reduction products 43 and 44,⁶⁹ which are synthetic intermediates for the synthesis of anthracyclines. The racemic prostaglandin analogue 45 has been treated with resting cells of *Saccharomyces cerevisiae* (ATCC-4125), and 30-40% of alcohol 46 and 40% of starting material have been obtained.⁷⁰ It was not possible to assign unambiguously the stereochemistry at position C-15. The resolution of racemic *estra*-4,9-diene-3,17-dione by 48

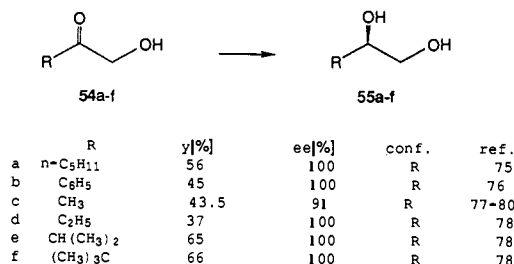
SCHEME 15



SCHEME 16



SCHEME 17

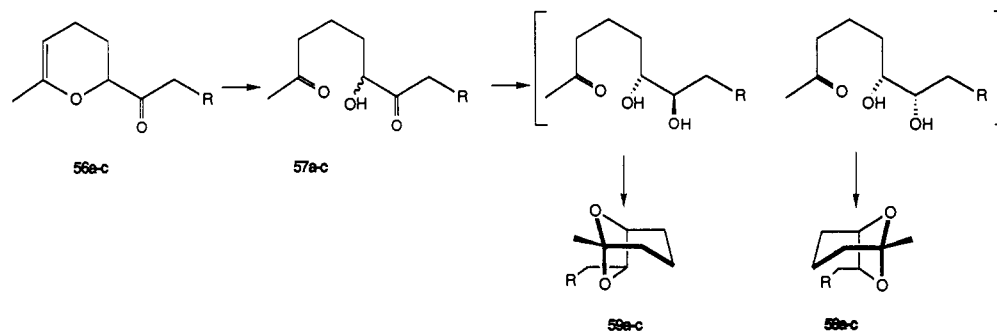


different microorganism has been investigated recently.⁷¹

Interestingly, the reduction of 47 (Scheme 14) gave crystalline 48,^{72a} which seems to be remarkably stable under these physiological conditions; however, (*R*)-49, as the main product, was obtained in 75% yield and 99.4% ee.^{72a} Recently, the enantioselective reduction of 4-acetylpyridine by nonfermenting BY has been reported. Thus, (*S*)-1-(4-pyridyl)ethanol (79% yield) has been obtained with an ee of 96%.^{72b} In addition, 1-(acyloxy)-3-azido-2-propanones (70–95% yield of (*S*)-1-(acyloxy)-3-azido-2-propanols, ee = 75–>95%)^{72c} and 3-(benzyloxy)-1-hydroxypropanone have been reduced (ee = 99% of (*S*)-3-(benzyloxy)-1,2-propanediol).^{72d}

The biodegradation of pentoxifylline (50) (Scheme 15) (used in the treatment of cerebrovascular and peripheral vascular diseases) has been investigated. Thus, *Saccharomyces cerevisiae* NRRL Y2034 reduced 50 to give alcohol 51 with 8% conversion within 72 h. The

SCHEME 18



R	y [%]	ee [%] 57	ee [%] 58	ratio 58/57
a CH ₃	29	0	99	1/16
b H	32	98	99	2/1
c C ₂ H ₅	9	98	99	1/2

ref. 81,82

highest conversion rates, however, have been obtained by using *Cryptococcus macerans*, *Curvularia falcata*, and *Rhodotorula mucilaginosa*.⁷³

Synthesis of masked 1,2- or 1,*n*-diols has been investigated by several groups. Slow addition of educts 52a,b (Scheme 16) (approximately 0.5 g of educt during 8–18 h) to a highly diluted yeast suspension (*Eridania* brand) gave access to the *S*-configured alcohols 53a,b in 90% and 95% ee, respectively.⁷⁴

More recent examples for the reduction of α -hydroxy ketones 54a–f to yield the corresponding 1,2-diols 55a–f are summarized in Scheme 17.^{75–80}

Older examples for the bioreduction of aliphatic (hydroxy) carbonyl compounds have already been compiled in the literature.^{40,54} In general, it was found⁴⁰ that larger amounts of yeast were required for the reduction of ketones than for the reduction of aldehydes.

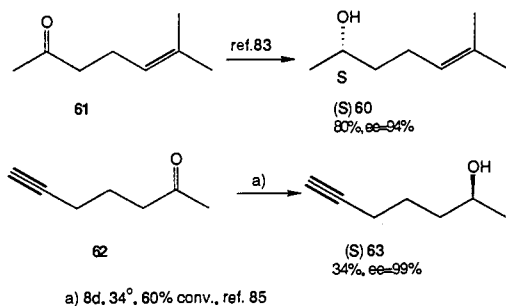
Recently, a kinetic resolution by BY has been used for the synthesis of *endo*-brevicomine.⁸¹ Reduction of racemic 56a (Scheme 18) with actively growing BY under anaerobic conditions afforded after initial formation of racemic 57a 27% of 58a (99% ee) and 2% of 59a (98% ee). 56a gave primarily *endo* product 58a via syn-selective reduction, whereas in the reduction of 56b the *exo* isomer 59b predominated (18%). The observation of the opposite diastereoselectivity in the reduction of 56a as compared to 56b was attributed^{81,82} to the loss of the precursor for the *endo* product by biodegradation. Finally, reduction of 56c gave after 7 days only 9% of reduction products 59c (3%) and 58c (6%), but the ratio of 59c to 58c can be increased to 1:30 by using acyclic intermediate 57c (yield 7.5%).⁸¹

The aggregation pheromone sulcatol ((*S*)-60) (Scheme 19) has been synthesized by reduction of 6-methylhept-5-en-2-one (61) in 80% yield and 94% ee.⁸³ This recent finding is in contrast to previous reports.⁸⁴ No reduction of the double bond was observed. Similarly, 62 was only reduced at the carbonyl moiety to give 34% of (*S*)-63 in 99% ee. (*S*)-63 has been used as a starting material in a Brefeldin A synthesis.⁸⁵

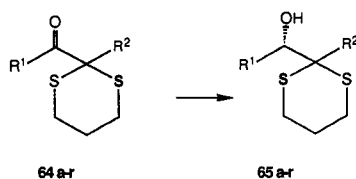
4. Reduction of Sulfur-Containing Molecules

a. Thiocarbonyl Compounds. Thiols from the corresponding thioaldehydes are formed in an analogous manner as reported for the reduction of aldehydes. Thioacetaldehyde was very readily reduced to ethyl mercaptan,^{86,87} thio-*n*-butyaldehyde as well as thio-

SCHEME 19



SCHEME 20



R ¹	R ²	t [d]	y [%]	ee [%]	ref.	
a	CH ₃	H	1	84	>96	91, 96
b	C ₂ H ₅	H	2	71	-	91, 96
c	n-C ₃ H ₇	H	4	92	-	91
d	n-C ₄ H ₉	H	3	71	-	91
e	n-C ₆ H ₁₃	H	8	38	-	96
f	CF ₃	H	2h	96	67	96
g	CH ₂ OH	H	5	28	96	96
h	CH ₂ OAc	H	1	58	87	96
i	CH ₂ OC ₂ H ₅	H	5	50	95	96
j	CH ₂ O (4-OCH ₃ C ₆ H ₄)	H	6	27	98	96
k	CH ₂ OTHP ^d	H	3	37	96	96
l	CH ₂ OCH ₂ OCH ₃	H	4	82	-	96
m	CH ₂ OSi(CH ₃) ₂ tC ₄ H ₉	H	7	<10	-	96
n	(CH ₂) ₃ OH	H	10	74	96	91
o	CH ₃	CH ₃	2.5	50	-	91
p	CH ₃	CH ₂ CH=CH ₂	4	31	-	91
q	(CH ₂) ₃ CO ₂ CH ₃	H	hydrolysis of ester	-	92	92
r	c	H	3	17	97	93
r	(CH ₂) ₃ CO ₂ ^t Bu	H	3	65	99	92

a) BY (Oriental); addn. of MgSO₄/glucose; ref. 91

b) BY (Red star); without sugar; 70h; 25°; ref. 92

c) SC 567; 1-7d; 28-58%; ee= 87-95%; ref. 96

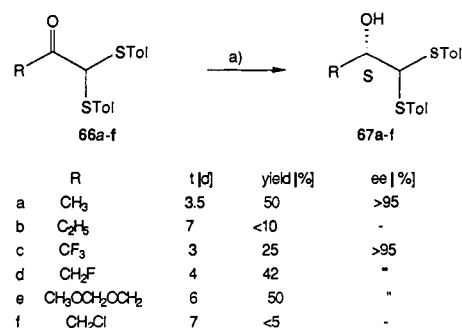
d) complete cleavage of THP-acetal

isobutyraldehyde gave the corresponding mercaptans;⁸⁸ diethyl thioether was reported to cleave to ethanethiol.⁸⁹

b. 2-Thio-Substituted Ketones. The reduction of carbonyl groups with adjacent sulfur substituents by actively fermenting BY is critically dependent upon the substituents attached to the sulfur-containing group and to the carbonyl group. In cases where these substituent groups are bulky, very little reduction occurs. The relative ease of reduction decreases from β -keto sulfones to β -keto sulfoxides to β -keto sulfides.⁹⁰ Asymmetric reduction of α -keto thioacetals was achieved by fermenting BY to afford optically active α -hydroxy thioacetals, which are synthetic equivalents to chiral α -hydroxy aldehydes or ketones. Reduction of **64a-r** (Scheme 20) afforded **65a-r** with high ee and proceeded predominantly to the *S*-configured alcohols; only the allyl-substituted educt **64p** afforded the *R*-configured product **65p**.⁹¹ The methyl ester **64q** was rapidly hydrolyzed with *Red-Star* BY, whereas the *tert*-butyl ester **64r** was stable and afforded **65r** in good yield.⁹² **65q** (ee = 97%) could be obtained, although in low yield (17%), from SC567.⁹³

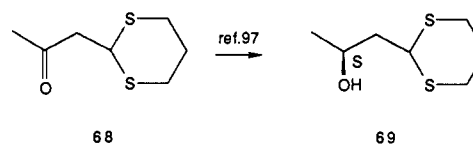
Somewhat lower yields but still high enantiomeric excesses could be achieved upon reduction of 1,1-bis-(*p*-tolylthio) ketones **66a-f** (Scheme 21) to the corre-

SCHEME 21

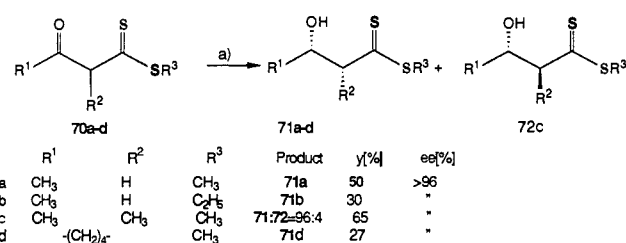


a) Educt in EtOH; 27-30°; ref. 94-96

SCHEME 22



SCHEME 23



a) Oriental yeast; glucose; 24h;

sponding alcohols **67a-f**. The rate of reduction was shown to depend on the length of hydrocarbon and the type of substituent.^{94,95} These results indicate that the use of the 1,3-dithione (as in **64**) instead of the bis(*p*-tolylthio)methane derivatives permits the synthesis of a broader range of α -alkoxycarbonyl compound equivalents.⁹⁶

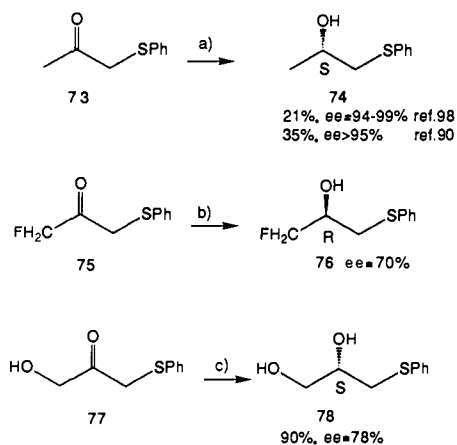
The β -keto thioacetal **68** (Scheme 22) was reduced in 99% ee to (*S*)-1-(1,3-dithian-2-yl)-2-hydroxypropane (**69**), a key intermediate for the synthesis of (*S,S*)-gramhamimycin A1.⁹⁷

Asymmetric reduction of the β -keto dithioester **70a-d** (Scheme 23) with BY produced mainly the corresponding optically pure (3*S*)-hydroxy thioester **71a-d**; **72c** was obtained as a low yield byproduct although with high ee (96%). The syn to anti ratio (**71c**:**72c**) is better than with the corresponding oxo isomers (cf. Scheme 81, compounds **357** and **358**), a fact that seems to be due to the enhanced enolization of the β -keto groups by the thiocarbonyl moiety. Thus, changing the oxygen atoms in an ester group of a β -keto ester to sulfur atoms can control the diastereoselectivity of the reduction quite efficiently.¹⁰⁰

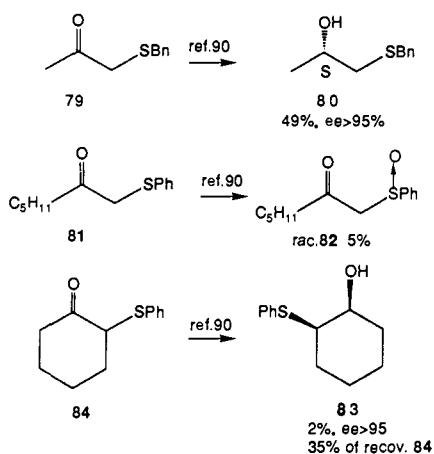
Many β -keto sulfides have been reduced by BY. Thus, (phenylthio)acetone (**73**) (Scheme 24) gave (*S*)-(+)-2-hydroxypropyl phenyl sulfide (**74**) with high ee but rather low yield;^{90,98} the corresponding 1,1,1-trifluoropropyl phenyl sulfide was not reduced at all by BY⁹⁹ whereas the 1-fluoro-2-oxopropyl phenyl sulfide (**75**) gave under similar conditions (*R*)-**76** with 70% ee.⁹⁹

Fair ee (78%) was achieved upon reduction of 1-hydroxy-3-(phenylthio)-2-propanone (**77**) to yield

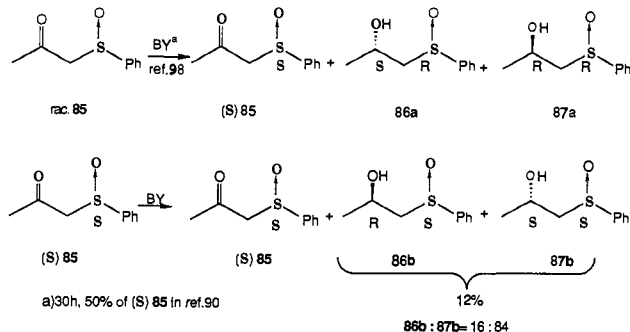
SCHEME 24



a) BY (Oriental), 5h, r.t., ref.98
b) BY, pH=7.3, 24h, 35°, ref.99
c) BY (Oriental), 24h, r.t., ref.101,102



SCHEME 25

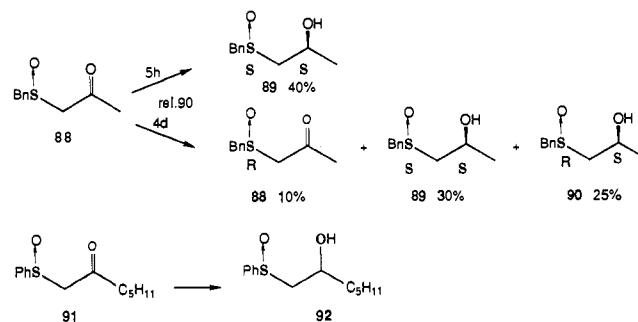


(*S*)-3-(phenylthio)-1,2-propanediol (78) (90%), which was successfully used for the synthesis of both enantiomers of the insect pheromone δ -*n*-hexadecanolid and for the synthesis of the deoxy sugars L-rhodinose and D-amicitose.^{101,102}

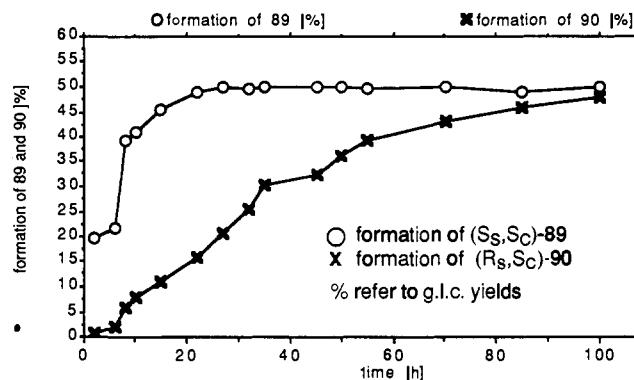
The structural analogue 79 gave 49% of *S*-configured 80 whereas 81 was not reduced but oxidized in about 5% yield to 82.⁹⁰ A very low yield of only 2% of *cis*-83 (and 38% recovery of starting material) was found for the cyclohexanone derivative 84.⁹⁰ Generally, the reduction of these β -keto sulfides proceeded with relative difficulties and only at low concentrations of the substrate.⁹⁰

Reduction of racemic 1-(phenylsulfinyl)acetone (85) (Scheme 25) resulted in formation of (*S*)-85 and a mixture of diastereomeric 86a/87a; the ratio of 85 to

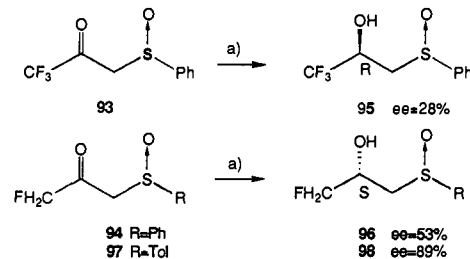
SCHEME 26



SCHEME 27



SCHEME 28



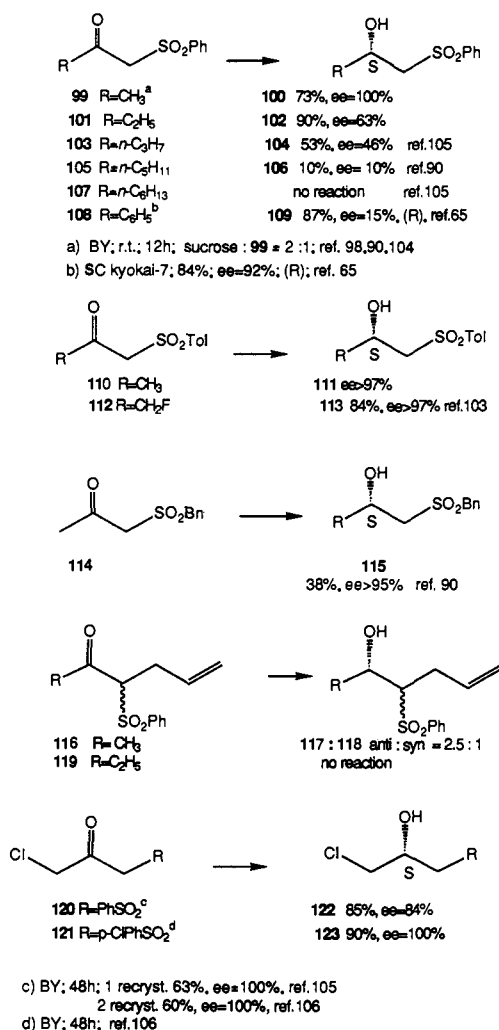
a) BY; 35°; 24h.

86a/87a (55:45 to 42:58) (86a/87a as a 79:21 to 89:11 mixture) depended on the supplied sucrose concentration. By means of recrystallization, optically pure (*S*)-85 was obtained in 28% yield, whose further reduction with BY afforded an 84:16 mixture of 87b and 86b.⁹⁸ Reduction of a mixture of 73 and (*S*)-85 showed that 73 is reduced much faster.⁹⁸ (*R*)-85 is reduced in high chemical and more than 95% optical yield whereas (*S*)-85 is reduced in both low chemical and optical yield (68%). The order of reducibility for these compounds was established to be (*R*)-85 > 73 > (*S*)-85,⁹⁸ which is for unknown reasons contradictory to the general rule.⁹⁰

Time dependency for the course of the reduction of 88 was encountered (Schemes 26 and 27). Thus, 88 gave after treatment with BY for 5 h 40% of (*S_S,S_C*)-89, whereas after 4 days 10% of (*R_S*)-88, 30% of (*S_S,S_C*)-89, and 25% of (*R_S,S_C*)-90 were isolated.⁹⁰ Reduction of 91 proceeded again very slowly (2.5% within 12 h) to give a mixture of the corresponding hydroxy sulfoxides 92, which could not be separated.⁹⁰

Reduction of the fluorinated compounds 93 and 94 (Scheme 28) afforded an 87:13 mixture of diastereomers (*R_C*)-95 and (*S_C*)-96 with low ee values of 28% and 53%, respectively.⁹⁹ The stereochemistry on the sulfur atom

SCHEME 29



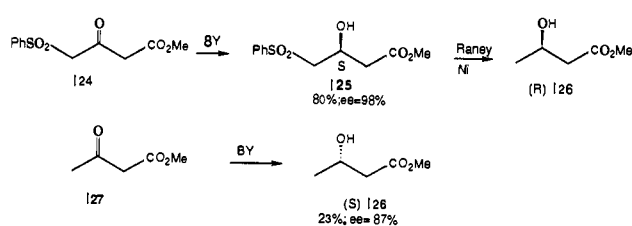
has not been assigned. The *p*-toluenesulfonyl analogue **97**, however, gave (*S*)-**98** of 89% ee.¹⁰³

Several β -keto sulfones have been reduced by BY. Thus, (phenylsulfonyl)acetone (**99**) (Scheme 29) afforded (*S*)-2-hydroxypropyl phenyl sulfone (**100**) in 73%⁹⁸ or 98%⁹⁰ yield, and 97%¹⁰⁴ or 100%⁹⁸ ee. The rate of conversion was shown to depend on the ratio of educt to sucrose. Thus, the yield of 73% (ee = 100%) was obtained at a sucrose to **99** ratio of 2:1, whereas only 8% of **100** was obtained with sucrose:**99** = 1:2.⁹⁸ Interestingly, when the same experiment was performed without any sucrose, only 28% of product was obtained.⁹⁸

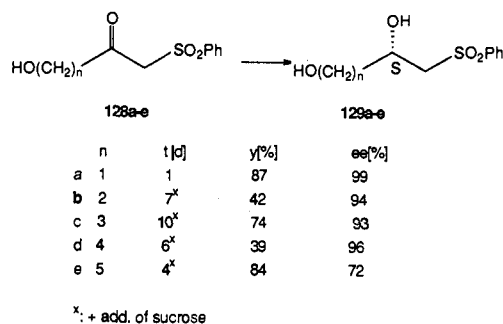
Decrease of the ee values resulted in an increase in the number of carbon atoms. Thus, **101** afforded (*S*)-**102** with 63% ee¹⁰⁴ and **103** gave (*S*)-**104** with 46% ee,¹⁰⁵ whereas reduction of **105** yielded only 10% of nearly racemic **106**.⁹⁰ No reduction by BY was observed for **107**.¹⁰⁵ **108** afforded upon reduction with BY (*R*)-**109** (87% yield and 15% ee).⁶⁵ Reduction with *Saccharomyces kyokai* 7, however, gave (*R*)-**109** with 84% yield and 92% ee.⁶⁵

In addition to phenyl, tolyl-substituted compounds have been investigated. Similarly **110** (Scheme 29) was transformed into (*S*)-**111** (ee > 97%); the fluorine-containing educt **112** afforded (*S*)-**113** in 84% yield with an ee of >97%.¹⁰³ Finally, (benzylsulfonyl)acetone **114** gave 38% of **115** (ee > 95%).⁹⁰

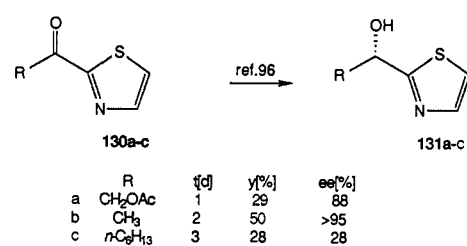
SCHEME 30



SCHEME 31



SCHEME 32



116 gave diastereomers *anti*-**117** and *syn*-**118** in a ratio of 2.5:1, whereas the structural analogue **119** did not react at all.¹⁰⁴

1-(Arylsulfonyl)-3-chloro-2-propanones **120** and **121** gave upon BY reduction 60% and 90% of enantiomerically pure (*S*)-**122** and (*S*)-**123**, respectively.^{105,106}

BY reduction of **124** gave 80% of **125** (Scheme 30), which afforded on treatment with Raney nickel W4 (*R*)-**126**, whereas BY reduction of the β -keto ester **127** afforded the enantiomer (*S*)-**126** but the reduction of the sulfonyl ketone was more easily performed than the reduction of the β -keto ester.⁶⁵

It was reported¹⁰⁸ that the introduction of a hydroxy group at the ω -position of a β -keto sulfone not only improved the enantioselectivity but also simplified conversion of the products into optically active lactones. Thus, β -keto sulfones **128a-e** (Scheme 31) gave the (*S*)- β,ω -dihydroxy sulfones **129a-e** with good to excellent ee.¹⁰⁸

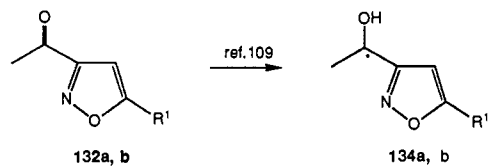
5. α -Heterocyclic Substituted Ketones

The reduction of 2-acylthiazoles **130a-c** (Scheme 32) gave poor yields of compounds **131a-c**. Only in the case of R = Me (**130b**) were yield and enantiospecificity satisfactory.⁹⁶

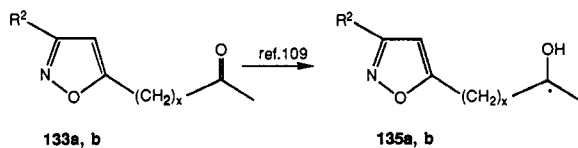
Better results, however, were obtained for the reduction of ketoisoxazoles **132a,b** and **133a,b** (Scheme 33) to carbinols **134a,b** and **135a,b**, respectively.¹⁰⁹

Two examples have been provided for the reduction of 5-acetyl-2-isoxazolines¹¹⁰ **136a,b**, which afforded at 35 °C (pH 3.3-8) **137a,b** (ee = 97-98%) and **138a,b** (ee > 98%). Alcohols **137** and **138** were formed at different rates, thus allowing partial kinetic resolution.¹¹⁰ The

SCHEME 33

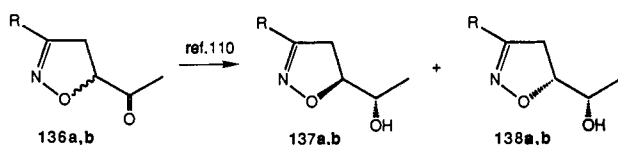


	R ¹	y[%]	ee[%]
a	CH ₂ Cl	78	n.d. ^a
b	n-C ₃ H ₇	78	100



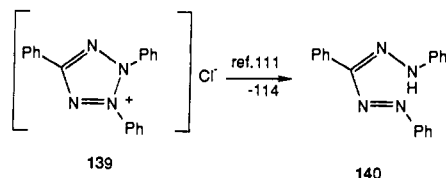
	x	R ²	y[%]	ee[%]
a	0	2,4,6-(CH ₃) ₃ C ₆ H ₂	75	100
b	2	C ₂ H ₅	10	n.d. ^{a,b}

BY eridania

^a n.d.= not determined^b incubation for 6d

	R	y[%]	ee[%]
a	2-furyl	95	97-98 for 137; >98 for 138
b	C ₆ H ₅	95	97-98 for 137; >98 for 138

SCHEME 34

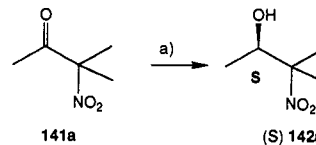


reduction of 2,3,5-triphenyltetrazolium chloride (139) (Scheme 34) to yield 140¹¹¹⁻¹¹⁴ is noteworthy in this context.

6. Nitrocarbonyl Compounds and Masked Amino Ketones

Although the reduction of aromatic nitro compounds is well documented,^{40,115,116} no reduction of aliphatic nitro compounds seems to occur. Thus, the reduction of ketones containing additional nitro or imide moieties has been performed to give the corresponding secondary alcohols with excellent optical purities, but the nitro alcohols were relatively unstable under the conditions of the BY reduction because of decomposition by retro nitro aldol reactions.^{117a} Despite these problems, 3-methyl-3-nitro-2-butanone (141a) (Scheme 35) gave 57% of the corresponding alcohol 142a with an ee > 96%.^{117a} 141b afforded under aerobic conditions 40% of (S)-142b, 141c gave 142c albeit in low yield and with low ee, and 141d gave nearly no reaction at all.^{117b} Due to the instability of the nitro alcohols under the reaction conditions and the fact that some amino ketones are difficult to isolate due to Schiff base formation but also because of the high solubility of the corresponding amino alcohols in the aqueous phase, masked amino ketones 143a-g have been investigated and the S-configured alcohols 144a-g could be isolated.^{117a}

SCHEME 35

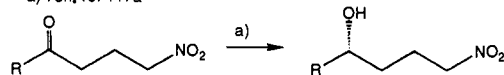
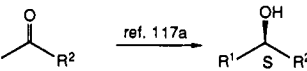
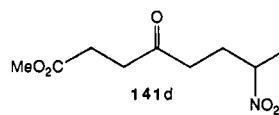


141a

(S) 142a

57%, ee>96%

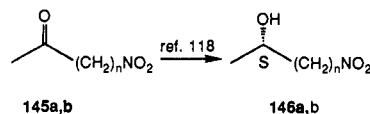
a) 75h, ref. 117a

141b R = CH₃, BY (Pleser), 37°, 41h141c R = C₂H₅, ref. 117b142b R = CH₃, 40%, ee=96%142c R = C₂H₅, 16%, ee=8%

143a-g

144a-g

	R ¹	R ²	[α] _D	y[%]	ee[%]
a	CH ₃	-CH ₂ -I	3	48	>96
b	CH ₃	-CH ₂ -III	1	81	>96
c	CH ₃	-C ₂ H ₄ -II	4	84	95
d	CH ₃	-C ₃ H ₆ -I	9	41	45
e	CH ₃	-C ₃ H ₆ -III	6	52	75
f	C ₂ H ₅	-CH ₂ -I	10	9	15
g	C ₂ H ₅	-CH ₂ -II	6	54	>96

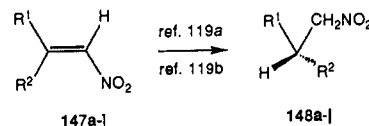


145a,b

146a,b

	n	y[%]	ee[%]
a	n=2	40-50	98
b	n=3	60	94

SCHEME 36



147a-j

148a-j

	R ¹	R ²	y (%)	ee (%)
a	C ₆ H ₅	H	26	n. d.
b	C ₆ H ₅	CH ₃	50	98
c	p-ClC ₆ H ₄	CH ₃	48	89
d	p-Br-C ₆ H ₄	CH ₃	57	94
e	p-NO ₂ -C ₆ H ₄	CH ₃	50	n. d.
f	C ₆ H ₅	C ₂ H ₅	64	97
g	C ₆ H ₅	C ₃ H ₇	23	89
h	C ₆ H ₅	C ₆ H ₁₃	--	--
i	C ₆ H ₁₃	CH ₃ (E)	58	83
j	CH ₃	C ₆ H ₁₃ (Z)	48	66

Similarly, 145a,b gave the S-configured alcohols 146a,b in about 40–60% yield, and good ee values of 98% and 94% could be achieved.¹¹⁸

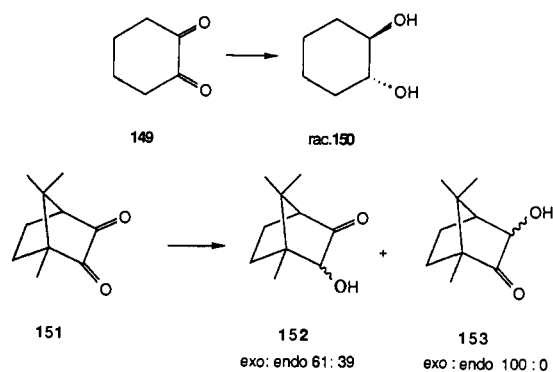
α,β-Unsaturated nitroalkenes 147a-j (Scheme 36) were reduced with moderate to excellent ee to yield nitroalkanes 148a-j.^{119a,b}

C. Reduction of Dicarbonyl Compounds

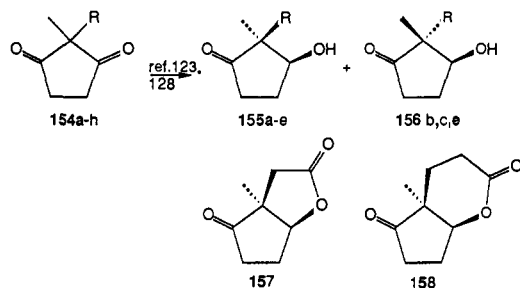
1. Cyclic Diketones

a. 1,2-Diketones. The reduction of cyclohexane-1,2-dione (149) (Scheme 37) gave racemic trans-cyclo-

SCHEME 37



SCHEME 38



Reduction of substituted cyclopentane-1,3-diones 154a-h

educt	R	recovered educt (%)	yield (%) and product	diastereo- ^a selectivity	ref.
154a	CH ₂ CH ₂ CH ₃	30	60	155a 100	123,124
154b	CH ₂ CH=CH ₂	15	75	155b/156b 90:10	123 ^b
154c	CH ₂ C≡CH	25	60	155c/156c 67:33	123,124
154d	CH ₂ C(CH ₃)=CH ₂	10	75	155d 100	124
154e	CH ₂ CH ₂ C≡N	71	n.r.	155e/156e 96:4	128
154f	CH ₂ CO ₂ CH ₃	80	9	157 100	128
154g	CH ₂ CH ₂ CO ₂ CH ₃	25	52	158 100	128
154h	C ₆ H ₅	n.r.	n.r.	155h >98	125

^a Assignments of previous works^{123, 124} have been corrected:^b additional references: 124, 126, 127.

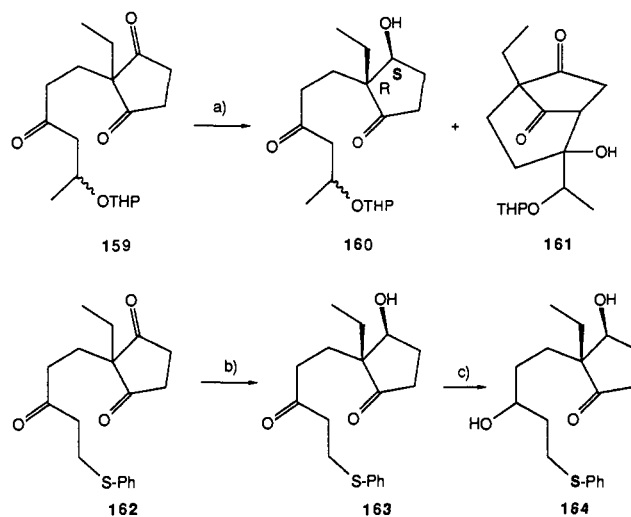
hexane-1,2-diol (150),¹²⁰ and under similar conditions camphorquinone (151) was transformed in 63% yield⁴⁰ into 3-hydroxycamphor (152) (exo:endo = 61:39) and exo-2-hydroxycamphor (153).

b. 1,3-Diketones. Similarly, asymmetric reductions of a series of 2,2-disubstituted 1,3-cycloalkanediones (Scheme 38) were investigated. First reports on these reductions were published in the mid-1960s^{121a-122} followed by extensive recent studies by Brooks et al. These reductions can be regarded as an example of an enzyme-catalyzed distinction of a substrate containing two trigonal carbonyl centers with stereotopic faces and one prochiral tetrahedral carbon center where mono-reduction generates two chiral centers. All of the products were obtained with ee > 98%.¹²³⁻¹²⁸

154f,g afforded lactones 157 and 158, respectively. 155b has been successfully used for the synthesis of the trichothecene mycotoxin anguidine^{126,127,129} or for coriolin¹²³ and 155d for the preparation of the diterpene zoapatanol.¹²⁴

More complex 2,2-disubstituted 1,3-cyclopentanediones are potential precursors in the steroid field, and their reduction by BY has been investigated by several groups. 159 (Scheme 39) was cleanly reduced to 160 in 73%¹³⁰ or 78%¹³¹ yield during 2¹³¹ or 3¹³⁰ days. 161 was observed as a byproduct in about 4%¹³¹ or 10%¹³⁰ yield. Similarly, 162 was reduced to (2*R*,3*S*)-163; 164 as a result of a second reduction was observed in approximately 13% yield. 164 was independently ob-

SCHEME 39

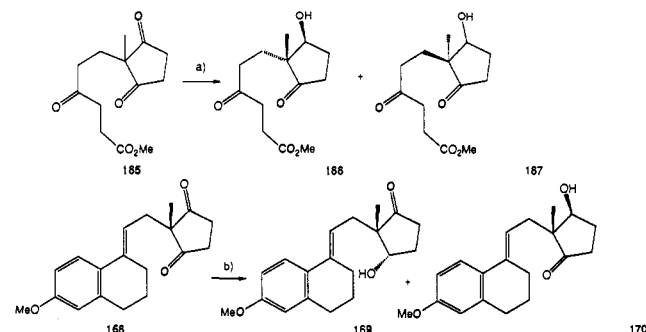


a) SC2346, 2-3d, 28-30°C, pH=6.7-7, educt in EtOH, ref. 130,131

b) SC2346, 25h, 30°C, pH=6.7-7, Tween80 added, educt in EtOH, ref. 132
13% of 164 isolated, too.

c) SC2346, 47h, 30°C, pH 6.7-7, 5%, ref. 132

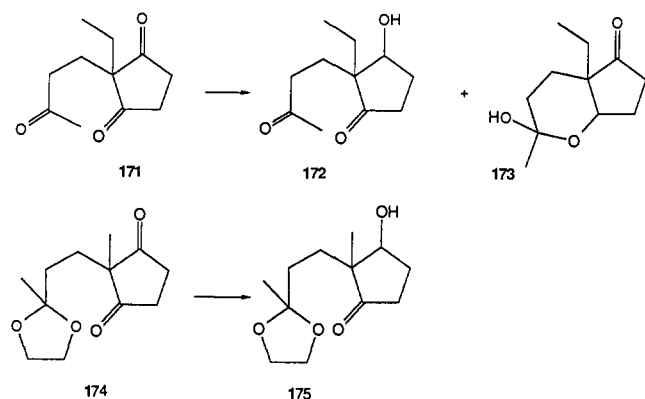
SCHEME 40

a) BY or *Schizosaccharomyces pombe* ATCC 2476, ref. 133,134b) *Saccharomyces cerevisiae* V2248 30 y[%] of 169 y[%] of 170 % direduction
Saccharomyces cerevisiae V2250 80 15 10
Saccharomyces cerevisiae NCYC 1203 40 4 10
ref.121a

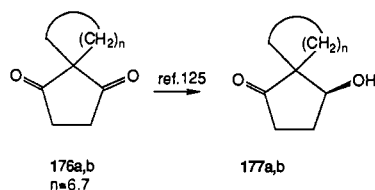
tained from 162 in 5% yield.¹³² Reduction of 165 (Scheme 40) or BY (or *Schizosaccharomyces pombe* ATTC2476) resulted in the formation of 166 as the main product and 167 as the byproduct. Addition of activators¹³³ enhanced the rate and the extent of product formation and reduced the level of byproduct formation.¹³⁴

As early as 1974 Lanzilotta¹³³ discovered activators for the reduction of cycloalkanediones. Allyl alcohol, acrylonitrile, methallyl alcohol, methacrylonitrile, acrylic aldehyde, α -methylacrylic aldehyde, and α,β -unsaturated ketones containing 3 to about 12 carbon atoms including cyclohex-1-en-3-one, methyl vinyl ketone, ethyl vinyl ketone, non-1-en-3-one, and dodec-1-en-3-one were shown to be activators. Typically, with *Saccharomyces cerevisiae* ATTC4097 or Y-147 NRRL, the reductions were performed for 24-120 h under aerobic conditions at 20-35 °C and pH 3.5-4.5; the activator to substrate ratio was about 1:1000 to 1:10 by weight.¹³³ A possible explanation for the effects of such activators could be that the activator compounds are suicide substrates for the oxidoreductases, affording undesired byproduct(s). Similarly, allylic alcohol was found to be a suicide substrate for the yeast alcohol de-

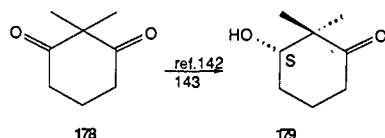
SCHEME 41



SCHEME 42



SCHEME 43



hydrogenase.¹³⁵ Unfortunately, application of these activator substances was up to now more or less only of theoretical interest but has only been scarcely used.¹³⁶

Reduction of 168 with different fungi, bacteria, and yeasts¹³⁷ afforded changing amounts of 169 and 170.^{121a} The best result (85% yield) for 169 was obtained with *Bacillus thuringiensis* and for 170 with *Saccharomyces uvarum* (75% yield).^{121a} A structural analogue has been reduced with acrylamide-*N,N*-methylenebisacrylamide immobilized *Saccharomyces cerevisiae*.¹³⁸

Reduction of triketone 171 (Scheme 41) afforded, along with the reduction product of ketone 172, cyclic hemiacetal 173,¹³⁹ a problem that could be circumvented by using the acetal-protected compound 174 to yield 175.^{140,141}

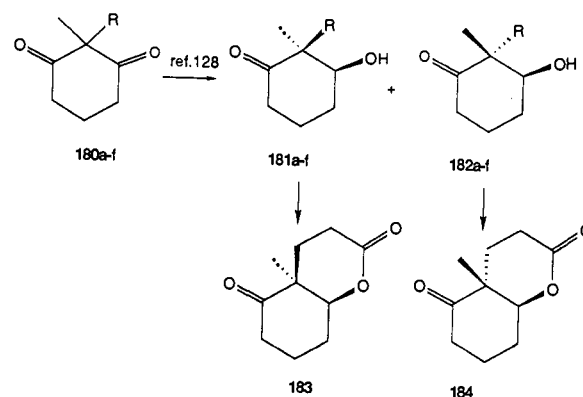
Spiro-fused diones 176a,b (Scheme 42) were efficiently reduced to ketols 177a,b.¹²⁵ The enantiomeric purity was >98% in each case, providing useful building blocks for both cyclopentanoid and cyclohexanoid natural products.

Cyclohexanoid 1,3-diketones 178 and 180a-f (Schemes 43 and 44) have also been reduced (products 179 and 181-184, respectively), with low diastereoselectivity as compared to the C₅ series (cf. Scheme 38).¹²⁸

The simplest member of this class of compounds, 2,2-dimethylcyclohexane-1,3-dione (178) (Scheme 43), was reduced to (*S*)-179 in 78% yield, and an excellent ee of 98.8% could be achieved upon addition of 0.2% Triton X to the fermentation broth with aeration.^{142,143} Previous experiments with *Kloeckera magna* (ATTC 20109) gave 179 with comparatively lower yields and with lower ee. (The optical rotations reported for enantiomerically pure 179 are controversial.^{143,144})

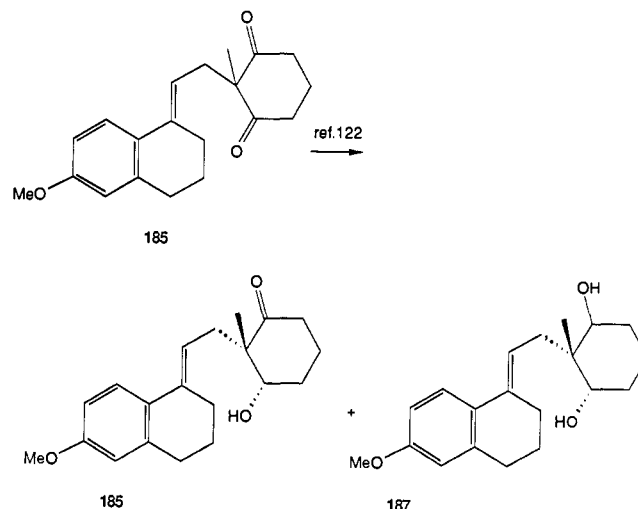
It is of interest to note that (*S*)-2,2-dimethyl-3-hydroxycyclohexane (179) is obtained in lower ee with

SCHEME 44



educt	R	recov. dione (%)	yield (%)	products
180a	CH ₂ CH ₂ CH ₃	15	80	181a:182a=22:78
180b	CH ₂ CH=CH ₂	15	80	181b:182b=45:55
180c	CH ₂ C≡CH	20	75	181c:182c=27:73
180d	CH ₂ C(CH ₃)=CH ₂	20	49	181d:182d=40:60
180e	CH ₂ CH ₂ C≡N	30	49	181e:182e=30:70
180f	CH ₂ CO ₂ CH ₃	60	49	183:184 =35:65

SCHEME 45



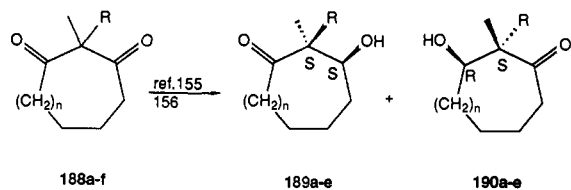
DMF/H₂O (1:38) as the solvent for this reaction.¹⁴⁵

179 is a valuable intermediate and has been used both for the synthesis of (*S*)-2-hydroxy-β-ionone (isolated as a metabolite of β-ionone from the broth of *Aspergillus niger* and known to have an improving effect on tobacco flavor¹⁴⁶) and for the synthesis of glycinolepin A (showing a significant hatch-stimulating activity for the soybean cyst nematode);¹⁴⁵ it has also been used for the synthesis of (-)-polygodial¹⁴⁷—a hot-tasting sesquiterpene from *Polygonum hydropiper*,^{148,149} which possesses antifeedant activity against African crop insects such as the army worm *Spodoptera exempta*.¹⁵⁰ Finally, 179 has been used for the synthesis¹⁵¹ of the monoterpenoid karahana compounds of 6-oxabicyclo-[3.2.1]octane structure, being constituents of the Japanese hop, *Kumulus lupulus* s.¹⁵²⁻¹⁵⁴ In addition, trimethyl-2-decalol was synthesized¹⁴² in a straightforward manner.

Reduction of the steroid analogue 185 (Scheme 45) resulted in formation of 186 and 187 as products of direduction.¹²²

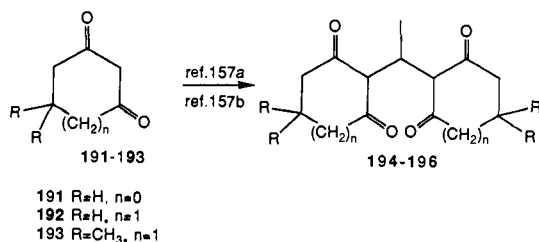
Reductions of cyclic 1,3-diketones being part of a medium-sized ring (Scheme 46) are not as effectively

SCHEME 46



educt	n	R	yield (%)	products (rel. %)	recovered educt (%)
188a	1	-CH ₂ CH ₂ CH ₃	10	189a:190a= 2:98	75
188b	1	-CH ₂ CH=CH ₂	20	189b:190b=100:0	60
188c	1	-CH ₂ C≡CH	60	189c:190c= 71:29	30
188d	1	-CH ₂ C(CH ₃)=CH ₂	40	189d:190d= 55:45	50
188e	2	-CH ₂ CH=CH ₂	5	189e:190e= 82:18	75
188f	3	-CH ₂ CH=CH ₂	0	---	80

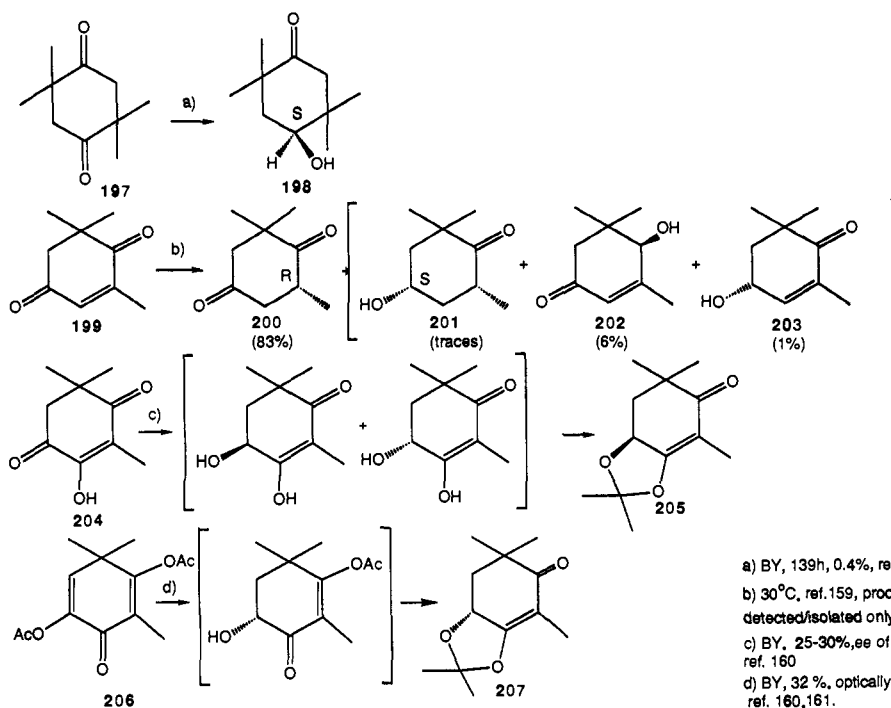
SCHEME 47



achieved as in the case of the five- or six-membered rings and resulted in high recovery rates. Of interest is the opposite diastereoselectivity in the yeast reduction of the propyl dione **188a** versus the allyl dione **188b** and the lack of stereoselectivity for the methyl allyl dione **188d**. The diastereoselectivity of the BY reduction parallels that of the NaBH₄ reduction of these compounds.^{155,156}

In contrast to these results, cyclohexane-1,3-dione (**191**), cyclopentane-1,3-dione (**192**), and 5,5-dimethylcyclohexane-1,3-dione (**193**) (Scheme 47) gave dimers **194–196**, respectively, resulting from a dimerization of

SCHEME 48

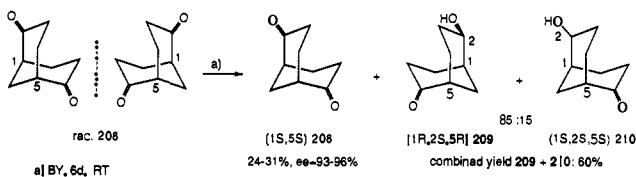


the 1,3-dione with acetaldehyde produced by fermenting yeast.^{157a,b}

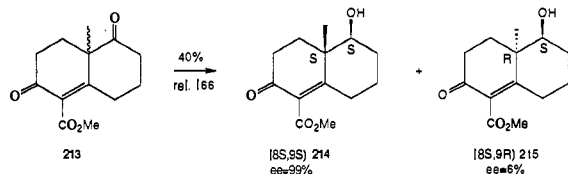
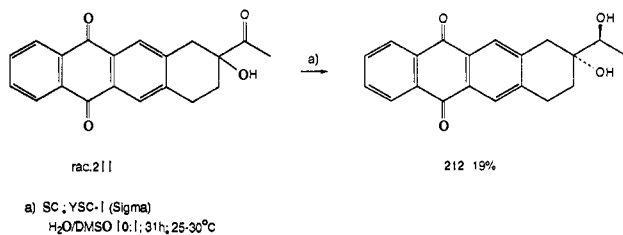
c. *1,4-Diketones*. The reduction of cyclic diketones has not been limited to the 2,2-disubstituted 1,3-diones but has also found extension and applications for cyclic 1,4-diones (Scheme 48). 2,2,5,5-Tetramethyl-1,4-cyclohexanedione (**197**) was relatively inefficiently (0.4%) reduced. The desired reaction product, 4-hydroxy-2,2,5,5-tetramethylcyclohexan-1-one (**198**), however, was efficiently obtained by employing *Curvularia lunata* NRLL2380 (75 h, 98.2% yield, ee > 98%).¹⁵⁸

Of more success was the reduction of oxoisophorone (3,5,5-trimethyl-2-cyclohexene-1,4-dione, **199**), a precursor for the straightforward synthesis of cryptoxanthin and zeaxanthin. This reduction afforded in principal four reduction products although formed at different rate. In general, the reduction of the C–C double bond is very quickly achieved, and a maximum of the main reaction product **200** is reached within the first 32 h (83%). Then, **201** is formed from **200**. **202** and **203** are formed very slowly and in minor concentrations of 6% and 1%, respectively. The rate of formation of **200** is strongly dependent on the concentration of **199**, and best results (with respect to the desired **200**) were obtained with a concentration of 5–6 g/L of **199**. Between 7 and 12 g/L of **199** the fermentation process slowed, and finally, at a value of 12 g/L, inactivation of the cells was found. Interestingly, the yeast cells were inactivated by increasing concentrations of **201–203**, but not by **200**, which precipitated from the fermentation broth. Under optimum conditions the BY cells could be recycled up to six times.¹⁵⁹ Reduction of the 4-hydroxyisophorone analogue **204** followed by acetonization gave 25–30% of **205** with an ee of 65%. The low-tolerated substrate concentration (1 g/L up to 3 g/L if semicontinuous substrate feeding was applied) is a drawback of this method.¹⁶⁰ Use of **206** afforded upon ester hydrolysis and enantioselective double-bond

SCHEME 49



SCHEME 50



hydrogenation of the less hindered enol ester group followed by hydrolysis and acetonization enantiomerically pure **207** in 32% yield.^{160,161}

Racemic bicyclo[3.3.1]nonane-2,6-dione (**208**) (Scheme 49), an intermediate for the synthesis of optically active adamantane compounds, was reduced by BY, and the (1*S*,5*S*)-**208** dione could be recovered in 24% yield (93% ee) on small preparative scale whereas in a larger scale preparation an ee of 66% was achieved (after 2 days), which increased to 83% after a total reaction time of 6 days (31% yield). (1*R*,2*S*,5*R*)-**209** (60%) and (1*S*,2*S*,5*S*)-**210** in an 85:15 ratio and 5% of diol were isolated. (1*S*,5*S*)-**208** of 60% ee (isolated after 18-h reaction time) was subjected to a second BY transformation (24 h) to yield (1*S*,5*S*)-**208** of 96% ee.¹⁶²

No reduction of the cyclic diketone was achieved with compound **211** (Scheme 50); reduction only occurred at the side chain to afford **212** in 19% yield. The asymmetric center involved did not provide any effect on the stereoselectivity of the microbial reduction.⁶⁹

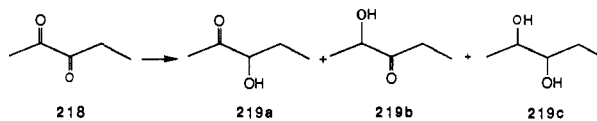
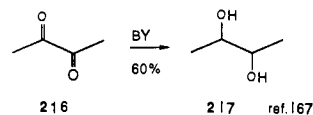
Very early attempts at reduction of quinones were successful although low yields were obtained. Anthroquinone yielded quinizarin,^{163,164} phenanthraquinone,¹⁶⁴ thymoquinone,¹⁶³ α -naphthoquinone,¹⁶³ and *p*-xyloquinone¹⁶⁵ gave the corresponding hydroquinones, and tetrabromo-*o*-quinone¹⁶³ and anthraquinone¹⁶³ proved to be resistant to attack.

Microbial reduction of the racemic diketone **213** afforded 40% of an inseparable mixture of (8*S*,9*S*)-**214** and (8*S*,9*R*)-**215** in a ratio of 77:23.¹⁶⁶

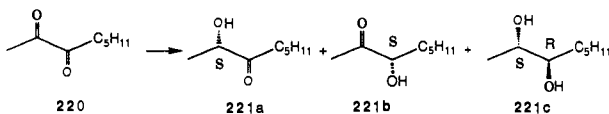
2. Acyclic Diketones

a. 1,2-Diketones. The reduction of acyclic 1,2-diketones by BY is a long-known reaction. Thus, butane-2,3-dione (**216**) (Scheme 51) gave 2,3-butanediol (**217**) in about 60% yield.¹⁶⁷ 2,3-Pentanedione (**218**) and 2,3-octanedione (**220**) gave mixtures of racemic mono- and direduction products **219a-c**^{54,120,168} and **221a-c**^{121b} in high yields. Similar results were obtained for 2,3-hexanedione,¹²⁰ and pentane-2,3,4-trione gave only 2.4% of pentane-2,3,4-triol.⁵⁴ Methylglyoxal was reduced

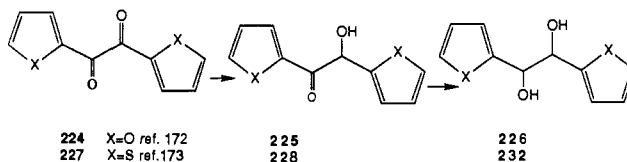
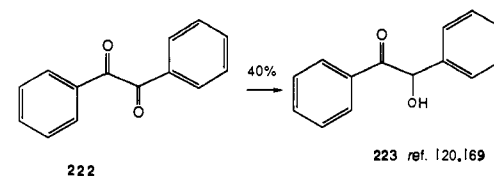
SCHEME 51



BY; SC Type I (Sigma), pH=5, 92%
219a:219b:219c=51:36:13, ref. 120,168



BY (Springer), 1h, 221a: 71%, 92% ee; 221b: 22%; 221c: 7%, 99% ee, ref. 121b



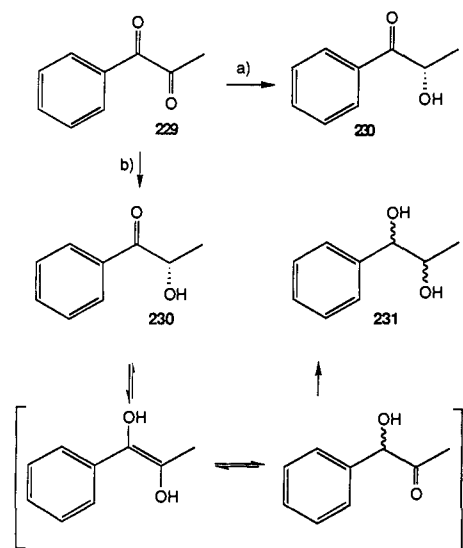
predominantly to D-1,2-propanediol in about 65% yield.⁵⁴

The BY reduction of benzil (**222**) stopped at the monoreduction stage with benzoin (**223**),^{120,169} whereas (S,S)-(94% ee)¹⁷⁰ or (R,R)-hydrobenzoin (96% ee)¹⁷¹ could be obtained by using *Saccharomyces montanus*,¹⁷⁰ *Rhodotorula glutinis*,¹⁷⁰ or *Candida macerans*,¹⁷¹ respectively. In contrast to these findings with **222**,¹⁶⁹ it is of interest to note that furil (**224**) was very quickly reduced via furoin (**225**) into hydrofuroin (**226**).¹⁷² Similarly, **227** and **228** have been reduced by BY to result in formation of **232**.¹⁷³ 1-Phenyl-1,2-propanedione (**229**) (Scheme 52) was reduced by BY at pH 5 to (S)-(-)-2-hydroxy-1-phenyl-1-propanone (**230**) whereas at pH >5 **230** together with the direduction product **231** were obtained. A possible explanation is depicted in the Scheme 52.¹²⁰

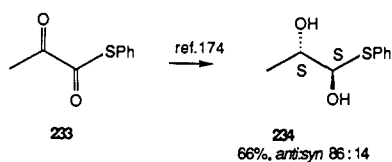
Although 1,2-diketones are good substrates for BY, the selectivity of the reductions is rather low. Introduction of a bulky sulfur-containing moiety (which can easily be removed) is an effective way to stereochemically control these reductions (cf. II.B.4.). Thus, 1-(phenylthio)-2,3-butanedione (**233**) gave mainly (2*S*,3*S*)-1-(phenylthio)-2,3-butanediol (**234**) (anti:syn = 86:14 with a total yield of 66%).¹⁷⁴

Similarly, 1-(1,3-dithian-2-yl)-1,2-propanedione (**235**) (Scheme 53) gave upon BY reduction after 2 h 60% of (S)-1-(1,3-dithian-2-yl)-2-hydroxy-1-propanone (**236**) whereas prolonged reaction time afforded the product of a direduction, (1*S*,2*S*)-**237**, with an ee of 97%; 5% of the syn-configured product **238** could be detected. The large difference between the reduction rates of the two carbonyl groups was attributed to the bulkiness around them. In comparison, reduction of **235** with diisobutylaluminum hydride (-90 °C) gave 74% of a

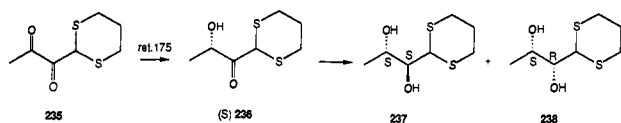
SCHEME 52



a) BY SC Type I/Sigma,
pH=5, 80%, ee>95%
b) pH>5; ref.120



SCHEME 53



mixture of **238** and **237** (syn:anti = 89:11); ZnBH_4 (-90°C) afforded 58% of the mixture of **238** and **237** (syn:anti = 86:16).¹⁷⁵

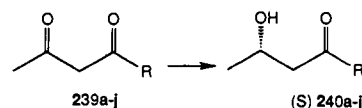
b. 1,3- and 1,4-Diketones. Similarly, reductions of acyclic 1,3-diketones have been attempted, but the results obtained were not satisfying. Thus, 2,4-pentanedione was hydrogenated only slowly and incompletely by fermenting yeast.¹⁷⁶ A comparative study for the reduction of different 2,4-diones **239a-j** (Scheme 54) showed for the reduction by BY excellent ee values with predominant formation of (S)-**240a-j**; only monoreduction was observed.¹⁷⁷⁻¹⁸¹ Reduction by the yeastlike fungus *Geotrichum candidum* or by *Aspergillus niger*, however, proceeded much faster and resulted for **239a,b,h,i** products of opposite configuration.

Analogue **241** (Scheme 55) was easily reduced within 3 days by BY (Hirondelle) in quantitative yield but only with low ee (30%) to (R)-**242**.¹⁷⁷ Reduction of the more lipophilic **243** gave after 100% conversion a 33:67 mixture of ketols **244** and **245**, each exhibiting an ee of 98%.¹⁸⁰

For the 3-methyl-branched compound **246** an 80:20 mixture of the syn-(3R,4S)-**247** and anti-(3S,4S)-**248** isomers was isolated in 30% yield (Scheme 56). This result suggests that there is high enantiofacial selectivity in reduction of the enantiotopic carbonyl groups but low diastereofacial selectivity since (3R)- and (3S)-methyl ketols were obtained.^{180,182a}

4-Methylheptane-3,5-dione (**249**) was found to be reduced only by *Geotrichum candidum*^{182b} to yield

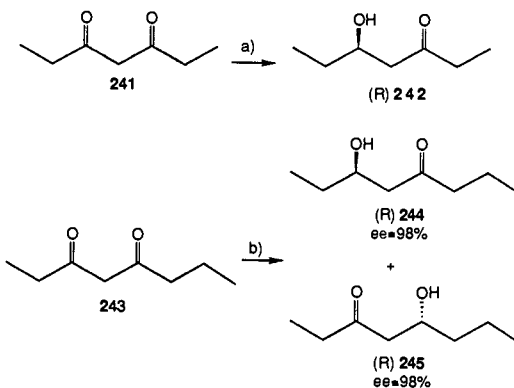
SCHEME 54



	R	t[d]	y[%]	ee[%]	ref.
a	CH ₃	6	90	>99	177,180
		2	18	>99	179
b	C ₂ H ₅	3	100	>99	177,180
		2	48	97	179
c	n-C ₃ H ₇	2	62	94	179
d	n-C ₄ H ₉	2	42	>99	179
e	n-C ₅ H ₁₁	2	25	96	179
f	n-C ₉ H ₁₇	2	23	97	179
g	i-C ₃ H ₇	2	24	92	179
h	C ₆ H ₅	2	21	94	179
		6	33	98	181
i	C(CH ₂) ₃ CH=CH ₂	3	90	98	180 ^a
		3	100	>99	177,180
j	(CH ₂) ₃ R ¹	2	n.r.	97.5	178

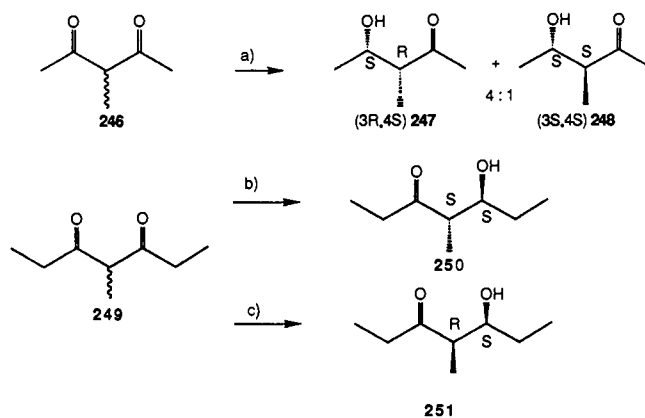
a) mixture of 2-hydroxy- and 4-hydroxyketone 85:15 R¹ =

SCHEME 55

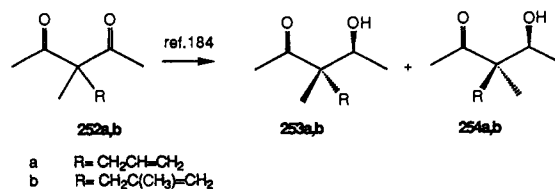


a) BY, 3d, 35°, 100% conversion, ee=30%, ref.177
b) BY, 3d, 35°, 100% conversion, ref.180

SCHEME 56



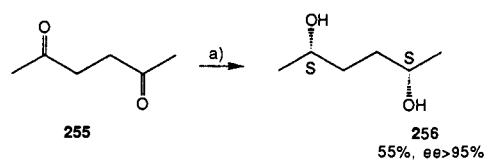
a) BY, 6d, 35°, 30%, ee=95%, ref.177,180
b) *Geotrichum candidum*, aerobic; c) anaerobic



a R = CH₂CH=CH₂
b R = CH₂C(CH₃)=CH₂

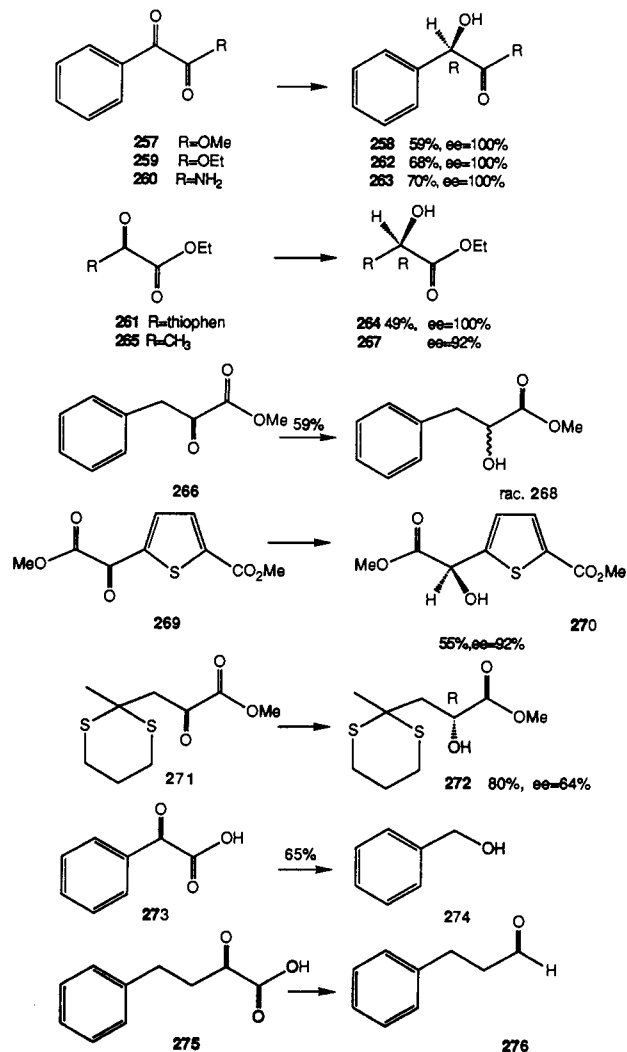
under aerobic conditions (4S,5S)-4-methyl-5-hydroxyheptan-3-one (**250**) or the 4R,5S stereoisomer **251** under anaerobic conditions.^{182b} The reduction by BY failed.

SCHEME 57



a) BY (Budweiser), 144h, ref.185a, 185b

SCHEME 58



251 (contaminated with 0.5% of 250) was found to be the aggregation pheromone sitophilure of the rice weevil *Sitophilus oryzae* L. and the maize weevil *Sitophilus zeamais* Motsch.¹⁸³

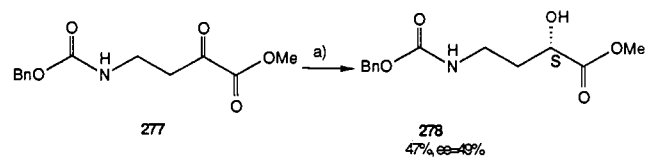
Yeast reduction of the 1,3-diketones **252a,b** bearing a quaternary carbon atom proceeded well and provided mixtures of diastereomeric ketols **253a,b** and **254a,b** with >98% enantiomeric purity.¹⁸⁴

In contrast to the exclusive monoreduction of these 1,3-diketones, 2,5-hexanedione (**255**) (Scheme 57) was cleanly reduced to (2*S*,5*S*)-hexane-2,5-diol (**256**).^{185a} HPLC analysis indicated the presence of *S,S*, *R,S*, and *R,R* diols in the ratio 49.8:1.04:1 (96% ee, 2% meso). This was upgraded to >98% ee and <1% meso by recrystallization.^{185b}

D. Reduction of α -Keto Esters

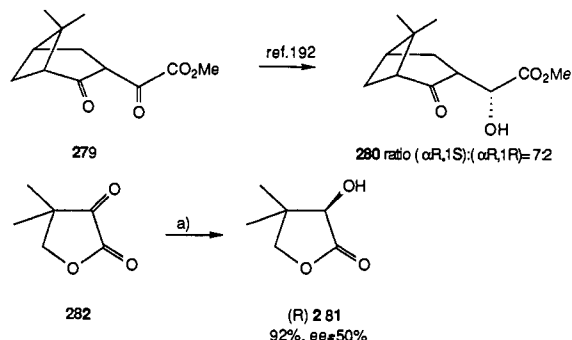
Although there are not as many examples as for the reduction of β -keto esters, α -keto esters have also been

SCHEME 59



a) BY, 12h, r.t., ref.187

SCHEME 60



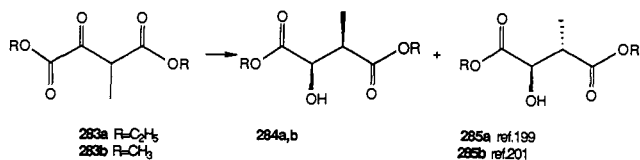
a) BY, 4h, 42%, ref.197, pH=3.2-3.5, aerated, 24h, 30°, ref.193

successfully reduced by BY. 2-Oxo-2-arylacetic acid derivatives **257**, **259**,^{186,187} **260**,¹⁸⁶ and **261**¹⁸⁶ (Scheme 58) gave optically pure α -hydroxy acid derivatives **258**, **262**,^{186,187} **263**,¹⁸⁶ and **264**,¹⁸⁶ respectively. The 2-oxoalkanoic esters **265** and **266** produced alcohols **267** (92% ee) and **268**; the latter product was found to racemize under the reaction conditions.¹⁸⁶ Ethyl pyruvate (**265**) is reduced to (*R*)-ethyl lactate¹⁸⁶ whereas pyruvate is reduced by purified yeast alcohol dehydrogenase in the presence of NADH into (*S*)-lactate.¹⁸⁸ The thiophene derivative **269** was reduced in fair yield to the corresponding alcohol **270**, a precursor in the synthesis of daucic acid, present in wheat, sugar beet, and sunflower.¹⁸⁹ Batyl alcohol, the key intermediate for the preparation of platelet activating factor, was synthesized from the keto ester **271**, which gave on BY treatment the corresponding (*R*)-(+)-alcohol **272** in high yield (80%), but only with a moderate ee of 64%. Use of *Saccharomyces cerevisiae* Kisato improved the ee (89%), although the yield dropped significantly (22%). The highest ee (99%) but very low yield (15%) were finally achieved, however, with *Torulopsis sp.* Jyozok-yokai 17,¹⁹⁰ keto acid **273**, however, decarboxylated under the same conditions to give 65% of benzyl alcohol (**274**),⁷⁶ whereas **275** afforded aldehyde **276**.¹⁹¹ **277** (Scheme 59) yielded after 12-h incubation with fermenting BY 47% of (*S*)-**278** (49% ee), which served as a precursor for the synthesis of the antibiotic butirosin.¹⁸⁷

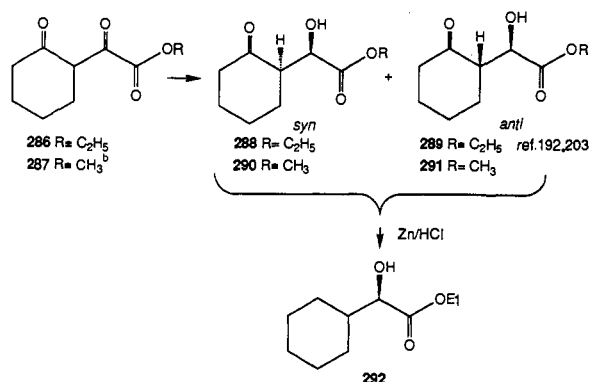
Regiodifferentiation has been achieved for the bicyclic educt **279** (Scheme 60), affording upon reduction a 7:2 mixture of diastereomers **280**.¹⁹²

The synthesis of enantiomerically pure (*R*)-pantolactone (**281**) was achieved via enantiospecific reduction of ketopantolactone (**282**). Among a broad variety of microorganisms¹⁹³ highest optical and chemical yields have been reported with *Rhodoturula minuta*,¹⁹⁴ *Candida parapsilosis* and *Aspergillus niger*,¹⁹⁵ or *Byssoschlamys fulva*¹⁹⁶ whereas the BY (Fleischmann's, Red Star, or Anheuser-Busch) mediated reduction gave low yields¹⁹⁷ or low ee values.¹⁹³ In addition, a 2-ketopan-

SCHEME 61



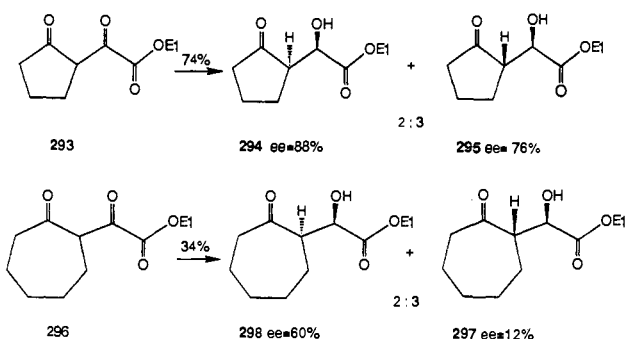
SCHEME 62



yeast/substrate	[t] ^a	y[%]	ratio 288:289
5.5	40	29	18:82
2.5	50	57	11:89
1.2	30	36	10:90

a) BY (Oriental), 32°, ee for both 100%; ee of 292 >99%, ref.203

b) 290 : 291 = 1 : 3, ee>95%, ref.192



tolactone reductase (optimum pH 7) and a 2-ketopantoic acid reductase (optimum pH 5) have been isolated from baker's yeast.¹⁹⁸

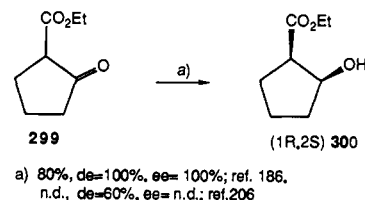
E. Reduction of α,γ -Diketo Ester and Keto α,γ -Diester

Diethyl 2-methyl-3-oxosuccinate (**283a**) (Scheme 61) gave a 43:57 mixture of *syn*-(2*R*,3*R*)-**284a** (79% ee) and *anti*-(2*S*,3*R*)-**285a** (31% ee). Higher ee's were obtained by use of *Candida albicans* instead of BY.¹⁹⁹ It was shown that formation of **284** decreases with increasing substrate concentration.

Low ee values (of 65 and 20%, respectively) were obtained for the reduction (57% yield) of dimethyl 2-methyl-3-oxosuccinate (**283b**), affording an inseparable mixture²⁰⁰ of dimethyl 2-methylmalates²⁰¹ **284b** and **285b** in a ratio of 53:47. A lower yield (22%) but a better ratio (**284b**:**285b** = 64:36) and higher ee values (95% and 58%²⁰²) were obtained with *Candida albicans*.

Several diketo esters of differing structural type have been reduced by BY. Optically pure (*R*)-(-)-hexahydroxymandelic acid has been synthesized by reduction of the cyclohexanone derivatives **286** and **287** under fermenting conditions (Scheme 62). Mixtures of *syn*-

SCHEME 63



288 and *anti*-**289** or of **290** and **291** (1:3, ee = 95% for both components) were obtained, which gave on subsequent treatment with Zn/HCl the optically pure (*R*)-(-)-hexahydroxymandelic ester **292** with an ee >99%.^{192,203}

It was found for **286** that the ratio of *syn* to *anti* depended on the relative amount of BY to substrate. The chemical yield and the *anti* selectivity increased as the ratio of the amount of yeast was decreased. No higher yield or better diastereoselectivity was obtained with immobilized yeast.²⁰³

The cyclopentanone analogue **293** gave higher yields (74%) of **294** and **295** (in a ratio of 2:3) although with reduced enantiomeric excess. With the cycloheptanone **296** both yields and ee's dropped. Only 34% of **297** (12% ee) and **298** (60%) were obtained (**297**:**298** = 3:2).¹⁹²

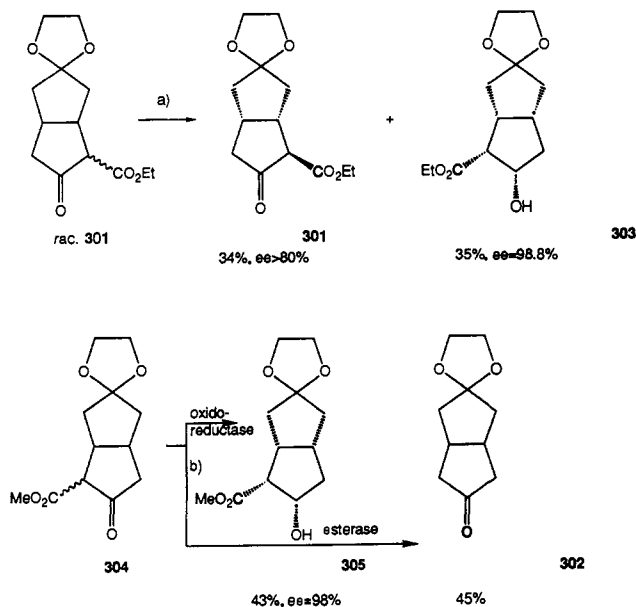
F. Reduction of β -Keto Esters

1. β -Keto Esters with the Keto Group Being Part of the Ring

The enantioselectivity of the reduction of cyclic keto esters seems to be higher than that of open-chain β -keto esters substituted at C-2 (cf. F.2., Scheme 69).^{204,205} There are several examples of the reduction of cyclic β -keto esters by BY. Thus, reduction of **299** (Scheme 63) is performed in 80% yield, leading to a de of 100% of *cis*-**300**,¹⁸⁶ in another experiment,²⁰⁶ **300** was obtained with a de of 60%. These reductions with BY produce 2-hydroxy esters of predominant 1*R*,2*S* configuration. In general, *Saccharomyces cerevisiae* gives for these types of compounds often mixtures of optically pure diastereomeric hydroxy esters, predominantly of 2*S* configuration, whereas mold strains often exhibit a very high dia- and enantioselection and give only *one* optically pure *cis* or *trans* stereoisomer.²⁰⁶

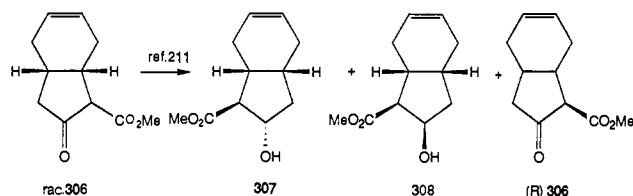
The 1*R*,5*S* enantiomer of racemic methyl (\pm)-3-oxo-7,7-(ethylenedioxy)bicyclo[3.3.0]octane-2-carboxylate (**304**) was reduced in high optical purity to the methyl (1*R*,2*R*,3*S*,5*S*) 3-hydroxy-7,7-(ethylenedioxy)bicyclo[3.3.0]octane-2-carboxylate (**305**), whereas the 1*S*,5*R* enantiomer of **304** gave achiral **302** (Scheme 64). Formation of the latter can be explained by a hydrolysis of (1*S*,5*R*)-**304** followed by a subsequent decarboxylation of the proposed intermediate β -keto acid.²⁰⁷ These transformations can be regarded as a simultaneous dual kinetic resolution performed by two different enzymes of BY. Mori et al.²⁰⁸ found the reaction of **301** with fresh BY (Oriental) to proceed smoothly and to give (+)-**301** in 34% yield (ee > 80%) together with 35% of *cis*-**303** (ee = 98.8%). In comparison, dry BY gave **301** in 61.8% ee. *Saccharomyces bailii* yielded **301** of 92–94% ee, but its use was more time-consuming due to the necessity of performing a precultivation of the microorganism.²⁰⁹ In addition, BY was found to remove the acetal group under fermenting conditions.²⁰⁹

SCHEME 64



a) BY, 30°C, 24h, pH=7; ref.208,209
 b) BY, ref.207

SCHEME 65



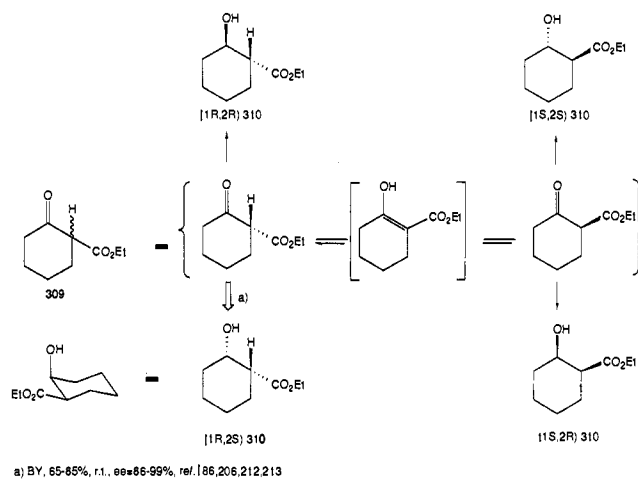
303 is an important precursor for the synthesis of pentalenolactone E, a sesquiterpene antibiotic isolated from cultures of *Streptomyces* 4C5319.²¹⁰ Enantiomerically pure **301** could be transformed into (+)-carba-PGI₂ (carbaprostaglandin). The reduction of racemic methyl 8-oxobicyclo[4.3.0]non-3-ene-7-carboxylate (*rac*-**306**) (Scheme 65) afforded only the *trans* isomer **307** although in low yield, but ee > 99% and 40% of starting material exhibiting an ee of 27% could be recovered whereas *Kloeckera saturnus* gave both *cis* and *trans* isomers (**307**:**308** = 73:27, ee (**307**) = 99%, ee (**308**) = 99%) and the recovered starting material (*R*)-**306** in high optical purity (ee = 96%).²¹¹

The reduction of 6-membered cyclic β -keto esters is more widespread. Reduction of the simplest compound of this class, racemic **309**, was performed by BY (Scheme 66)^{186,206,212a,b} in 65–85% yield and resulted in formation of ethyl (+)-(1*R*,2*S*)-2-hydroxycyclohexanecarboxylate (**310**) in 86% ee²¹³ or with an ee of 96–99%^{206,212a} and a diastereoselection giving rise to a de of 76%,²⁰⁶ 86%^{212b} or 99%.^{212a} One of the possible diastereomers (of *cis* configuration) was produced in excess—a fact that was due to an equilibrium by enolization (with concomitant racemization) of the educt followed by kinetic resolution.

The allyl-branched derivative **311** (Scheme 67) was found to be a less suitable substrate for BY. It was reduced only to an extent of about 10% to (1*S*,2*S*)-**312**, and 20% of starting material was recovered, comprising an ee of approximately 27%.²¹³

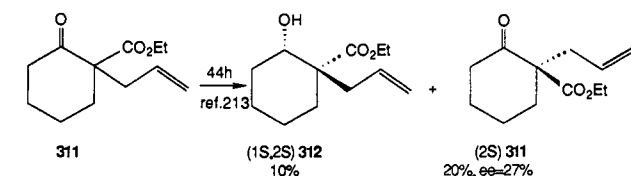
Reduction of racemic ethyl 2-oxo-4,4-(ethylenedioxy)cyclohexanecarboxylate (**313**) (Scheme 68) by BY

SCHEME 66

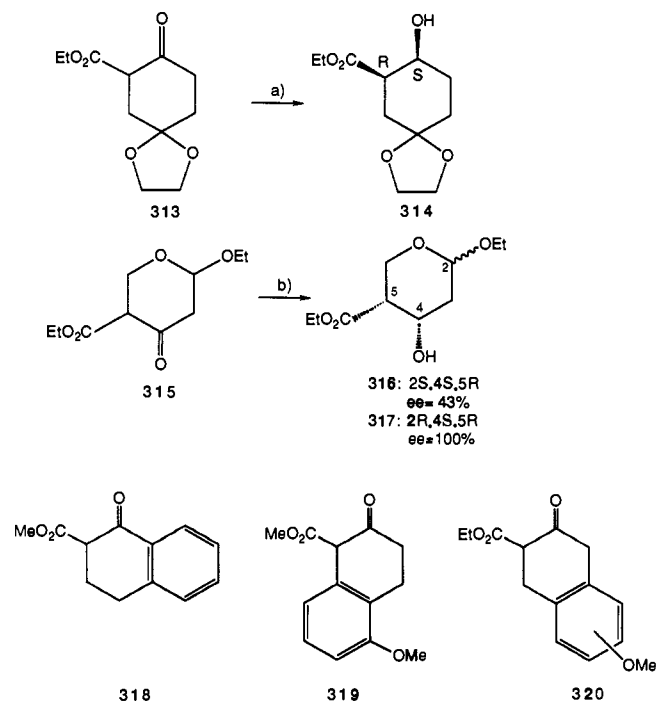


a) BY, 65–85%, r.t., ee=86–99%, ref.186,206,212,213

SCHEME 67



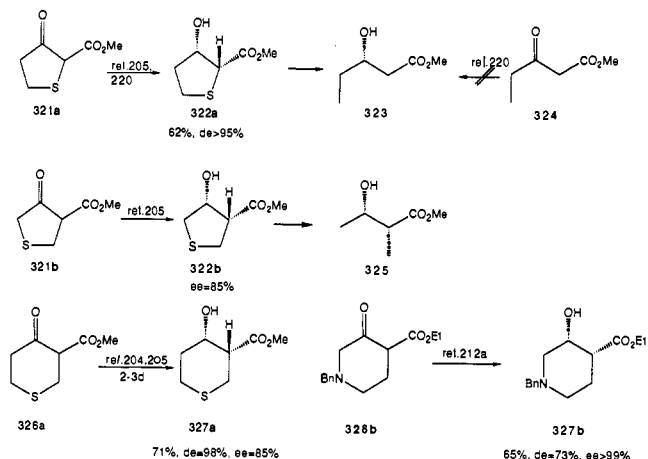
SCHEME 68



a) 74–86%, 30°C, 12h, aeration, ee=98.4%, recovered ca. 15%; ref.212,214,215
 b) 81%, 1h, 30°C, pH=8, Triton X, ref.219

afforded in 74% yield the corresponding ethyl (1*R*,2*S*)-4,4-(ethylenedioxy)-2-hydroxycyclohexanecarboxylate (**314**),^{214,215} which was used²¹⁵ for the synthesis of sporogen-AO-1, a sporogenic sesquiterpene from *Aspergillus oryzae*.^{210,216–218} The aerated fermentative reduction of **315** afforded a mixture of diastereomers **316** and **317** in a ratio of 63:27. Interestingly, (2*S*,4*S*,5*R*)-**316** was obtained only with an ee of 43% whereas the minor (2*R*,4*S*,5*R*)-**317** was found to exhibit an ee of 100%. Both *Saccharomyces bailii* and *Pichia*

SCHEME 69



terricola were found to be unsuitable for this reduction of **315**.²¹⁹

Several benzo-annulated oxo esters, e.g., of type **318–320**, were successfully reduced to their corresponding cis hydroxy esters under “starving conditions” in fair to excellent yield, de, and ee.^{212a}

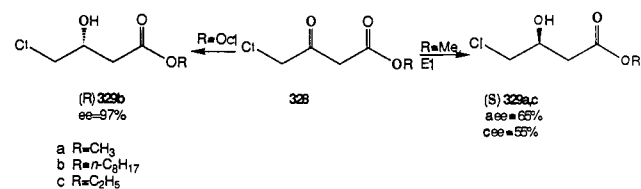
The reduction of such cyclic β -keto esters can be extended to 5- and 6-membered rings containing one additional ring heteroatom instead of a carbon. Reduction of methyl tetrahydro-3-oxothiophene-2-carboxylate (**321a**) (Scheme 69) yielded in high diastereomeric excess (>95%) the corresponding (2*R*,3*S*)-3-hydroxy derivative **322a**.²⁰⁵ **322a** afforded upon treatment with Raney nickel (3*S*)-**323**, which cannot be obtained by BY reduction of the keto ester **324**.²²⁰

Similarly, **321b** gave with an ee of 85% **322b**;²⁰⁵ the analogous reduction of the methyl thiacyclohexanone-2-carboxylate (**326a**) is the key step in a synthesis of (4*RS*,6*S*,7*S*)-serricornine, a pheromone of the cigarette beetle.²⁰⁵ **327a** is obtained in 98% diastereomeric purity, 85% ee, and a yield of 71%.²⁰⁴ No higher ee was obtained for this transformation with the corresponding acid instead of the ester or by working in a more diluted solution.²⁰⁴ The piperidone derivative **326b** afforded **327b** in 65% yield (de = 73%, ee > 95%).^{212a}

2. Aliphatic Keto Esters

a. General Remarks. Reductions of β -keto esters by BY are well documented and have been reviewed recently.^{26,55,56,221–223} The general feature for these reductions is for most cases well explained by Prelog’s rule.³⁰ When an exception to this rule is found, it is generally assumed that an enzyme system other than alcohol dehydrogenase is used for “anomalous” biohydrogenation.²²⁴ Since discussions of stereochemistry in terms of involved enzymes have already taken place,⁵⁵ this will be omitted here. However, it was established that the absolute configuration and the optical purity of the products depend strongly both upon the nature and the size of the substituents adjacent to the carbonyl group and of the ester moiety but also—in some instances—upon the substrate concentration,²²⁵ the pH,^{226a} the concentration of glucose,^{226b} and the cultivation conditions of the yeast.²²⁷ Thus, low concentrations of substrate often give better enantiospecificity because at low concentrations specificity is determined by the relative V_{max}/K_m , while at higher concentrations

SCHEME 70



it is the relative V_{max} that determines specificity. The stereochemistry of the reduction can be influenced to some extent by the addition of certain α,β -unsaturated carbonyl compounds. These additives tend to shift the stereochemistry to the production of the D-hydroxy ester.^{226b}

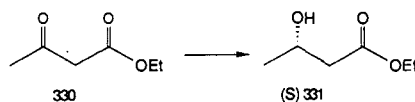
The changes in the stereochemical course caused by different physiological states of the yeast cells (e.g., glucose-grown versus methanol-grown cells) are probably due to the induction of different oxidoreductases.^{227,228} A simulation for quantitative mathematical treatment of the kinetics of competing enzymes as for the reduction of **328** (Scheme 70) has been performed.²²⁹ The low ee’s obtained for several educts have very often to be explained by invoking the participation within the reduction process of two or more different enzymes^{228a,b,230} acting with opposite stereochemistry. It was the merit of C. J. Sih to establish that the stereochemistry of BY reductions of β -keto carbonyl compounds may be influenced by substituents at both ends of the molecule by the action of different oxidoreductases operating with different rates.^{29,228b} Thus, the preferred substrates for the enzymes leading to *R*-type products are those with a large hydrophobic substituent at position C-4 whereas enzymes yielding *S*-type products should prefer substrates bearing large hydrophobic ester moieties.^{226,228b}

Three oxidoreductases capable of actively reducing β -keto esters have been isolated from the cytosolic fraction of Red-Star BY. These enzymes of molecular weight 2 400 000 (a fatty acid synthetase), 74 000 (the L enzyme, leading to carbinols of L configuration), and 38 000 (the D enzyme) were purified to homogeneity, and their respective Michaelis constants and turnover numbers were measured.^{228b}

Sih and co-workers^{29,228b,231} investigated the BY reduction of γ -chloroacetoacetates **328** (Scheme 70) in more detail. It was shown that the stereochemical course for this reduction can quite efficiently be altered by changing the size of the ester grouping. No significant difference in the rates of the reductions were observed for educts containing esters up to eight carbons, whereas the C₁₆ ester was not reduced at all. The methyl ester **328a** was reduced to (S)-**329a** with an ee (S) of 65% whereas the octyl ester **328b** was reduced to the *R* enantiomer **329b** in high yield (70%) and with an excellent ee (*R*) of 97%.^{29,230} The reduction of **328c** gave (S)-**329c**, although with low yield (30%) and low ee (50–60%).²³² It is of interest to note that there was a change in the stereochemistry of the carbinols derived from the butyl and the pentyl esters.²⁹

b. Reduction of Ethyl Acetoacetate. The simplest case, the reduction of ethyl acetoacetate (**330**) (Scheme 71) to the corresponding (S)-hydroxybutanoate **331** has been performed many times although with differing yields and ee’s.^{76,136,225,233–240} **331** has been starting material for many syntheses: e.g. for the main com-

SCHEME 71



Reduction of 330

yield (%)	ee (%)	ref.	yield (%)	ee (%)	ref.
54	92	233	n.r.	90	236
57	84	234	59-76	85	237
n.r.	88	241	67	86	84
36	97	243	70	94	238
n.r.	90	245	32	96	239
64	>97	246	60-80	>98	225
56	82	248	80	n.r.	76
n.r.	95	250	66	77	136
64	>97	235	62	95	240

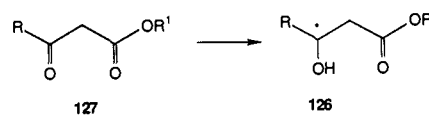
ponent of the cephalic segregation of *Andrena wilkella*, 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane,²⁴¹ for phoracantholides,²⁴² for (S)-(-)-citronellol,²⁴¹ for (6R,11R,12R,14R)-colletodial (a metabolite of the plant pathogen *Colletotrichum capsici*),²⁴³ for the carbapenem thienamycin,^{244,245} for the C-2-C-5 building block of griseoviridin,^{246,247} for (S)-2-methyloxetane,²⁴⁸ for 2-methyl-1,7-dioxaspiro[5.6]dodecane (the volatile secretion from the mandibular glands of *Andrena haemorrha* F.),²⁴⁹ for (2R,5S)-2-methylhexanolide,²⁵⁰ for (3S,11S)-3,11-dimethyl-2-nonacosanone (the sexual pheromone of the German cockroach),²⁵⁰ for serricornin,²⁵⁰ for (S)-(+)-sulcatol,⁸⁴ and for the synthesis of nystatin²⁵¹ and amphotericin B.²⁵¹ Unfortunately, an unacceptable broad bias for the quoted optical rotations of 331 is found, and the reported ee values determined by NMR vary from 70% to >97%.²⁴⁸

It was shown that this reduction strongly depends on the reducing conditions. Thus, replacement of the carbon source in the medium by other nutrients (fructose, (R)-lactate, (S)-lactate, acetate, glycerol, mannitol, glucuronolactone) showed that only glucuronolactone completed the conversion of 330. "Best conditions" were claimed to be "starving conditions",^{212a} a 4-day treatment of the yeast with 5% ethanol prior to the addition of 330.²³⁸ Another group claimed that interrupting the fermentation after 4 h will lead to 94-96% pure material.⁸⁴ Several of the results obtained for this reduction are summarized in Scheme 71, although some of the procedures given seem not to be reproducible with respect to yield and/or ee. The activating effect of allylic alcohol has been demonstrated.¹³⁶

Leuenberger et al.²⁵² have investigated this reduction very carefully under controlled conditions even on a large industrial scale, and these authors found a decrease of optical purity with increasing concentration of the educt (up to 1 g/L for ee = 95-97%, 20 g/L for ee = 58%). Performing the reduction with *Geotrichum candidum* led to (R)-331 in 36% yield and 90% ee. The latter reduction was found to depend strongly on the physiological state of the cells.²⁵² The reduction of 330 by many other microorganisms has been compared and investigated.^{67,68}

c. *Reduction of Alkyl-Substituted β -Keto Esters.* Some reductions of β -keto esters are worthwhile to comment on in more detail. The results for "simple" alkyl-substituted β -keto esters^{29,136,186,224-227,235,236,239,253-258} are summarized in Scheme 72. It seems noteworthy that while acetoacetates were reduced predominantly to (S)-3-hydroxybutanoates, β -ketovalerates (and all other β -ketoalkanoates 127 with R > CH₃) gave predominantly their respective (R)-3-hydroxyalkanoates

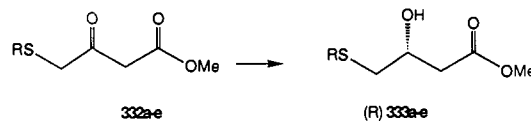
SCHEME 72



R	R ¹	y (%)	conf.	ee (%)	ref.
CH ₃	CH ₃	23	S	87	253
CH ₃	n-C ₄ H ₉	58	S	90	254
CH ₃	t-C ₄ H ₉	61	S	85	254
		45	S	77	239
C ₂ H ₅	CH ₃	60-80	R	5	225
		47	R	54	227
		56	R	96	136 ^a
C ₂ H ₅	C ₂ H ₅	67	R	40	236 ^b
C ₆ H ₅	C ₂ H ₅	70	S	100 ^c	224, 186
C ₂ H ₅	C ₈ H ₁₇	75	R	95	255
		67	R	100	29
C ₃ H ₇	C ₈ H ₁₇	12	S	71	29
C ₄ H ₉	C ₂ H ₅	32	R	90-99	236, 256
CH ₂ CH ₂ CH=CH ₂	H	35	R	99	257
n-C ₁₅ H ₃₁	K	40	R	98	258 ^a
CH ₂ CH ₂ CH=CH ₂	K	38	R	>99	239
"	CH ₃	30	R	92	239
"	C ₂ H ₅	54	R	80	239
"	t-C ₄ H ₉	66	R	81	239
CH ₂ CH ₂ C(CH ₃)=CH ₂	K	55	R	>99	239
"	CH ₃	18	R	67	239
"	C ₂ H ₅	15	R	18	239
"	t-C ₄ H ₉	0	-	-	239
CH ₂ CH ₂ CH=C(CH ₃) ₂	K	59	R	>99	239
"	CH ₃	73	R	92	239
"	C ₂ H ₅	12	R	50	239
"	t-C ₄ H ₉	0	-	-	239

^a on addition of 1g/L of allylic alcohol; without activator an ee of 59% was reported.¹³⁶ ^b 18h, room temperature; material of higher enantiomeric purity was obtained by acidic depolymerization of natural heteropolymer (R-CH(OH)-CH₂-C(=O))_nOH with R: 20% Et, 80% Me 254; ^c at high yeast:substance ratio.

SCHEME 73



entry	R	y (%) ^a	ee (%) ^a	y (%) ^b	ee (%) ^b
		(R)-333	(R)-333	(S)-333	(S)-333
a	C ₂ H ₅	55	70	41	85
b	n-C ₃ H ₇	49	65	36	85
c	n-C ₄ H ₉	67	70	53	80
d	n-C ₅ H ₁₁	30	58	28	85
e	p-Cl-C ₆ H ₄	40	50	30	80
f	C ₆ H ₅	42	73	50	50

a) by redn. using SC NCYC1765
b) --- candida guilliermondi

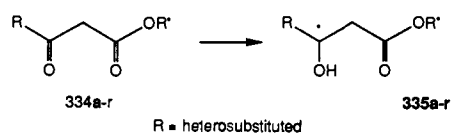
126. A better access to (R)-3-hydroxybutanoate was found by the reduction of ethyl acetoacetate with *Thermoanaerobium brockii*.²⁵⁹

d. *Reduction of δ -Heteroatomic Substituted β -Keto Esters.* The reduction of δ -thio-substituted keto esters 332a-e (Scheme 73) with *Saccharomyces cerevisiae* (NCYC1765) afforded the corresponding hydroxy esters (R)-333a-e²⁶⁰ whereas upon treatment with *Candida guilliermondi* (S)-333a-e could be obtained.²⁶⁰

The results for reduction of β -keto esters^{136,224-226,232,239,260-262} containing a further heteroatom-substituted moiety attached to C-4 are summarized in Scheme 74.

Dramatic differences in the reduction of 334q (Scheme 74) (to yield 335q) have been found that were related to the incubation conditions in general and the yeast to substrate ratio in particular. Addition of 334q as an ethanolic solution over 6 h to a suspension of yeast (38 g/mmol of 334q) afforded (R)-335q after 1 day of incubation with 71% ee and 73% yield. The stereo-

SCHEME 74



entry	R	R'	y (%)	conf.	ee (%)	ref.
a	-CH ₂ Cl	CH ₃	60-80	R	a	225, 232
			60	R	70	260
b	-CH ₂ Cl	C ₂ H ₅	60-80	R	50-60	232, 136
			30	S	b	225
c	-CH ₂ -Br	C ₂ H ₅	40-50	S	100 ^c	226
d	-CH ₂ -Br	CH ₂ -C ₆ H ₅	40-50	S	100 ^d	226
e	-CH ₂ -Br	C ₃ H ₇	40-50	S	100 ^d	226
f	-CH ₂ -Br	C ₇ H ₁₅	40-50	S	100 ^d	226
g	-CH ₂ -Br	C ₈ H ₁₇	40-40	S	100 ^d	226
h	-CCl ₃	C ₂ H ₅	70	S	84-88	261
i	-CF ₃	C ₂ H ₅	75	R	49-51	261 ^e
j	-CH ₂ -N ₃	C ₂ H ₅	70-80	R	80	226 ^f
k	-CH ₂ -N ₃	CH ₂ -C ₆ H ₅	70-80	R	95	226 ^f
l	-CH ₂ -N ₃	CH ₂ CH ₂ -C ₆ H ₅	70-80	R	95	226 ^f
m	-CH ₂ -N ₃	(CH ₂) ₆ -C ₆ H ₅	70-80	R	100	226 ^f
n	-CH ₂ -N ₃	(CH ₂) ₇ -C ₆ H ₅	70-80	R	100	226 ^f
o	-CH ₂ -O-t-C ₄ H ₉	CH ₃	72	R	97	262
p	-CH ₂ -O-t-C ₄ H ₉	C ₂ H ₅	70	R	82	262
q	-CH ₂ OCH ₂ -C ₆ H ₅	C ₂ H ₅	73	R	71	224 ^g
			58	R	56	262
r	-(CH ₂) ₃ -O-CH ₂ -C ₆ H ₅	K	51	R	>99	224 ^h
				S	48	239 ⁱ

a the ee was shown to depend on the concentration of the educt: 10 mM gave 31 % ee, 20 mM gave 12% ee.²²⁵; b the ee was shown to depend on the concentration of the educt; 10 mM concentration of educt afforded product of 42 % ee whereas 20 mM gave 27% ee and 50 mM only 15% ee.²²⁵; c at pH=8; with pH=6 an ee of 60% was achieved; d at pH=8; e at a concentration of 15g/L of educt, 30^o, 44h; 2 recrystallizations of the 3,5-dinitrobenzoate afforded product with ee>98%; data reported in refs. 263, 264 are erroneous.²⁶¹; f at pH=7.5-8; g educt was added as a solution in EtOH during 6h; ld, 38 g yeast per mmol of educt. h educt added at once, 6d; 0.75 g yeast per mmol of educt; i the corresponding derivatives with R'-Me, Et, or t-Bu gave no reaction at all.²³⁹

SCHEME 75

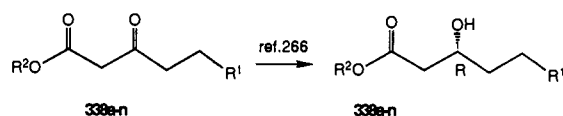


entry	R	y (%)	config.	ee (%)	ref.
a	O-CH ₃	58;65	n.r.;R	30;60	265, 266
b	O-C ₂ H ₅	60	S	56	265
c	O-i-C ₃ H ₇	40	S	34	265
d	O-n-C ₄ H ₉	55	S	70	265
e	O-CH ₂ CH(CH ₃) ₂	70	S	90	265
f	O-n-C ₅ H ₁₁	40	S	92	265
g	O-CH ₂ CH ₂ CH(CH ₃) ₂	35	S	54	265
h	O-CH ₂ C(CH ₃) ₃	35	S	96	265
i	O-C ₆ H ₁₃	10	S	96	265
j	O-C ₈ H ₁₇	0	-	--	265
k	OK	10	S	n.r.	265
l	S-C ₄ H ₉	40	S	68	265
m	S-CH ₂ CH(CH ₃) ₂	30	S	74	265

chemical outcome of this reduction is reversed when **334q** is added at once and only 0.75 g/mmol of yeast is added. After 6 days of incubation (S)-**335q** is obtained with 48% ee.²²⁴

Enantioselectivity of the yeast-mediated reduction of 5-(benzyloxy)-3-oxopentanoate esters **336a-m** (Scheme 75)^{265,266} was influenced by changes in the ester alkoxy group in a way that the enantioselectivity increases with increasing chain length for the *n*-alkyl esters. However, the amount of conversion of substrate to product **337a-m** decreased with increasing chain length. For branched hydrocarbon groups, there seems

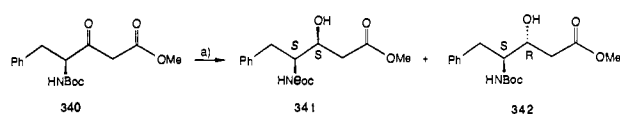
SCHEME 76



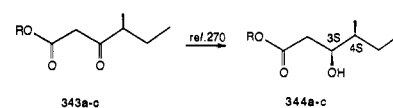
entry	R ¹	R ²	y (%)	config.	ee (%)	of 339	ref
a	OCH ₃	CH ₃	11	S	33.3		266
b	OCH ₃	C ₂ H ₅	41	S	66.1		266
c	OCH ₃	C ₃ H ₇	40	S	80.0		266
d	OCH ₃	C ₄ H ₉	48	S	82.1		266
e	OCH ₃	C ₅ H ₁₁	26	S	80.2		266
f	OCH ₃	C ₆ H ₁₃	15	S	76.7		266
g	OCH ₃	C ₇ H ₁₅	9	S	71.4		266
h	OH	C ₄ H ₉	33	S	79.8		266
i	OH	C ₅ H ₁₁	59	S	86.7		266
j	OH	C ₆ H ₁₃	3	S	77.8		266
k	O(CH ₂) ₂ Si(CH ₃) ₃	CH ₃	41	R	33.3		266
l	SCH ₃	CH ₃	28	S	20.0		266
m	SC ₆ H ₅	CH ₃	88	R	41.2		266
			42	R	73.0		260
			32	R	88		267
			36	S	65		268 ^a
n	SC ₆ H ₅	K	8	S	100		268 ^b

a using *Saccharomyces SCNCYC240*; b isolated as methylester

SCHEME 77



a) 30-50%, de=60%, ref.269



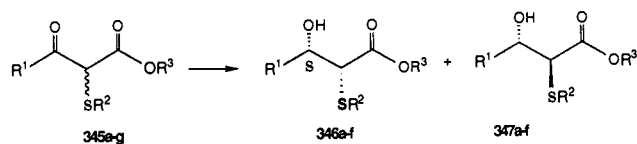
a	R	de (%)
a	K	98
b	CH ₃	96
c	C ₆ H ₅	89

to be an optimum of enantioselectivity with one methylene group spacer before branching but this trend seems unclear. A change of the electronic environment with thioesters (entries l, m) had little effect, and no change in the enantioselectivity of this reduction was observed.²⁶⁵

For **338a-g** (Scheme 76) both enantiomer ratio and the chemical yield increased on going from the methyl ester (entry a) to the butyl ester (entry d) and then fell off toward the heptyl ester (entry g). The esters with yet a smaller and more hydrophilic OH group (entries h-j) exhibited a similar trend, but in this series the maximum was obtained in the reaction of the pentyl ester (entry i). These results²⁶⁶ are in contrast to the results obtained by Sih et al.^{29,231} on the reduction of **328**. In this case, the preferred enantiomer has the same configuration when R¹ is sufficiently large in both series.²⁶⁶ The results for **338m** are somewhat controversial^{260,266-268} with respect to yield and ee. Use of *Candida guilliermondii* (NCYC973 or NCYC1399) for some of these educts gave predominantly (S)-**339** in comparable yields and ee values between 83 and 90%.²⁶⁰

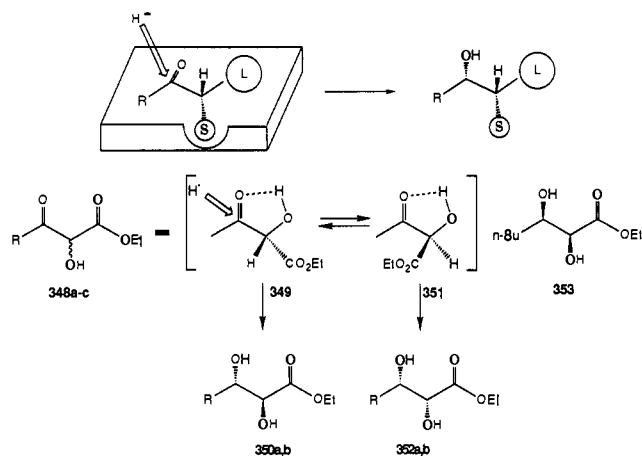
e. *β*-Keto Ester with an Additional Center of Chirality. An additional center of chirality in the side chain as in **340** (Scheme 77) gave upon treatment with BY 30-50% of (3S,4S)-**341** (major) and (3R,4S)-**342** (minor); the de of 60% could be improved either by 2-fold recrystallization (de = 99%) or by use of *Hansenula anomala* to yield (3S,4S)-**341** with de = 92% whereas *Candida boidini* gave predominantly

SCHEME 78



entry	R ¹	R ²	R ³	y (%) of 346	y (%) of 347
a	CH ₃	CH ₃	CH ₃	52	20
b	CH ₃	CH ₃	C ₂ H ₅	37	25
c	CH ₃	CH ₃	C(CH ₃) ₃	31	44
d	CH ₃	C ₆ H ₅	CH ₃	33	7
e	CH ₃	C ₆ H ₅	C ₂ H ₅	36	13
f	C ₂ H ₅	CH ₃	C ₂ H ₅	21	23
g	CH ₂ CH ₂ OCH ₂ -C ₆ H ₅	C ₆ H ₅	C(CH ₃) ₃	decomposition	

SCHEME 79



R	y [%] of 350 : 352
a CH ₃	56 : 13
b C ₆ H ₅	62 : 11
c n-C ₄ H ₉	48 : 12 of 353

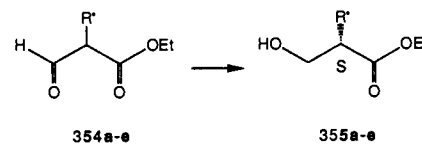
rel. 272

(3*R*,4*S*)-342 with 90% de.²⁶⁹ Similarly, reduction of 343a-c gave 344a-c although with low yields but excellent de values.²⁷⁰

f. α -Substituted β -Keto Ester. The reduction of α -thio-substituted β -keto esters 345a-g (Scheme 78) has been suggested as an alternative to the reduction of β -keto esters 127²⁷¹ since α -sulfenyl esters 346 or 347 can smoothly be desulfenylated by oxidation (*m*-chloroperbenzoic acid, dichloromethane, -78 °C, followed by treatment with amalgamated aluminum). The yields of 346 and 347 are fair, but ee values >96% were found for all compounds investigated. Although these reactions yielded both *syn*-346 and *anti*-347, it should be noted that the *S* configuration in the C-3 position was exclusively obtained in all cases.²⁷¹

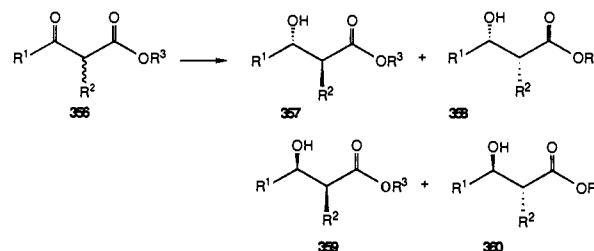
For the reduction of the α -hydroxy keto esters 348a-c a mechanism as depicted in Scheme 79 has been proposed²⁷² to explain the predominant formation of 2*S*,3*S* isomers. If BY prefers *re* face reduction by the Prelog rule, reduction of 349 to form a 5-membered ring with a hydrogen bond between the 2-hydroxy and the carbonyl oxygen at C-3 with less hindered *re* face site to give the 2*S*,3*S* products 350 is more favorable than that of 351 to give 2*R*,3*S* products 352. An equilibrium between 349 and 351 is possible as well as degradation of the 2*R* isomer by BY to cause the predominant formation of the 2*S*,3*S* isomers.²⁷² From 348c (2*S*,3*R*)-353 is formed. Chiral (2*S*,3*S*)- and (2*R*,3*S*)-2,3-dihydroxybutanoic acids have been shown to be

SCHEME 80



entry	R ¹	y (%)	ee (%)	ref.
a	CH ₃	70-80	60-65	282
b	CH ₂ CH ₃	88	91	238, 282
c	CH(CH ₃) ₂	50-70	60	238, 282
d	CH ₂ -C ₆ H ₅	50-70	46	282
e	C ₆ H ₅	50-70	83	282

SCHEME 81



R ¹	R ²	R ³	y (%) of 357	y (%) of 358	ee (%) of 357	ee (%) of 358	ref.
CH ₃	CH ₃	CH ₃	13	58	100	100	283
CH ₃	CH ₃	C ₂ H ₅	16	49	100	100	283, 285, 286
							291, 292
CH ₃	CH ₃	<i>t</i> -C ₄ H ₉	18	22	100	100	283
CH ₃	CH ₃	CH ₂ CH(CH ₃) ₂	10	46	100	100	283
CH ₃	CH ₃	<i>n</i> -C ₅ H ₁₁	11	59	100	100	283
CH ₃	CH ₃	<i>n</i> -C ₈ H ₁₇	4	78	100	100	283
CH ₃	Cl	C ₂ H ₅	34	34	100	n.r.	290
CH ₃	CH ₃	CH ₂ C ₆ H ₅	30	15	97	84	284 ^a

R ¹	R ²	R ³	y (%) of 357	y (%) of 358	ee (%) of 357	ee (%) of 358	ref.
CH ₃	CH ₂ CH=CH ₂	C ₂ H ₅	63	21	100	70	285, 286
CH ₃	CH ₂ -C ₆ H ₅	C ₂ H ₅	15	7	100	85	285
CH ₂ OCH ₂ C ₆ H ₅	CH ₃	CH ₃	45	5	6	68	288

R ¹	R ²	R ³	y (%) of 358	y (%) of 360	ee (%) of 358	ee (%) of 360	ref.
CH ₂ OCH ₂ C ₆ H ₅	CH ₃	C ₂ H ₅	25	21	36	24	288 ^b
CH ₂ OCH ₂ C ₆ H ₅	CH ₃	<i>i</i> -C ₃ H ₇	15	49	96	50	288
CH=CH-C ₆ H ₅	CH ₃	CH ₃	7	-	95	-	289 ^c

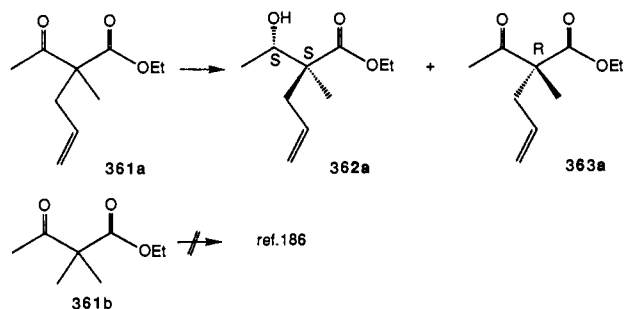
^a better yields were obtained with *Candida albicans* (92%, *syn/anti*-ratio 26/74 with ee's of 97 and 95%, respectively, highest *syn/anti*-ratio 94/6 (ee 97% and 87%) with *Rhodotorula glutinis*, highest ee-values (99%, 99%) with *Pichia farinosa* [*syn/anti* = 50/50]).
^b to obtain the different stereoisomers with higher ee values, *Rhodotorula mucilaginosa*, *Curvularia minuta* and *Geotrichum candidum* have been used²⁸⁸; ^c use of *Candida albicans* gave 35% of 359 with 97% ee.

versatile key intermediates in a variety of natural product syntheses.²⁷³⁻²⁸¹

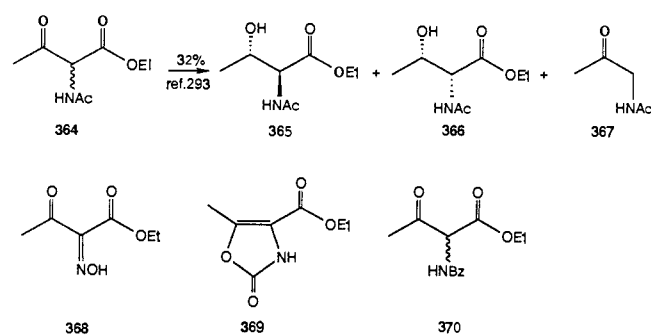
A general model for predicting the diastereoselectivity in yeast reductions has been suggested^{228b} following similar reasoning as in the formulation of Prelog's rule. Thus, size and hydrophobicity of the α -substituent are compared to that of the ester ligand. Such microbiological reductions allow the production predominantly of a single diastereoisomer; these reductions are at the same time both enantioselective and stereospecific.^{53b} This model explains the high *syn/anti* selectivity observed for the reduction of α -substituted β -keto esters.^{228b}

g. Reduction of Formyl Derivatives. Although of great synthetic potential, the reduction of formyl derivatives 354a-e (Scheme 80) has only scarcely been performed;^{238,282} however, 355a-e were obtained in 70-83% yield and ee values ranging from 46 to 91%. As exemplified by 355a the ee could improved to 100%

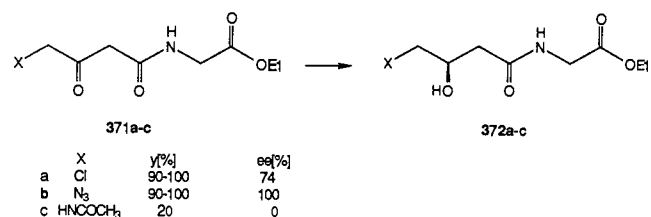
SCHEME 82



SCHEME 83



SCHEME 84



by 6-fold recrystallization of the corresponding 3,5-dinitrobenzoate (yield 40%).²⁸²

h. Miscellaneous Aliphatic β -Keto Esters. β -Keto-butanates **356** (Scheme 81) substituted at the α -position²⁸³⁻²⁹⁰ have been reduced and afforded compounds **357-360**.

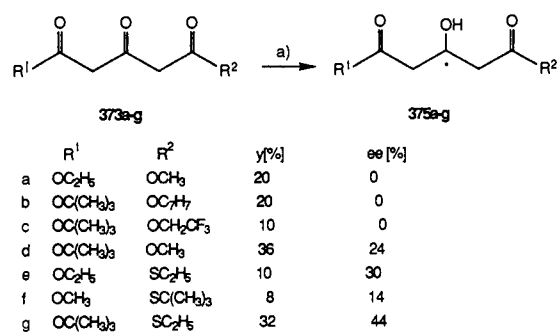
Introduction of a quaternary carbon as in **361a** (Scheme 82) afforded upon BY reduction (*S,S*)-**362a** (20%, ee = 100%) and 38% of (*R,R*)-**363a** of 28% ee,^{285,286} whereas 2,2-dimethylacetoacetate **361b** was not reduced at all.¹⁸⁶

During an L-threonine synthesis, **364** (Scheme 83) was reduced in 32% yield, leading to the diastereomers **365** and **366** in a ratio of 60:40; in addition, 5% of **367** (as a product of hydrolysis and decarboxylation) was identified. Better yields than with BY were obtained with *Saccharomyces rouxii* (60%). No reaction was observed for compounds **368-370**,²⁹³ contrary, other oximes have already successfully been reduced.¹⁶³

BY reduction of 4-substituted 3-oxobutanamides **371a-c** (Scheme 84) gave **372a-c**.²⁹⁴

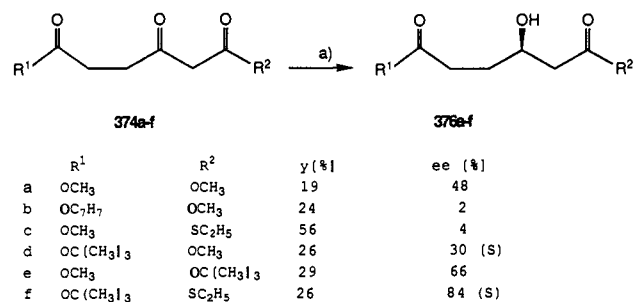
The enantioselectivity of the BY-mediated reduction of prochiral 3-ketoglutarates **373a-g** (Scheme 85) and 3-ketoadipates (Scheme 86) **374a-f** to the corresponding 3-hydroxy esters **375a-g** and **376a-f**, respectively, was influenced by the simple differences in the ester group,²⁹⁵ but for **373a-g** no readily differentiation (by changing in the size of the ester group) was found. For the reduction of **374f**, the best result with 84% ee was obtained.²⁹⁴ Enantiomerically enriched 3-hydroxy-

SCHEME 85



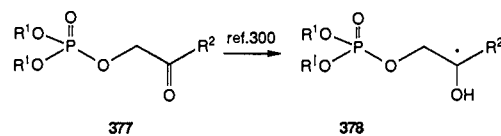
a) 25-30°, pH=7, 24h

SCHEME 86



a) 25-30°, pH=7, 24h

SCHEME 87



glutarates have been synthesized by hydrolysis of the corresponding diesters with the esterase from porcine liver,^{296,297} α -chymotrypsin,²⁹⁸ and *Arthrobacter*²⁹⁹ and *Acinetobacter sp.*²⁹⁹

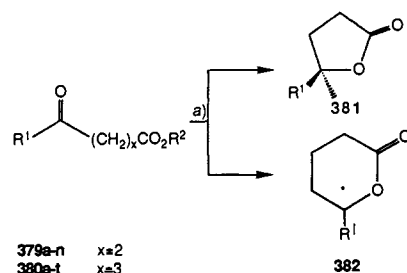
The heteroanalogous keto esters **377** (R¹ and R² alkyl substituents) (Scheme 87) have been reduced by BY to yield the corresponding β -hydroxy phosphates **378**.³⁰⁰ The reactions proceeded well, but due to partial racemization the optical purities of the products were low (0-52%).

G. Reduction of γ - and δ -Keto Acids and Esters

BY reduction of keto acids or esters **379** or **380** (Scheme 88) affords the corresponding γ - or δ -lactones **381** or **382**, respectively. Some of these lactones are insect pheromones. As for the esters it was supposed that first the ester is hydrolyzed by some nonspecific esterase(s) to the corresponding acid, which is the true substrate for the bioreduction.²²⁴ In addition, it was suggested³⁰¹ that the acids are not reduced as such but that their corresponding CoA thioesters are.

Reduction of the simplest compound, namely ethyl levulinate (**379a**, R¹ = Me), proceeded only to a modest extent under a variety of conditions. Approximately 70-85% of starting material was recovered after 48 h of incubation and 10-15% of the reduction product was obtained, which gave on chemical cyclization (*S*)-**381a**.²²⁴ Reduction of an ethanolic solution of potassium 4-oxo-4-phenylbutanoate (**379n**, R¹ = Ph) yielded

SCHEME 88



a) ref.224

Reduction of 379

entry	R ¹	R ²	y (%)	t (h)	ee	conf.	ref.
a	CH ₃	C ₂ H ₅	0	10-15	48	n.r.	S 302
b	CH ₃	H	10	24	46	S	224 306
c	CH ₃	CH ₃	30	24	85.6	S	306
d	C ₂ H ₅	H	16	24	>98	R	306
e	C ₂ H ₅	CH ₃	44	24	83	R	302, 308
f	C ₃ H ₇	H	39	24	>98	R	306
g	C ₄ H ₉	H	44	24	>98	R	302, 306
h	C ₅ H ₁₁	H	77-82	24	>99	R	302-306
i	C ₆ H ₁₃	H	85	24	>99	R	303, 304
j	C ₇ H ₁₅	H	72-90	24	>99	R	302-306
k	C ₈ H ₁₇	H	60-71	24	>99	R	302, 303 304, 306
l	C ₈ H ₁₇	CH ₃	41	24	94	R	305
m	C ₁₁ H ₂₃	K	50	48	>98	R	302
n	C ₆ H ₅	K	31	160	>95	S	224

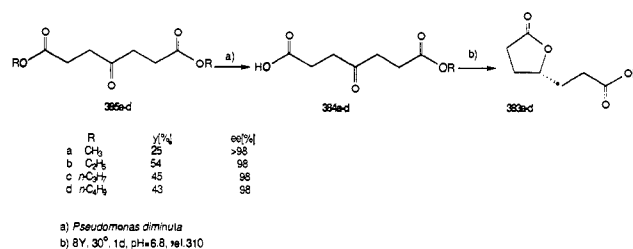
reduction of 380

entry	R ¹	R ²	y (%)	t (h)	ee/de	conf.	ref.
a	CH ₃	H	0	48			302
b	CH ₃	C ₂ H ₅	0	48			302
c	C ₂ H ₅	H	6	48	>98	R	302
d	C ₂ H ₅	C ₂ H ₅	11	48	>98	R	302
e	C ₃ H ₇	H	13-30	24	83	R	224, 303
f	C ₃ H ₇	C ₂ H ₅	54-67	48	>98	R	302, 304
g	C ₄ H ₉	H	35	24	95	R	302
h	C ₄ H ₉	K	55-68	48	>98	R	303, 304
i	C ₄ H ₉	C ₂ H ₅	67	24	98	R	307
j	C ₅ H ₁₁	H	71	48	>98	R	302
k	C ₅ H ₁₁	H	30-47	24	>98	R	224, 303
l	C ₆ H ₁₃	H	45-75	48	>98	R	224, 303
m	C ₆ H ₁₃	H	30	24	>98	R	224
n	C ₇ H ₁₅	H	63	24	>98	R	303, 304
o	C ₇ H ₁₅	H	35-56	24	>98	R	224, 303, 304
p	C ₈ H ₁₇	H	54	48	>98	R	302
q	C ₈ H ₁₇	K	54	48	>98	R	307
r	C ₈ H ₁₇	C ₂ H ₅	21	48	>98	R	302
s	C ₁₁ H ₂₃	CH ₃	29	24	57	R	305
t	C ₁₁ H ₂₃	H	40	48	>98	R	302
u	C ₁₁ H ₂₃	H	40	48	39	R	309
v	C ₁₁ H ₂₃	K	40	48	>98	R	302
w	C ₁₁ H ₂₃	C ₂ H ₅	0	48			302
x	C ₁₃ H ₂₇	K	17	48	>98	R	302

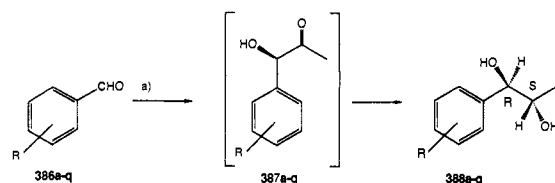
31% **381n** (with an ee > 95%) after 7 days.²²⁴ It appears noteworthy that longer chain alkyl-substituted 4-oxocarboxylates **379**³⁰²⁻³⁰⁵ gave better yields and higher ee values (in favor of *R*-configured products) as compared to derivatives possessing short alkyl groups.³⁰⁶ 4-Oxo-3-methyloctanoate gave a mixture of *cis*-**4R** (major, ee > 99%) and *trans*-**4R** (minor, ee > 92%) substituted 3-methyl lactones.³⁰⁶

For **380f** a more detailed investigation has been undertaken,³⁰² and it was revealed that the optical purity of **382f** is always >98% irrespective of conditions used. It must be noted that the results obtained in this investigation^{302,307} are controversial to previous results.³⁰⁸ The yields observed indicate that 5.5 g of dry yeast/mol of substrate was sufficient while smaller amounts were insufficient due to poisoning of the yeast³⁰² and larger amounts of yeast gave lower yields due to trapping of the keto acid by the yeast within the first 6-h period of the reaction.³⁰² As an alternative for the preparation

SCHEME 89



SCHEME 90



a) dry yeast (Oriental), r.l., 3.5 h, rel.320

R	yield (%)	ee (%)	anti/syn	R	ref.
a H	30	97	97/3	m o-CH ₃ OC ₆ H ₄	317
b p-CH ₃	22	97	97/3	n m-HOC ₆ H ₄	318
c p-CH ₃	28	99	98/2	o p-HOC ₆ H ₄	318
d p-Cl	27	98	98/2	p 3-CH ₃ O, 4-HOC ₆ H ₃	319
e p-F	28	97	97/3	q (3,4-OCH ₂ O)-C ₆ H ₃	319
f o-CH ₃	7	97	99/1		
g o-Cl	32	97	99/1		
h o-F	31	97	99/1		
i m-F	30	97	93/7		
j o-CF ₃	---	---	---		
k m-CF ₃	---	---	---		
l p-CF ₃	---	---	---		
1	---	---	---		

for entry a see rel. 313,321,326; for entry b and g: see rel. 317; for entry c and i see rel.322; for entry d, e,h-l see rel. 320

of **382f**, the use of an acylase from *Aspergillus sp.* has been proposed.³⁰⁹

It seems noteworthy that the material deficit and hence the yield seem to depend upon the length of the alkyl chain whereas the pH was not found to be critical as long as it was kept between 4.7 and 7.0.³⁰² This is in good accord with the reported value of pH 5 for the reduction of **380c**.³⁰¹ Generally, δ -keto acids are more rapidly reduced than the corresponding δ -keto esters. Retardation in the reduction of the long-chain alkyl keto esters was assumed to arise from their low solubility.³⁰²

Optically active (*R*)-(+)-(γ -butyrolactonyl)-propionates **383a-d** (Scheme 89) were prepared by reducing 3-ketoheptane-1,5-dicarboxylic monoesters **384**. Whereas diesters **385a-d** were hydrolyzed to **384** with *Pseudomonas diminuta* (IFO13181) as the best microorganism for partial hydrolysis of compounds of such type, **384a-d** were reduced to **383a-d** with fermenting BY in large-scale quantities, fair yields, and excellent ee. The yeast cells could be employed repeatedly several times for the reductions.³¹⁰

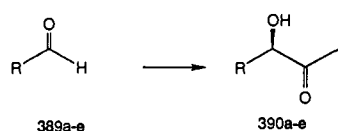
III. C-C Bond-Forming and -Breaking Reactions

A. α,β -Unsaturated Systems

1. Acyloln-Type Condensations and Reductions of α,β -Unsaturated Compounds

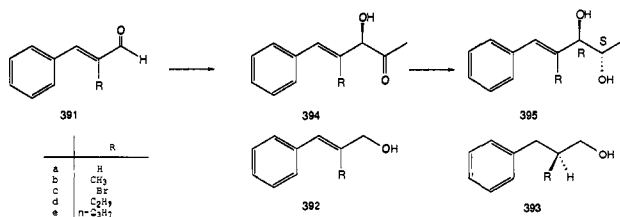
First reports on this condensation reaction have been published 60 years ago by von Liebig (for the reaction of furfural),³¹¹ and later Neuberg^{312,313} and Dirscherl³¹⁴ investigated this type of reaction for benzaldehyde (**386a**) (Scheme 90) in more detail. Broader synthetic applications³¹⁵ have been brought about by Fuganti and

SCHEME 91



a	R	ref.
b	CH ₃	324,325,326
c	(CH ₃) ₂ CH	327
d	n-C ₄ H ₉	328
e	n-C ₇ H ₁₅	329
	n-C ₁₂ H ₂₅	329

SCHEME 92



co-workers since the mid-1970s.³¹⁶ The results for substituted benzaldehyde derivatives **386b-q** (leading via **387b-q** to products **388b-q**) are summarized in Scheme 90.³¹⁷⁻³²² Mainly, formation of *anti*-1-aryl-1,2-dihydroxypropanes **388a-q** has been observed.³²⁰

The acyloin-type condensation³²³ of benzaldehyde (**386a**) found its industrial application very early in the synthesis of D-(-)-ephedrine, thus being one of the first industrial processes combining microbiological and chemical synthesis.³²¹ These investigations have been extended to aldehydes **389a-e**, leading to products **390a-e** (Scheme 91).^{313,317,318,322-329}

This reaction type can be considered the result of two subsequent transformations: Addition of a C₂ unit equivalent of acetaldehyde in *statu nascendi* by means of 2-(α -hydroxyethyl)thiamine pyrophosphate onto the *si* face of the carbonyl group to form (*R*)- α -hydroxy ketones.^{330,331} Subsequent reduction of this intermediate on the *re* face by (an) alcohol dehydrogenase(s) results in the formation of predominantly *anti*-configured diols.

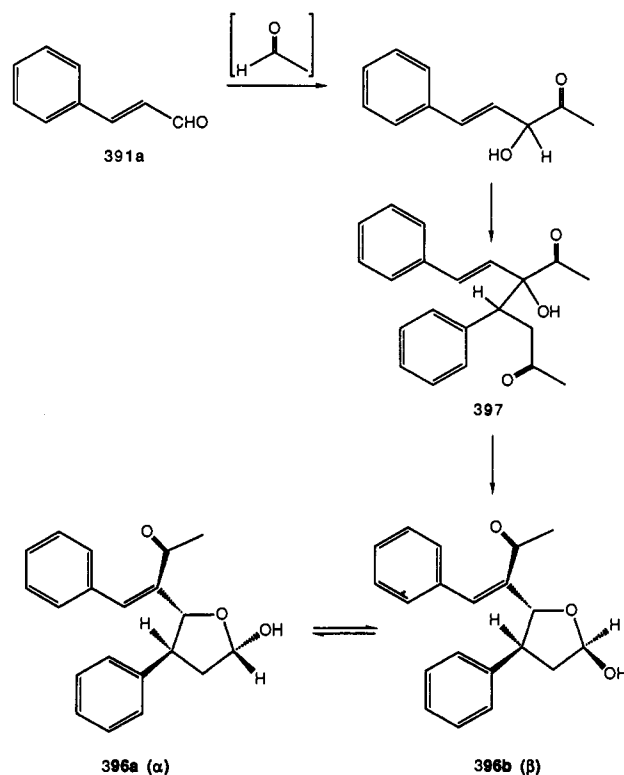
The reaction sequence was found to be dependent on pH. In acidic medium, the formation of ketols predominates whereas under neutral or basic conditions (optimum pH 9.5) only diols were observed.³²⁰

In addition, it seems noteworthy that upon addition of salicylaldehyde the fermentation immediately stops;^{329,332,333} this result has been interpreted in terms of toxicity. The highest cell toxicity in the aliphatic series has been found for heptanal.³²⁹

There are some severe limitations for these reactions: Whereas a broad structural tolerance for the first aldehyde to be used in the enzymic system is found, only acetaldehyde is accepted as the second terminus. With other aldehydes (e.g., propion- or butyraldehyde), no incorporation of these added aldehydes could be found.³³⁴ The other limitation investigated in more detail³¹⁶ in the case of α,β -unsaturated carbonyl compounds,³¹⁵ e.g., cinnamaldehyde (**391a**) (Scheme 92), is that the acyloin condensation is dominated by simple biohydrogenation leading to the corresponding alcohols **392** and **393** (70–75%) instead of a chain extension reaction leading to **394** (25–30%).¹⁹¹

Compounds **394** and **395** have successfully been used for the synthesis of deoxy sugar analogues,^{273-277,331,335,336}

SCHEME 93



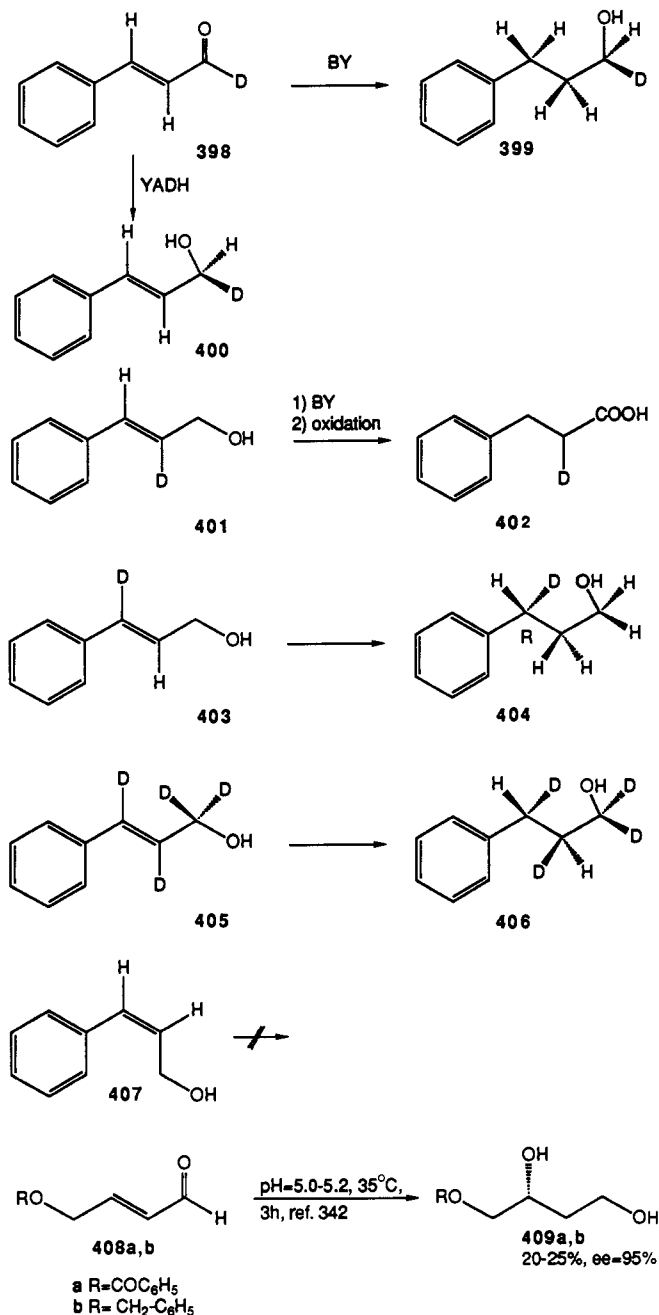
LTB₄ intermediates,²⁷⁹ D-(-)-*allo*-muscarine,³³⁷ (+)-*exo*-brevicomin,^{256,277} hexanolides,^{278,281} octanolides,²⁷⁷ frontalin, and other pheromones.³³⁸

Reaction of **391d** and **391e** afforded under these conditions **392d,e** and **393d,e**.^{274,330} Small amounts of **391a** were converted into **396**.²⁷³ (Scheme 93). As shown by NMR studies,³³⁹ **396a** (possessing α -configuration when regarded as a substituted furanoid carbohydrate) underwent mutarotational isomerism, reaching an equilibrium value of about 60% α -anomer after 1 day. Intermediate **397** is expected to arise upon Michael addition of the anion derived from the α -ketol from cinnamaldehyde and a molecule of cinnamaldehyde followed by ring closure to yield **396**.³³⁹ It is noteworthy that no analogous byproduct of this type was formed from **391b**.

A further application of the enzymic acyloin-type condensation to mono- and sesquiterpenes has been reported by *Diplodia gossypina* and *Corynespora cassicola* for the synthesis of norterpene.³⁴⁰ A similar reaction of 3,4-dimethoxybenzaldehyde has been reported with use of *Aerobacter aerogenes* instead of BY.³¹⁹

Since the acyloin condensation reaction was found to be followed more or less always by reduction of the carbonyl and/or the C–C double bond, some deuteration studies have been performed (Scheme 94) in order to establish the stereochemical course of the reduction of α,β -unsaturated carbonyl compounds.³⁴¹ Thus, [²H]formyl-labeled **398** gave (1*S*)-3-phenyl[1-²H]-propanol (**399**), whereas treatment of **398** with purified yeast alcohol dehydrogenase afforded **400**. Analogue **401** gave upon treatment with BY followed by CrO₃ oxidation **402** without any loss of deuterium. From **403** was obtained (3*R*)-[²H]-**404**. [1,1,2,3-²H₄]Cinnamyl alcohol (**405**) afforded (1*S*,2*S*,3*R*)-3-Ph [1,2,3-²H₃]-**406**,³⁴¹ (*Z*)-cinnamyl alcohol (**407**), however, was re-

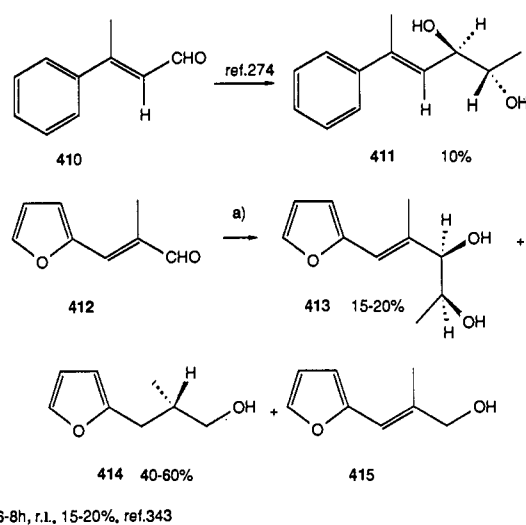
SCHEME 94



covered unchanged from the reaction mixture.³⁴¹ These results indicate a formal anti stereospecific addition of hydrogen across the double bond; this reduction is the result of an introduction of a *pro-R* hydrogen substituent at position 1. Deuterium labeling has also been used to get first insights for the analogous water addition and transformation of 408a,b into (*R*)-409a,b in approximately 25% yield and proceeding with 90–95% ee for the products.³⁴²

Many different α,β -unsaturated carbonyl compounds have been used for this kind of BY-mediated C–C bond formation. 410 (Scheme 95) gave 10% of 411 (conjectural stereochemical assignment).²⁷⁴ Enantiomerically pure vitamin E has been synthesized in a convergent manner from a single precursor, α -methyl- β -2-furyl-acrolein (412), which gave upon BY-mediated acyloin condensation followed by reduction diol 413 in 15–20% yield. Yields of 40–60% of reduction product 414 and traces of unsaturated alcohol 415 were also isolated.³⁴³

SCHEME 95



BY treatment of a 1:1 mixture of (*Z*)- and (*E*)-3-methyl-5-phenyl-2,4-penta-2,4-dien-1-ol (416) (Scheme 96) afforded ca. 10% of desired 417 and a 6:4 mixture of isomers 418.²⁷⁴ *E*-Configured 419 gave 15% of 420, which was further transformed into 2,6-dideoxy-3-*C*-methyl-*L*-arabino-hexopyranose (olivomycose, 421), thus ascertaining the stereochemical assignments for 420.²⁷⁴

The same group also used 419 and (*Z*)-416 as starting materials for another synthesis of α -tocopherol. Thus, 419 gave 87% of 422 whereas BY treatment of (*Z*)-416 gave an inseparable mixture of 423 and (*Z*)-418.³⁴⁴

Reduction of 424 under modified conditions afforded 15–20% of 4-phenylbut-3-en-2-ol (425) (Scheme 97) of 90% ee, a valuable starting material for the synthesis of (*S*)-*O*-benzylaldehyde.³⁴⁵ 425 was found to be contaminated with 5–10% of inseparable 426.³⁴⁵

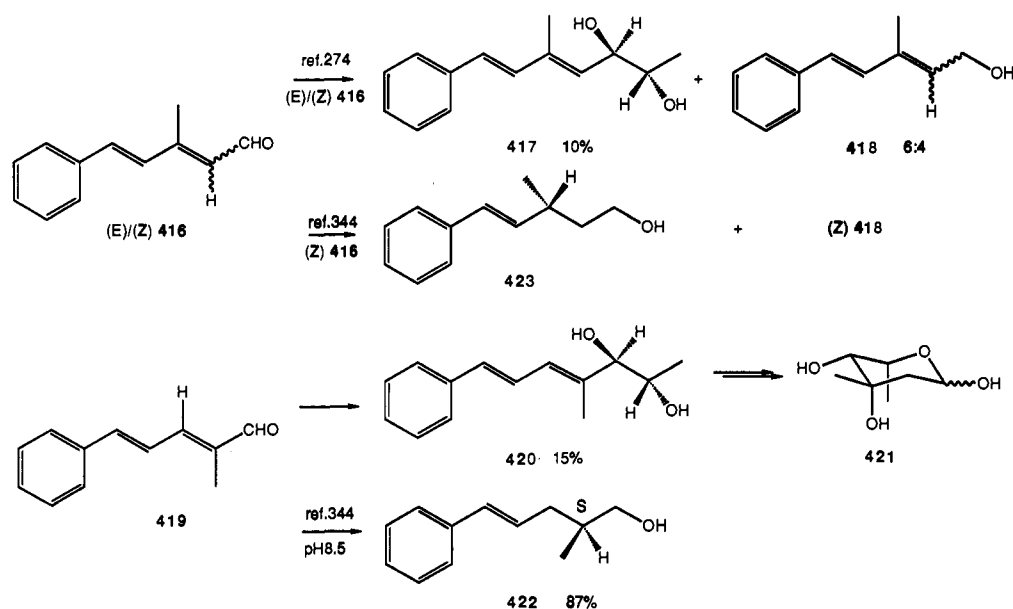
α,β -Unsaturated ketone 427 gave upon reduction with fermenting BY 12% of (*S*)-428 of ca. 95% ee and 1.5% of 429. Reduction of 430 with fermenting *Saccharomyces cerevisiae* or resting cells of *Saccharomyces fermentati* gave different results; the former yielded racemic 431, the latter (*S*)-431 of ca. 50% ee. Reduction of 432 gave only 4% of 433 (ee 95%) and 1% of 434.³⁴⁶ The absolute stereochemistry of these products has not been determined.

It seems that the condensing enzyme(s) are very specific whereas the substrate specificity of the subsequent reduction step is not so restricted. Thus, the racemic and synthetically prepared hydroxy ketones 435a–d (Scheme 98) (all of them *not* formed by BY-mediated acyloin-type condensation) were clearly reduced by BY under usual conditions. 435a gave 70–80% of a 6:4 mixture of (2*S*,3*R*)-436a and (2*S*,3*S*)-437a. The same is true for 435b (leading to products 436b and 437b) whereas 435c,d gave only 20% of anti-configured 436c,d accompanied by about 10% of the corresponding syn isomers 437c and d, respectively.³³⁴

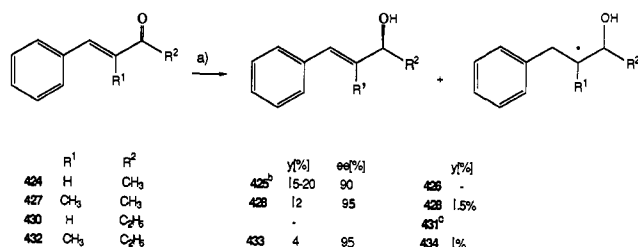
Reduction of 438 gave 70% of a 6:4 mixture of (2*S*,3*R*)-439 and the syn analogue (*R,R*)-437b.³³⁰ The latter is the enantiomer of (*S,S*)-437b (obtained by BY reduction of 435b).³³⁴

Branched hydroxy ketones of similar type were also submitted to BY reduction. Thus, 440 (Scheme 99) gave 80% of a 1:1 mixture of 441 and 442 whereas upon reduction of 443 excess (4*S*)-443 and 15³³⁶–20%³³⁴ of

SCHEME 96



SCHEME 97

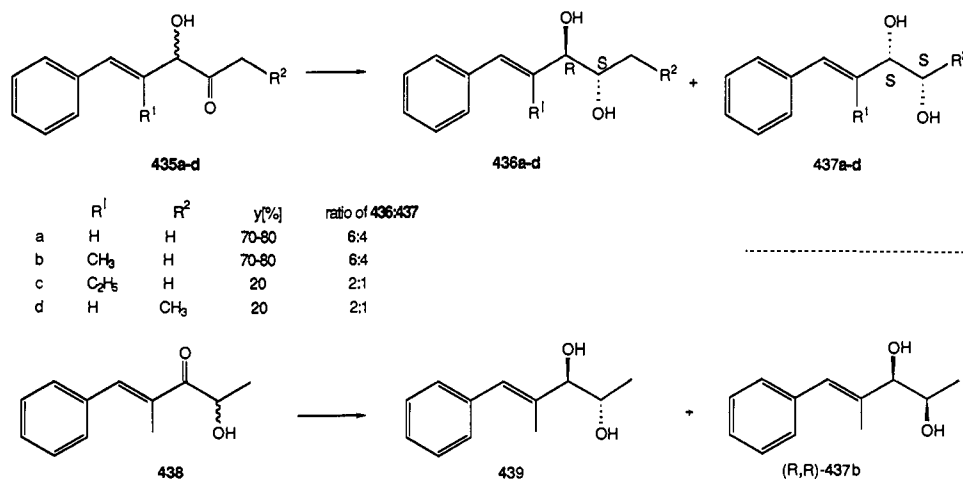


a) educt in EtOH, 28-27°C, 3h,
 b) 72% of 424 recovered, ref.345
 c) racemic with BY

(3*S*,4*R*)-444 were obtained.³³⁶

Some conclusions can be drawn from these experiments. For 435*a,b* and 440 the hydride addition to the carbonyl grouping occurred on the *re* face regardless of the configuration of the adjacent center. For 435*c,d* and 443, however, only the *R* enantiomers were reduced to a significant extent.³³⁰ In contrast to 435*a,b* and to 440 for 438 hydride addition took place onto the *si* face irrespective of the configuration of the adjacent center. Chain elongation (as in 443 as compared to 440) resulted in decreased yields.³³⁴

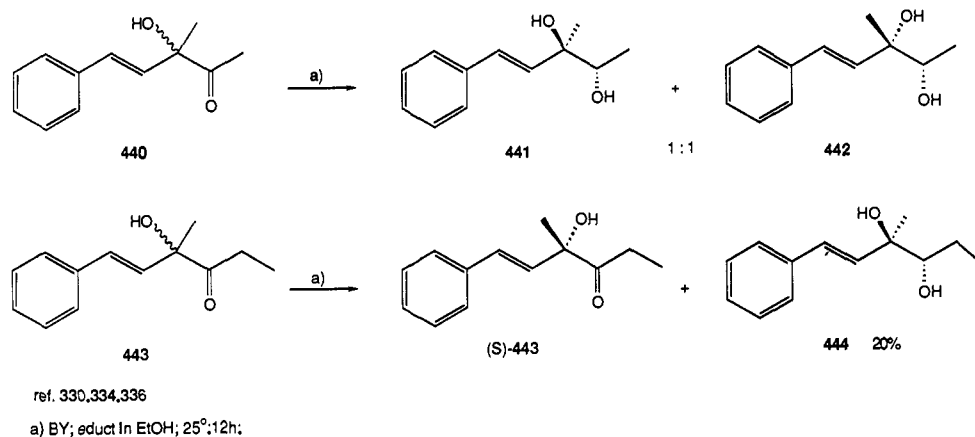
SCHEME 98



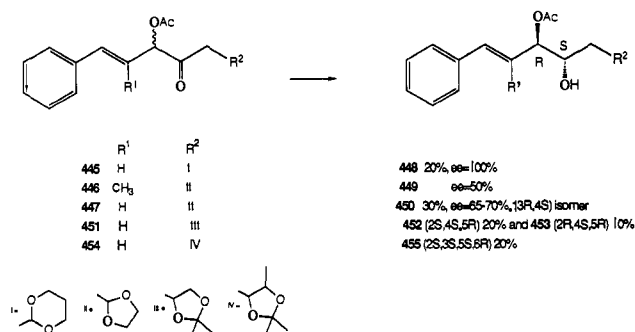
Direction of the stereocontrol of the reduction was performed for 445-447 (Scheme 100). 445 gave 20% of 448 in enantiomerically pure form, and 30-40% of 4*S*-configured starting material (of 90% ee) could be recovered;³⁴⁷ 446 gave mainly (3*S*,4*R*)-449 with 50% ee. Reduction of 447 resulted in predominant formation of (3*R*,4*S*)-450. For 447 the hydrogen addition occurred preferentially on the *si* face. The stereochemical course of these reactions parallels³³¹ the mode of reduction of 4-heterosubstituted 3-oxobutanoate esters by yeast (due to its five-membered acetal in 1,3-relationship to the carbonyl group).³³¹

Similarly, 451 was reduced in about 20% yield to 452, and 10% of 453 and 70% of starting material could be recovered. While (2*S*,4*S*,5*R*)-452 served as a valuable starting material for the synthesis of 4-deoxy-D-*lyxo*-hexopyranose, (2*R*,4*S*,5*R*)-453 was used for the preparation of 2,3-di-*O*-acetyl-4-deoxy-D-*lyxo*(L-*ribo*)-hexopyranose; 454 gave upon reduction 20% of 455.³⁴⁸ Apparently for this compound the absolute configuration at C-3 determined which of the components of the racemic mixture is accepted as a substrate for reduction by the enzyme(s) at C-5. (2*R*,3*R*,6*R*)-454 was not reduced by this system.³⁴⁸

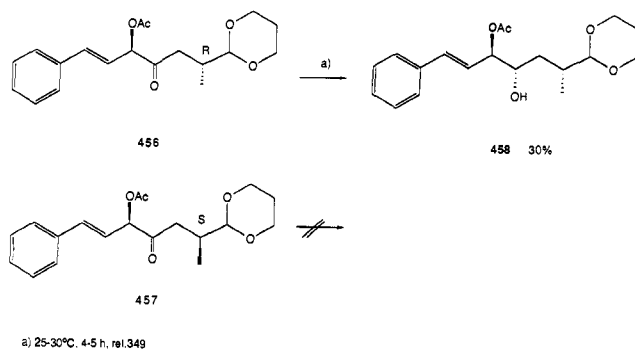
SCHEME 99



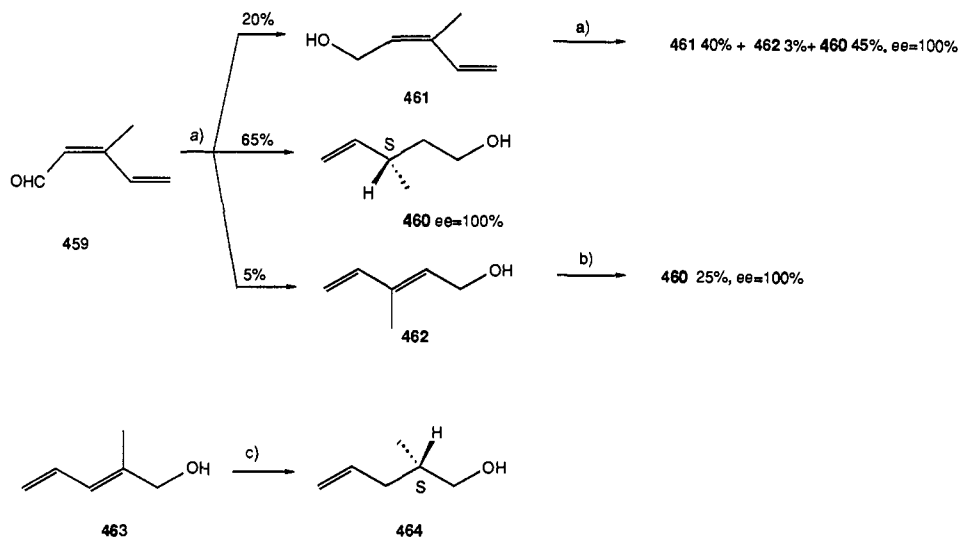
SCHEME 100



SCHEME 101



SCHEME 102

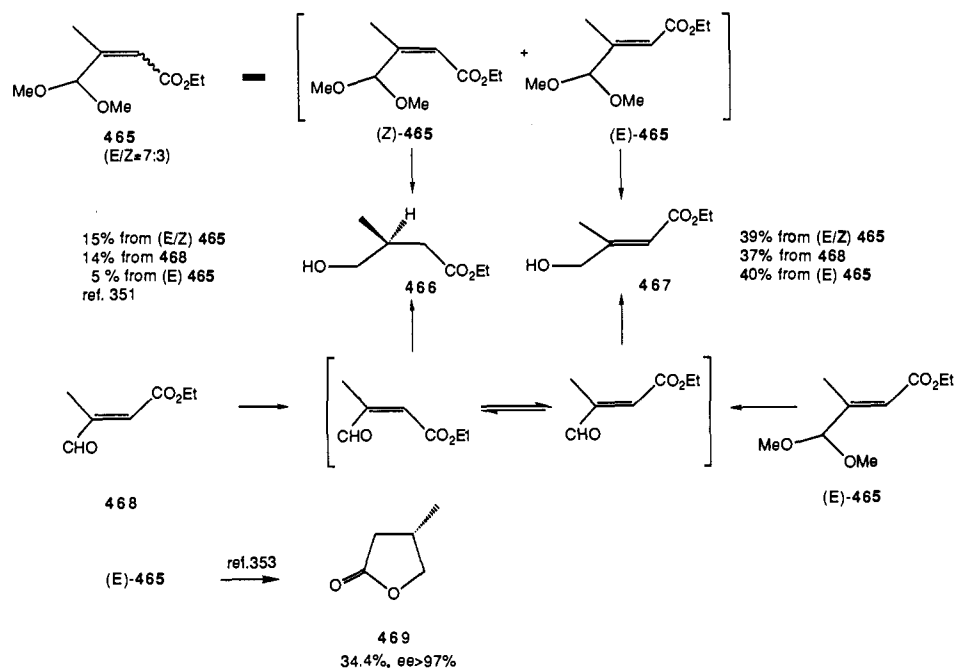


The same phenomenon was observed when the structural analogues (2*RS*,5*RS*)-456 and (2*SR*,5*RS*)-457 were treated with BY (Scheme 101). Only (2*RS*,5*RS*)-456 gave 30% of (2*R*,4*S*,5*R*)-458—an intermediate in the synthesis of (-)- α -multistriatin. (2*SR*,5*RS*)-457 gave no reaction at all.³⁴⁹ It appears that the yeast's enzyme(s) involved in the reduction of these α -acetoxy carbonyl compounds are quite sensitive to the stereochemistry of remote (here β) centers. Hydrogen addition onto the carbonyl grouping occurred from the *re* face of the 5*R* or 6*R* enantiomer, but of the two diastereomers the one with a (2*R*)-methyl group was reduced at much higher rate.^{348,349}

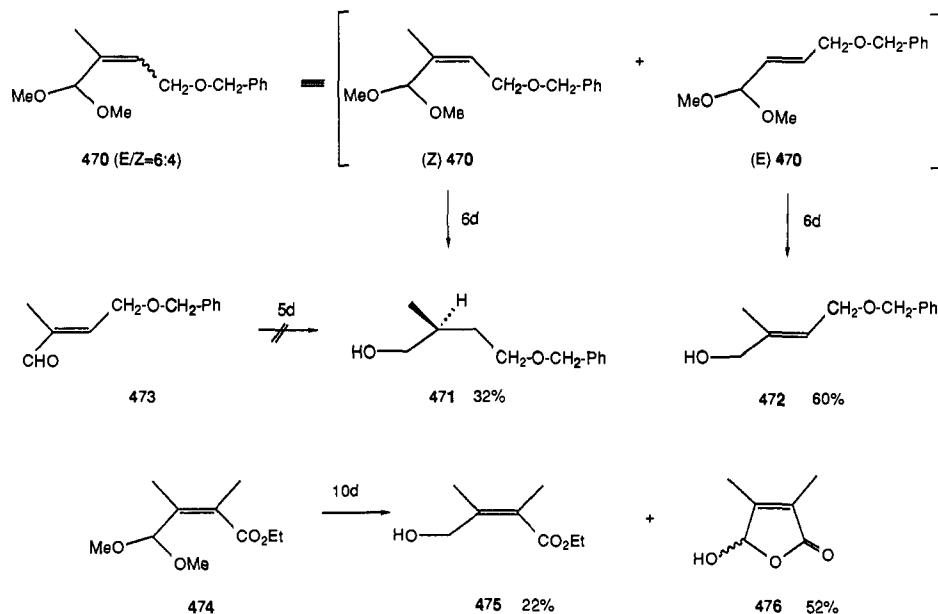
The reduction of (*Z*)-3-methyl-2,4-pentadienal (459) (Scheme 102) gave after 10 days of reaction with BY 65% of (*S*)-3-methyl-4-penten-1-ol (460), 20% of (*Z*)-3-methyl-2,4-pentadienol (461), and 5% of (*E*)-462 whereas reduction of 461 afforded after 10 days 40% of recovered starting material, 45% of 460, and 3% of (*E*)-462, which upon further BY reduction at pH 8 for another 10 days gave 25% of enantiomerically pure (*S*)-460.³⁵⁰

The structural analogue 463 afforded 30% of enantiomerically pure (*S*)-2-methyl-4-penten-1-ol (464) under similar conditions.³⁵⁰ It was established for all of these transformations that only the double bond con-

SCHEME 103



SCHEME 104



tiguous to the alcoholic or aldehydic function is hydrogenated by BY.³⁵⁰

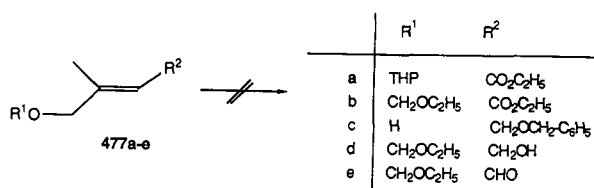
Reduction of the acetal-masked aldehyde but no reduction of the ester moiety were observed for a *Z/E* mixture of ethyl 4,4-dimethoxy-3-methylcrotonate (465) (Scheme 103). Thus, 465 (*E/Z* = 7:3) gave 15% of 466 and 39% of (*E*)-467,³⁵¹ a valuable synthon for the synthesis of cholesterol derivatives.³⁵² The *Z* isomer 465 was the best substrate for this biohydrogenation. The acetal group, however, is not completely equivalent to the aldehyde group, since slow hydrolysis of (*Z*)-465 allowed the isomerization to the (*E*)-aldehyde ester, which was reduced to the corresponding (*E*)-alcohol more rapidly than any possible isomerization. Slow addition of aldehyde 468 to BY afforded within 1 day 37% of 467 and 14% of 466. With the acetal group the formation of the unsaturated hydroxy ester 467 is favored over formation of the saturated product.³⁵¹ If 467

was subjected to a further subsequent BY reduction, it was recovered unchanged. Similarly (*E*)-465 afforded upon aerobic reduction within 56 h at 30 °C and pH 3–4 a mixture of 466 (49.2%, ee > 97%) and 467 (46.9%). Approximately 2–5% of (*S*)-3-methyl- γ -butyrolactone (469) was formed during the reaction and could be obtained from 466 by acidic workup (34.4%, ee > 97%).³⁵³

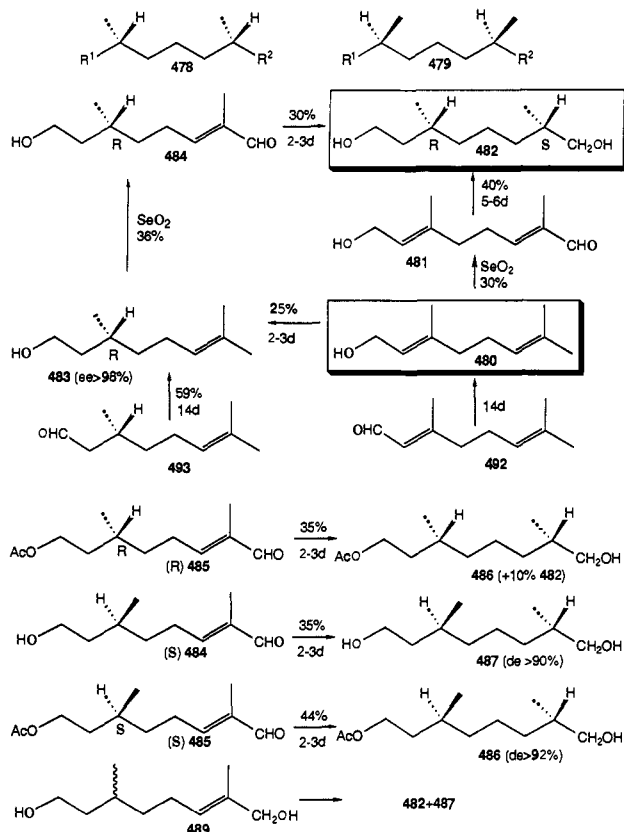
Treatment of the analogue 470 (*E/Z* = 6:4) (Scheme 104) showed that (*Z*)-470 or its equivalent aldehyde was the substrate for the biohydrogenation to yield 471 in 32% yield, whereas (*E*)-470 was only hydrolyzed and reduced to the corresponding alcohol 472 (60%). No reaction was observed for 473. 474 gave after 10 days 22% of 475 and 52% of lactone 476.

The inability to biohydrogenate the respective *E* isomers seems not to be a general rule since such transformations are known to proceed with structural analogues.³⁵⁴ Furthermore, no reaction upon BY

SCHEME 105



SCHEME 106



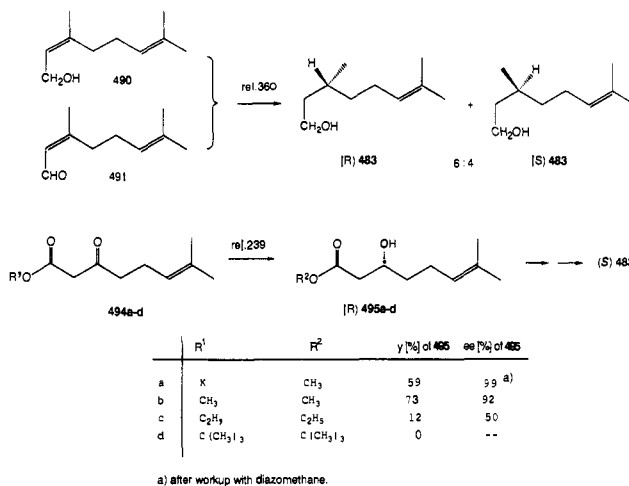
treatment was observed for **477a-e** (Scheme 105) differing in the O substituent R¹ or in R². This may be attributed to a too dramatic change in the electronic and stereochemical demand of the biohydrogenation.³⁵¹

A noteworthy access to the valuable C₁₀-synthons for building up molecules containing the 1,5-dimethylated acyclic units **478** or **479** has been reported (Scheme 106).³⁵⁴ These units are present in tocopherol, phylloquinones, insect pheromones,³⁵⁵⁻³⁵⁸ and the marine sponge sesquiterpenoid fasciculatin.³⁵⁹

Thus, in a very elegant way geraniol, 3,7-dimethyl-2,6-octadien-1-ol (**480**), was oxidized to the aldehyde **481**, which gave by a one-pot double hydrogenation diastereomerically pure **482**. The same compound was prepared by BY hydrogenation of **480** to afford enantiomerically pure (*R*)-citronellol ((*R*)-**483**),^{354,360} which was oxidized to (*R*)-**484**; (*R*)-**484** gave on subsequent BY treatment again **482**. In a similar way and with comparable yields (*R*)-**485**, (*S*)-**484**, and (*S*)-**485** were hydrogenated to afford **486-488**, respectively.³⁵⁴

Racemic **489** afforded an equimolar mixture of diastereomers **482** and **487**. It was shown that introduction of the asymmetric center at C-2 is highly stereoselective and the same absolute configuration at C-2 resulted when starting from both (*R*)- or (*S*)-citronellol (**483**). Both (*R*)-**483** and (*S*)-**483** were precursors for the

SCHEME 107



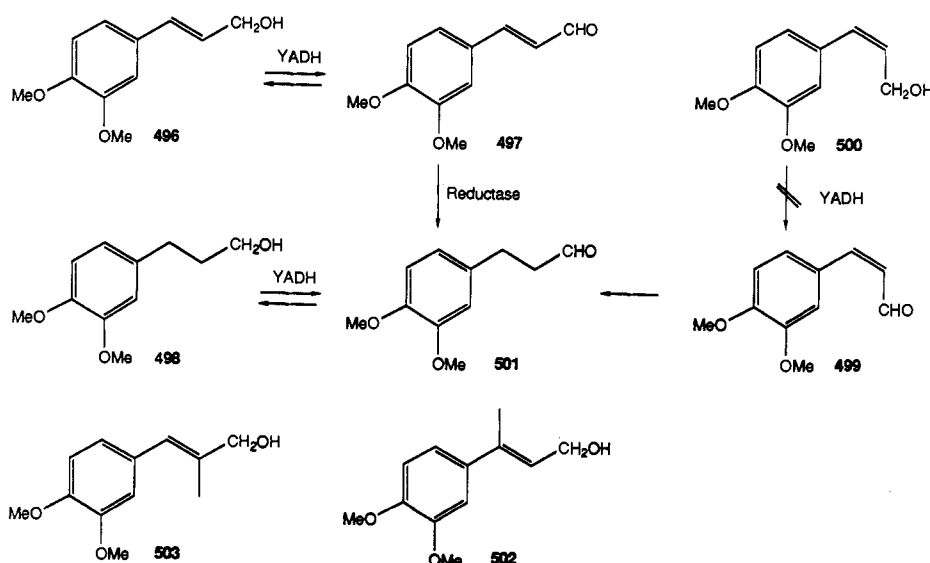
preparation of (*R*)- or (*S*)-**484**. No epimerization took place at C-6 of the α,β -unsaturated aldehydes or alcohols during microbial reduction.³⁵⁴ It is interesting to note that nerol (**490**) and neral (**491**) (Scheme 107) each afforded a mixture of the two enantiomers of citronellol with a ratio of (*R*)-**483** to (*S*)-**483** of 6:4. This is due to a potential *Z/E* isomerization of neral (**491**), which appears to be an obligatory intermediate in the BY-mediated conversion of nerol into citronellol.³⁶⁰ The same *R* to *S* ratio was found for the reduction performed with *Beauvaria sulfurescens*.³⁶¹

Even the starting materials for these syntheses have been prepared by BY-mediated biohydrogenations or reductions. Thus, **492** (Scheme 106) gave on BY treatment³⁶² for 2 weeks **480**, and **493** afforded under similar conditions 59% of (*R*)-**483**.³⁶³ BY reduction²³⁹ of the β -keto ester **494a-d** gave (*R*)-**495a-d**, which were successfully transformed into (*S*)-**483** (Scheme 107).³⁶⁴ The intermediates of this elegant approach to chiral C₁₀ synthons have been used for the synthesis of natural (*E*)-(7*R*,11*R*)-phytol.³⁵⁴

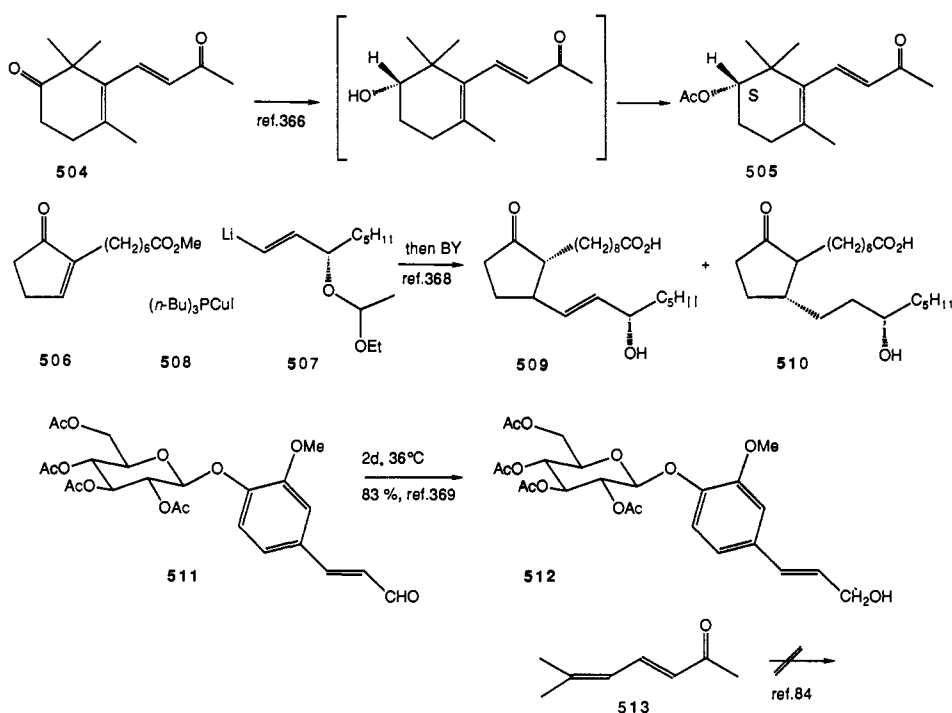
Reduction of α,β -unsaturated alcohols was investigated in more detail for substituted cinnamyl alcohols.³⁶⁵ It was shown that the reduction of **496** (Scheme 108) proceeded through the corresponding aldehyde **497** to yield finally **498**. In analogy to the decarboxylation of (*E*)-cinnamic acid (cf. III.A.b), only (*E*)-cinnamyl alcohol but not the *Z* type was reduced. Since (*Z*)-3',4'-dimethoxycinnamylaldehyde (**499**)—which could not be obtained from **500**—was rapidly reduced to **501**, it was concluded that in this two-step enzymic system consisting of one (or two) alcohol dehydrogenases and a reductase it is the alcohol dehydrogenase showing a specificity with respect to *E* versus *Z* configuration.³⁶⁵ Gramatica and co-workers observed that an inductive alcohol dehydrogenase (ADH-II, of ethanol-grown cells) showed the same specificity toward the double-bond configuration as the constitutional dehydrogenase ADH-I. Although no (*Z*)-alcohol was reduced by ethanol-grown cells, these cells were able to reduce the (*E*)-alcohol faster than glucose-grown cells.³⁶⁵ The same results were found for cell homogenates, thus allowing a more direct comparison between ADH-I and ADH-II. A blockage of the reductase but not of the alcohol dehydrogenase was established for **502** but not for **503**.³⁶⁶

Slower reaction as compared to α,β -unsaturated aldehydes or alcohols or even no reaction at all was ob-

SCHEME 108



SCHEME 109

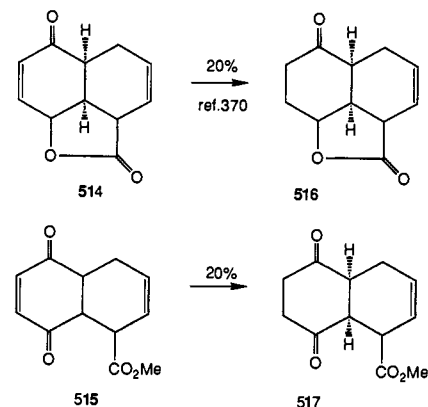


served for α,β -unsaturated ketones. Thus, no reaction of α,β -unsaturated ketone **504** could be achieved (Scheme 109). Instead, reduction of the cyclic keto function occurred; subsequent acetylation afforded (*S*)-**505** in 60% yield.³⁶⁶ **505** is a valuable building block for the synthesis of carotenoids with a 2-hydroxylated β -ring.³⁶⁷ Side-chain reduction but no reduction of the cyclanone were found during the synthesis of prostaglandins. Thus, reaction of cyclopentanone **506** with **507** and **508** followed by hydrolysis and subsequent BY treatment gave a mixture of unsaturated **509** and saturated **510**.³⁶⁸

Upon reduction of glycoside **511**, 83% of tetra-*O*-acetylconiferin (**512**) was obtained.³⁶⁹ Interestingly, no deacetylations were found to occur during this transformation. No reaction occurred with **513**.⁸⁴

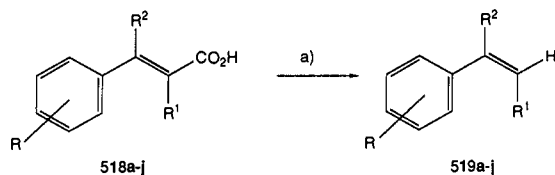
In addition, Woodward's lactone (**514**) (Scheme 110) and the analogue **515** have been reduced in about 20%

SCHEME 110



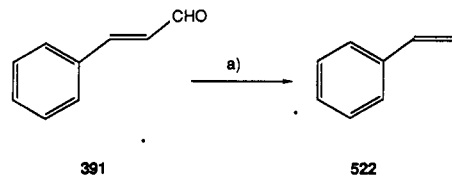
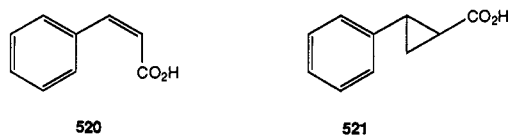
yield to give **516** and **517**, respectively.³⁷⁰ Further examples for the reduction of C=C double bonds are

SCHEME 111



a) 24h, pH=4-5, ref.371

No	R	R ¹	R ²	reaction
a	H	H	H	-
b	3,4-dihydroxy	H	H	-
c	3,4-methylenedioxy	H	H	-
d	4-hydroxy	H	H	+
e	4-methoxy	H	H	+
f	4-hydroxy-3-methoxy	H	H	+
g	3,4-dimethoxy	H	H	+
h	3,4-dimethoxy	CH ₃	H	+
i	3,4-dimethoxy	H	CH ₃	+
j	3,4-dimethoxy	CH ₂ CH ₃	H	+



a) for conditions, cf ref. 374

found in Schemes 48, 92, 95, 97, 120, and 123.

2. Decarboxylations

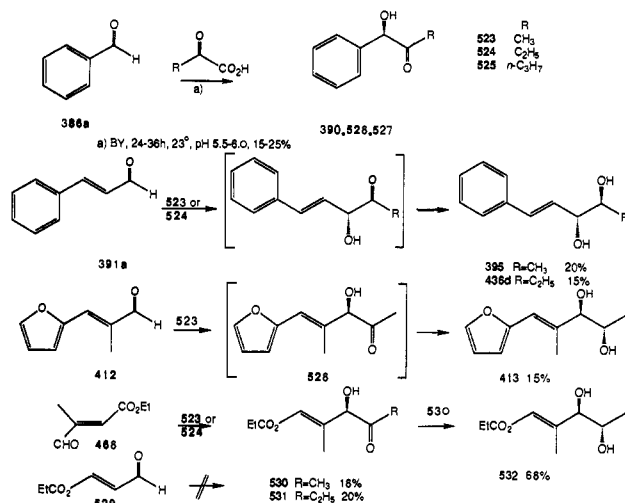
The decarboxylation of substituted cinnamic acids 518a-j by BY has been investigated extensively by Gramatica et al.³⁷¹ and as shown by NMR investigation of deuterated compounds to proceed with retention of configuration to yield 519d-j (Scheme 111). The results obtained for BY parallel earlier findings obtained for *Bacillus pumillus*.³⁷² Thus, to account for the overall stereochemistry, it has been suggested that a syn-1,2-Michael-type addition has been followed by an anti-1,2-decarboxylative elimination.

The *E* configuration of the double bond is necessary since neither 520—for geometric reasons—nor 521—for electronic and/or geometrical reasons—was reduced at all. This pronounced enzymic specificity with respect to the double bond might also be due to a conformational effect at the transition-state level of the decarboxylation step.³⁷³ The presence of remote hydroxy or methoxy groups in the aromatic ring seems to be necessary for attachment to the enzyme.³⁷¹

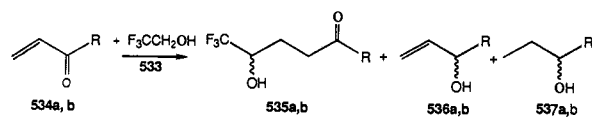
Similar to the decarboxylation of aromatic α,β -unsaturated carboxylic acids upon treatment with BY, there is one report describing the transformation of (*E*)-cinnamylaldehyde (391) to styrene (522) by means of *Saccharomyces cerevisiae* SC3212³⁷⁴ instead of its reduction to cinnamyl alcohol.¹⁹¹

Recently, decarboxylative incorporation of linear C₃, C₄, and C₅ α -oxo acids into (*R*)- α -hydroxy ketones was accomplished when benzaldehyde was incubated with BY.³⁷⁵

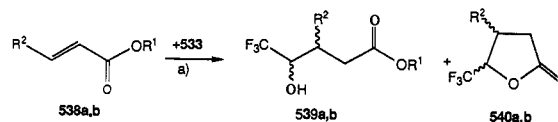
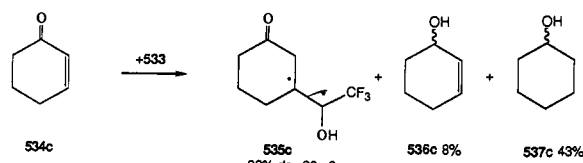
SCHEME 112



SCHEME 113



R	y(%) of 535	ee(%)	y(%) of 536	y(%) of 537
a CH ₃	26	93	18	38
b C ₂ H ₅	41	91	14	36



R ¹	R ²
a C ₂ H ₅	H
b C ₂ H ₅	CH ₃

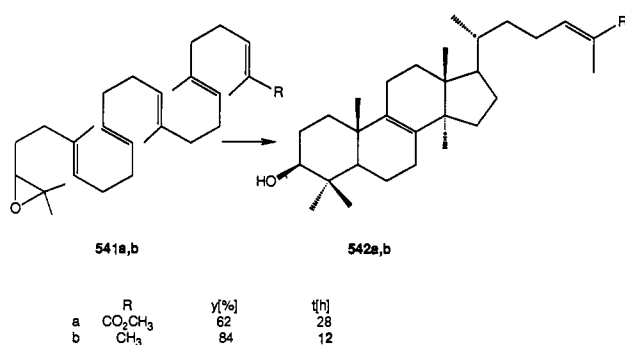
a) BY (Oriental); 35°:4d;

Thus, BY-mediated reaction of benzaldehyde (386a) (Scheme 112) with 523-525 afforded 15-20% of 390, 526, and 527, respectively. Similarly, cinnamyl aldehyde (391a) afforded 20% of 395 or 15% of 436d on incubation with 523 or 524, respectively. The furfural derivative 412 gave 528, which afforded on subsequent BY reduction 15% of 413; 529 gave no reaction at all whereas 468 yielded 18-20% of 530 and 531, respectively, the former of which was reduced by BY to optically pure 532 in 68% yield.³⁷⁵

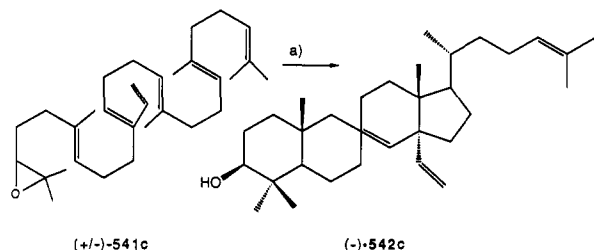
B. Miscellaneous C-C Bond-Forming Reactions

C-C bond formation has been reported to occur during the reaction of 2,2,2-trifluoroethanol (533) (Scheme 113) with α,β -unsaturated ketones 534a-c. Products 535a-c are the results of a conjugate 1,4-addition, although obtained in lower yield (26-41%) but with high ee (91-93%) or de (92:8). These reactions were accompanied by a reduction process, thus yielding 536a-c and 537a-c. The α,β -unsaturated esters 538a,b gave products 539a,b, which lactonized very easily to 540a,b. In addition, it was found that the reduction of

SCHEME 114

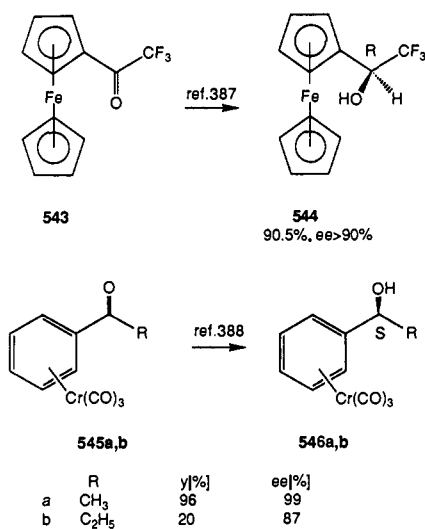


activation of BY: 25 g BY, pH 7.4, irradiated ultrasonically 0°, 2h, ref.377a



a) 23°, 48h, 62% conversion, ref. 377b

SCHEME 115

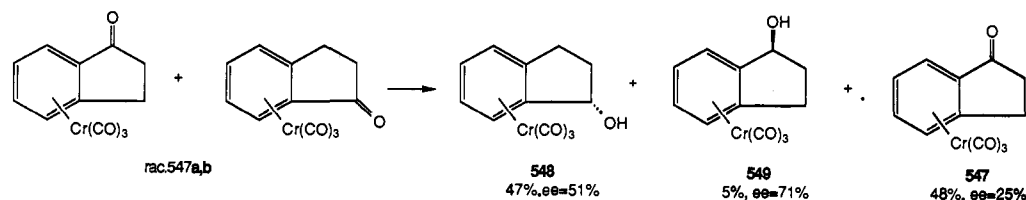


educts **534** and **538** is slower if **533** is absent.³⁷⁶

Cyclization of **541a** to **542a** (Scheme 114) by an sterol cyclase was achieved in an enantioselective manner by treatment of **541a** with ultrasonically activated BY. Similarly, 2,3-oxidosqualene (**541b**) gave lanosterol (**542b**) in 83% yield whereas using unstimulated BY only 19% of product could be obtained. It was shown^{377a} that the cyclase operates only on the *S* enantiomer of the racemic starting material.

On examination of the time course dependence of sterol production on ultrasound, it was shown that for

SCHEME 116



incubations conducted with whole cells there was a significant increase in sterol formation when cells were first sonicated for at least 0.5 h, reaching maximum conversion efficiency at 2 h of sonication.^{377a} Since a cell-free cyclase system was unaffected and was completely insensitive to ultrasound irradiation, it was suggested that the ultrasound effect is more likely associated either with facilitating substrate diffusion by removing the obstructing outer membrane (rather than activating the cyclase) or by liberating membrane-associated sterol-carrier protein factors.^{377a} A vinyl group rearrangement was observed in the BY oxido-squalene-lanosterol cyclase mediated cyclization of racemic squalendoid **541c** to afford (-)-**542c**.^{377b} However, an attempt to apply this cyclization method to an isomeric substrate possessing a vinyl appendage at C-15 in the squalene backbone was not successful.^{377b} In addition, imidazo-fused quinazolinones have been prepared from *N*-(allylcarbonyl)anthranilonitriles by BY-mediated cyclization.^{377c}

IV. Reduction of Organometallic Compounds

Although the reduction of porphyrins and hemoglobins³⁷⁸⁻³⁸⁰ by BY is long known, there are only a few works dealing with inorganic material³⁸¹⁻³⁸⁴ and with the reduction of metal-containing organic molecules or organometallic species. The first reports³⁸⁵ on that topic described the reduction of ferrocenyl-type molecules.³⁸⁶ Thus, **543** (Scheme 115) gave on treatment with BY 90.5% of (*R*)-**544** (ee > 90%).³⁸⁷ It is also possible to reduce aryl ketone-Cr(CO)₃ complexes **545a,b** with BY.³⁸⁸ This reaction seems to be dependent on the bulkiness of the substituents since reduction of **545a** afforded **546a** within 1 day, **545b** gave **546b** within 2 weeks, but 2,4,6-trimethylacetophenone-Cr(CO)₃ or acetophenone-Cr(CO)₂-PPh₃ gave no reaction at all. Reduction of racemic indanone-Cr(CO)₃ (*rac*-**547**) (Scheme 116) afforded a mixture of alcohols (*S*)-*endo*-**548** (47%, ee = 51%) and (*S*)-*exo*-**549** (5%, ee = 71%) together with unreacted starting material **547** (48%, 25% ee).³⁸⁸

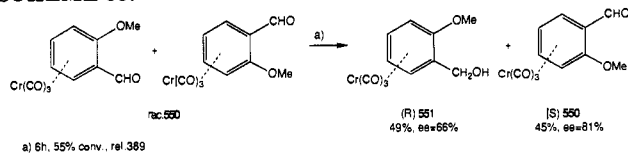
Similarly, enantioselective microbial resolution of the planar chiral metallocenic aldehyde **550** (Scheme 117) afforded after 55% conversion (+)-(*1R*)-tricarbonyl[2-methoxy-1-(hydroxymethyl)phenyl]chromium (*R*)-**551** in 49% yield (66% ee) and the optically active starting material (*S*)-**550** (45%, ee = 81%).³⁸⁹

V. Reduction of Fluorine-Containing Compounds

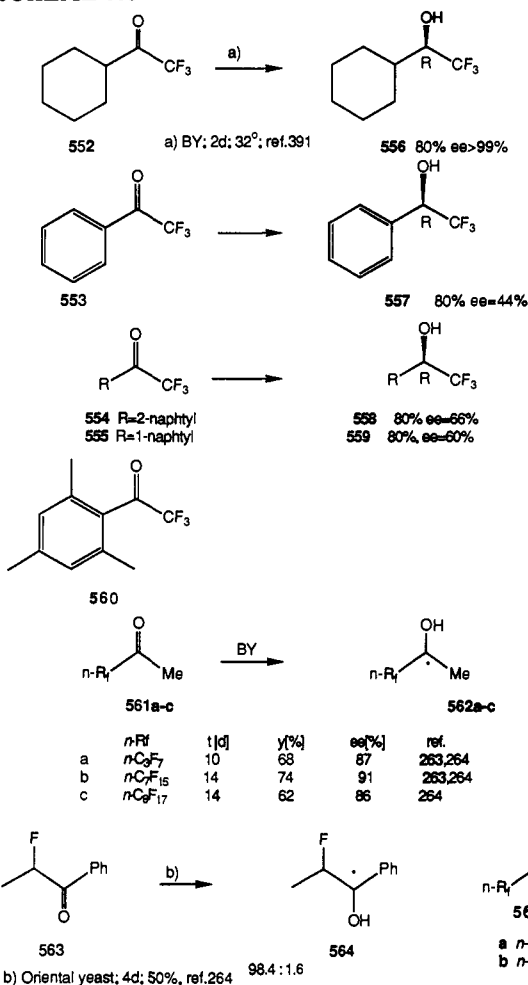
A. Ketones

Fluorine-containing compounds are only scarcely found in nature.^{107,390} Nevertheless, due to their potential use as drugs and valuable tools for metabolic studies, the number of syntheses of mono- and poly-fluorinated natural product analogues has increased

SCHEME 117



SCHEME 118

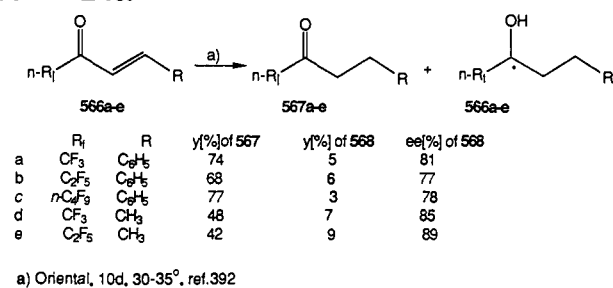


tremendously within the last 10 years. Recently, the reduction of such compounds by means of enzymic or microbial systems has gained much interest as tools for the preparation of enantiomerically pure fluorinated compounds. The trifluoromethyl ketones 552–559 (Scheme 118) were reduced in good yield to the corresponding (*R*)-carbinols 556–559, respectively,³⁹¹ but no reduction was observed for 560.³⁹¹

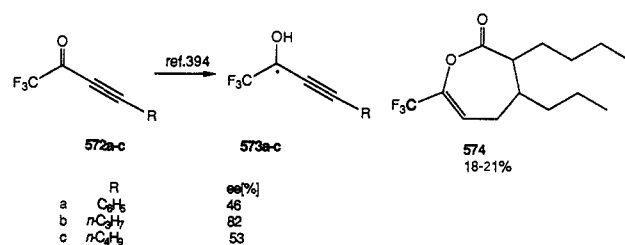
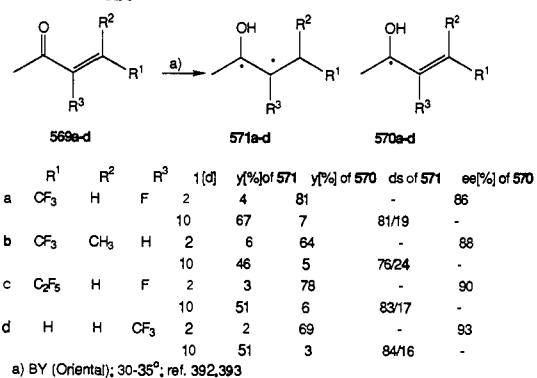
561a–c gave after a quite long reaction time of 10–14 days between 62 and 74% of (–)-562a–c; the absolute configuration of these compounds has not been determined. Although the reactions were inconvenient to handle, particularly in the case of compounds with long perfluoroalkyl chains, the optical purity of the products was high.^{263,264} (–)-563 gave in 50% yield a 98.4:1.6 mixture of diastereomers 564. Bis(perfluoroalkyl) ketones were strongly resistant to the action of yeast; thus, trifluoromethyl-substituted ketones 565a,b gave no reaction at all within 7 days.²⁶⁴

Further examples have been provided,⁶⁴ and it was shown that the stereochemistry of the reduction and the ee value can be rationalized due to steric effects of the adjacent groups rather than to electronic effects.

SCHEME 119



SCHEME 120



Trifluoromethyl ketones are faster reduced than the corresponding methyl ketones but slower than their bromomethyl analogues.⁶⁴ Differences were encountered for the reduction with or without addition of carbohydrates.

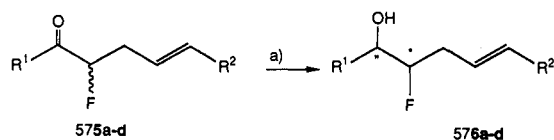
Investigations on the reduction of these fluorinated carbonyl compounds were extended to unsaturated analogues. Thus, compounds 566a–e (Scheme 119) gave within 10 day of reaction 567a–e (major) and 568a–e was found as a minor byproduct although of high optical purity; the absolute stereochemistry of the products has not been established.^{392,393}

When the reductions were applied to ketones 569a–d (Scheme 120) containing a perfluoroalkyl group attached on the carbon–carbon double bond, BY was found to reduce these particular ketones, producing first the optically active carbinols 570a–d; diastereomers 571a–d were obtained after incubation for 10 days.^{392,393} These results seem to indicate that the C=O group of fluoroalkenyl ketones is more easily reduced than the C=C bond with actively fermenting BY.³⁹²

Fluoroalkynones 572a–c have been reduced by BY to give fluoroalkynols 573a–c; (*E*)-fluoroalkenones and fluoroalkanones were observed as the byproducts. Interestingly enough, for the reduction of 572b,c, 574 was obtained in 18–21% yield.³⁹⁴

The olefinic α -fluoroolefins 575a–d (Scheme 121) gave in 38–64% yield mixtures of diastereomers 576a–d without any reduction of the C=C double bond. The respective diastereomeric ratio was determined by ap-

SCHEME 121

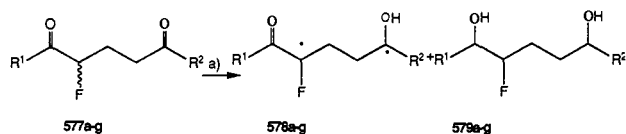


a) BY(Oriental); 35°; pH 7.3; ref.395

	R ¹	R ²	y[%]	threo/erythro	ee[%] ^a
a	CH ₃	H	58	72/28	86/76
b	C ₂ H ₅	H	64	68/32	75/54
c	n-C ₃ H ₇	H	38	56/44	79/45
d	n-C ₄ H ₉	H	42	58/42	64/47

a) of threo and erythro compound, respectively.

SCHEME 122

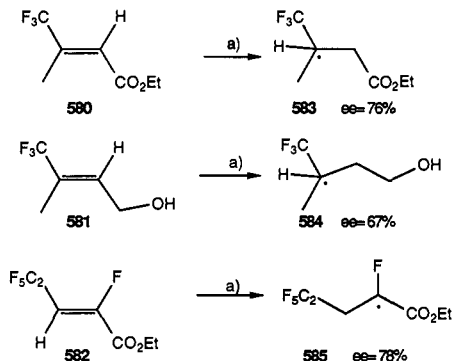


a) BY(Oriental); 35°; pH 7.3; ref. 395

	R ¹	R ²	t[d]	y[%] of 578	y[%] of 579	ee[%] of 578 ^a
a	CH ₃	CH ₃	1	61	4	73/27
			5	2	65	
b	CH ₃	C ₂ H ₅	1	58	2	66/34
			5	5	49	
c	C ₂ H ₅	CH ₃	1	62	7	68/32
			5	2	57	
d	C ₂ H ₅	C ₂ H ₅	1	54	5	58/42
			5	7	51	
e	n-C ₃ H ₇	CH ₃	1	48	16	71/29
			5	6	52	
f	n-C ₃ H ₇	C ₂ H ₅	1	51	8	63/37
			5	6	46	
g	n-C ₄ H ₉	CH ₃	1	62	2	55/45
			5	4	48	

a) of the corresponding stereoisomers, whose configuration was not assigned

SCHEME 123



a) BY; 7d; ref. 263

plication of ¹⁹F NMR spectroscopy.³⁹⁵

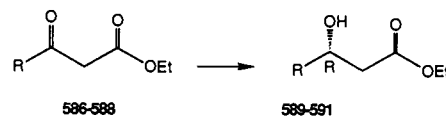
Fluorinated 1,5-diketones **577a-g** (Scheme 122) gave after a 1-day lasting reduction mainly the products of a monoreduction remote of the fluorine substituent **578a-g**, whereas on prolonged reaction time (5 days) the corresponding diols **579a-g** were obtained.³⁹⁵

Clean reduction of the double bond even occurred with compounds **580-582** (Scheme 123) after 7-day incubation with fermenting BY and gave products **583-585**, respectively.²⁶³

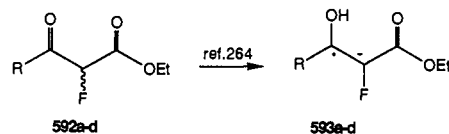
B. Keto Esters

Reduction of polyfluorinated β -keto esters **586-588** (Scheme 124) gave the desired reduction products

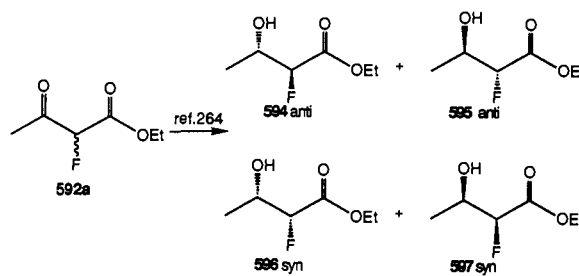
SCHEME 124



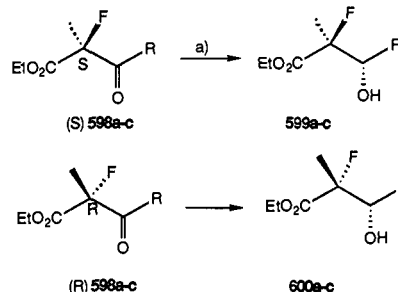
	R	y[%]	ee[%]	ref.
586	CF ₃	75	49-51	261,263,264
587	C ₂ F ₅	87	94	263,264
588	n-C ₇ F ₁₅	88	93	264



	R	t[d]	y[%]	syn / anti
a	CH ₃	5	78	81/19
b	C ₂ H ₅	5	69	78/22
c	n-C ₃ H ₇	7	74	84/16
d	n-C ₄ H ₉	7	71	82/18



SCHEME 125



	R	y[%] of 599	y[%] of 600
a	CH ₃	52	39
b	C ₂ H ₅	43	54
c	n-C ₃ H ₇	37	41

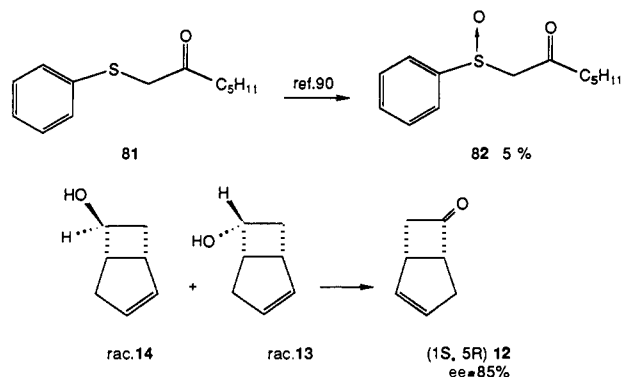
a) BY(Oriental); 35°; 3d; ref. 396

589-591 in good yields.^{261,263,264} Previously reported optical purities^{263,264} for **589** are in contrast to more recent findings.²⁶¹ With lower yields, but still with a fair diastereomeric ratio, monofluorinated compounds **592a-d** were reduced to afford mixtures of diastereomers **593a-d**.²⁶⁴

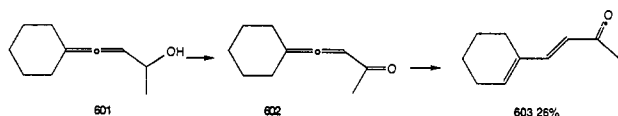
Reduction of the racemic monofluorinated ethyl acetoacetate **592a** (giving rise to the four products **594-597**) was investigated in more detail by extensive NMR studies. The ratio of syn (**596 + 597**) to anti (**594 + 595**) was 81:19 with **596:597 = 4:96** corresponding to an ee of 92%, whereas the ratio between the anti-configured products **594** and **595** was determined to be 28:72 corresponding to a significantly lower ee of 44%.²⁶⁴

Reduction of the optically active (*S*)- α -fluoro- α -methyl- β -keto esters (*S*)-**598a-c** (Scheme 125) gave *anti*- β -hydroxy esters **599a-c** (ee > 99%) while (*R*)-

SCHEME 126



SCHEME 127

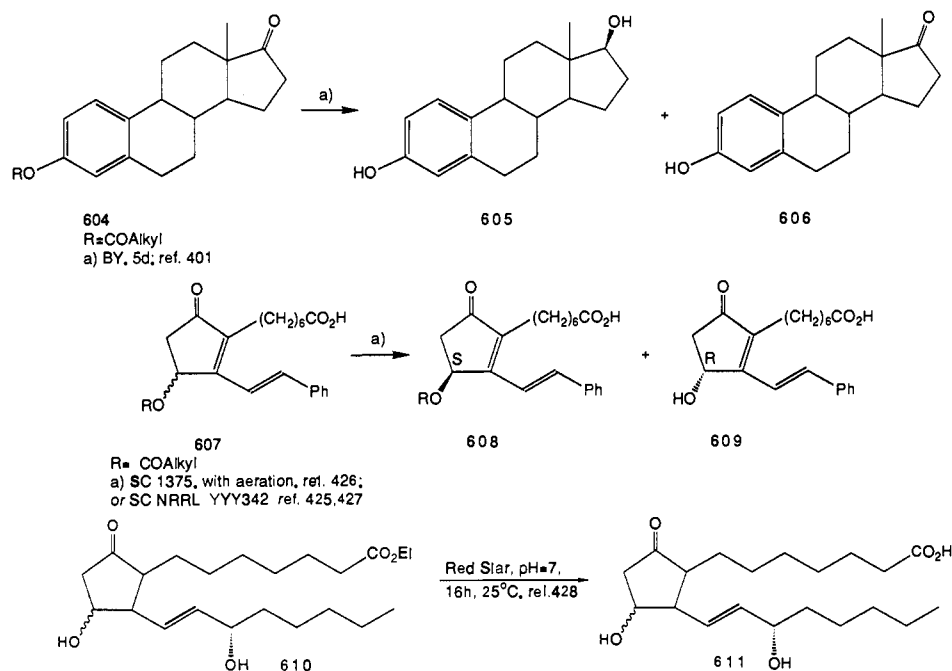


598a-c were reduced to give *syn*- β -hydroxy esters 600a-c (ee > 98%).³⁹⁶

VI. Oxidations

Oxidations by means of BY have scarcely been described in the literature obviously due to two reasons: On the one hand, the microbiological oxidation of alkanols to alkanones is not of very great interest except where polyols are to be selectively oxidized since chemical methods are often adequate.³² On the other hand, the oxidational capabilities of BY seem to be very limited.³⁹⁷ For example, chiral sulfoxidation became an important tool in organic synthesis and many fungi including *Aspergillus niger* and *Rhizopus arrhizus* and fungi from the *Penicillium* and *Rhodotorula* species as well as bacteria were used for these oxidations, but there are no examples performed by BY.³⁹⁸ It seems there is only one oxidation at a sulfur substituent known, namely the oxidation of 81 to racemic 82 (Scheme 126), which proceeded in only about 5% yield.⁹⁰

SCHEME 128



Due to these reasons, only a few examples can be provided. Thus, a 1:5 mixture of racemic 6-*exo*-bicyclo[3.2.0]hept-2-en-6-ol (14) and 6-*endo*-bicyclo[3.2.0]hept-2-en-6-ol (13) was treated with BY for 4 days at pH 6 to give (1*S*,5*R*)-12 in 85% optical purity; 13 was recovered in 90% optical purity.^{45,399}

Finally, α -allenic alcohol 601 (Scheme 127) was oxidized to 602, which underwent an isomerization of the allenic moiety. Thus, 26% of 603 was obtained and 72% of the starting material could be recovered.⁴⁰⁰

VII. Hydrolyses of Esters

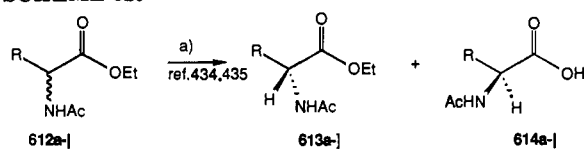
A. General Remarks

Although discovered inadvertently as an undesired side reaction,⁴⁰¹ the deacylations have only recently been a focus of thorough studies. These deacylations have been regarded for a longer period of time just as simple and more or less useless reactions happening only to annoy chemists and to complicate the workup procedure. The first report of such hydrolyses was given by Mamoli⁴⁰¹ in the steroid field. Esters 604 (Scheme 128) gave upon treatment with BY mainly 605 as products both of a reduction at position C-17 and a hydrolysis at position C-3. As byproduct, 606 was observed.⁴⁰¹

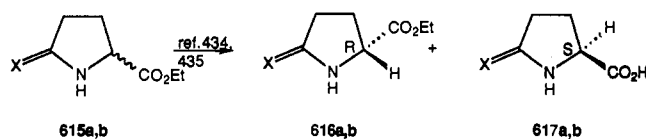
Several groups investigated the enzymes involved in hydrolysis reactions. A very comprehensive review on proteinases^{402,403} has been published recently,⁴⁰⁴ and it has been shown that all compartments of the cells are possible locations for these enzymes. Enzymes characterized as esterases have been isolated from *Saccharomyces cerevisiae*,⁴⁰⁵⁻⁴⁰⁹ and their hydrolytic as well as their synthesizing activities have been probed.^{405,410-414} In addition, phospholipase,⁴¹⁵⁻⁴¹⁷ lipase,^{415,418,419} tributyrinase,^{420,421} and triacylglycerol-lipase activities^{419,422-425} have been detected and investigated.

Such hydrolysis reactions were found to be very suitable in the synthesis of prostaglandins and their

SCHEME 129



	R	y[%] of 613	ee[%] of 613	cond.
a	CH ₃	47	100	an
b	C ₂ H ₅	48	96	an
c	i-C ₃ H ₇	75	13	an
c	i-C ₃ H ₇	38	80	ae
d	i-C ₃ H ₇	48	92	an
e	CH ₂ C ₆ H ₅	38	>97	an
f	CH ₂ CO ₂ Et	49	86	an
g	(CH ₂) ₂ CO ₂ Et	48	89	an
h	(CH ₂) ₂ NHAc	0 ^c	-	an
i	CH ₂ OAc	37	43	an
i	CH ₂ OAc	36	47	ae
j	CH(OAc)CH ₃	64	3	an



	X	y[%]	ee[%]
a	H ₂	10	25
b	O	59	0

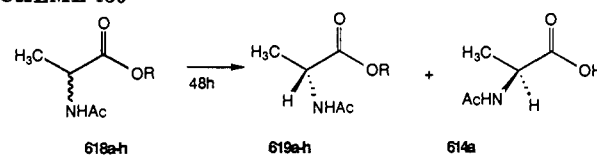
- a) SC Hansen 48h, r.t.
 b) an= anaerob, ae= aerob
 c) 94% of starting material recovered

precursors. Thus, esters of racemic 7-(2-*trans*-styryl-3-hydroxy-5-oxocyclopentenyl)heptanoic acid (607) were stereoselectively hydrolyzed by fermenting BY during 120 h to yield 608 and 609,^{426,427} but no ee values have been reported. Ester cleavage has been reported for 610 to yield 611⁴²⁸ and other prostaglandin precursors.⁴²⁹⁻⁴³¹ An excellent approach for the mathematical treatment of such biochemical kinetic resolutions of enantiomers has been proposed by Sih et al.^{431b}

B. Esters of Amino Acids

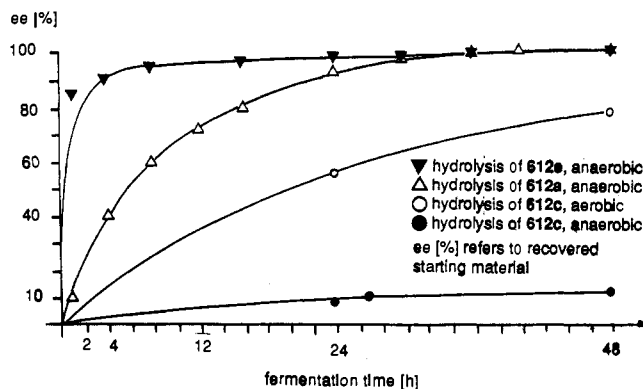
Although the enzymic de-N-acetylation of racemic N-acetyl amino acids by an aminoacylase (E.C. 3.5.1.14) from pork kidney^{432,433} has gained high industrial potential, the hydrolytic abilities of BY have also been used for the synthesis of amino acid derivatives. Thus, racemic esters of N-acetyl amino acids 612a-j (Scheme 129) were hydrolyzed by BY (*Saccharomyces cerevisiae* Hansen) to yield the unreacted D-configured esters 613a-j; acids 614a-k were not isolated.⁴³⁴ Generally the ee's were high for educts containing unbranched alkyl or arylalkyl substituents (e.g., 613a,b,d,e), but the reactions were inhibited by a branching in the β -position, thus resulting in high recovery rates and therefore low ee values (e.g., 613c,j); a substituent in the γ -position showed no effect, whereas introduction of additional polar groups as in 612h-j lowered the ee of the obtained products. Ethyl 3-(N-acetylamino)butanoate was a nonsubstrate for the insertion of an additional CH₂ group between the center of chirality and the ester moiety. Enzymic regioselection, however, was found for diesters 612f,g; only the ester moiety α to the center of chirality was hydrolyzed. The cyclic derivatives

SCHEME 130



	R	y[%] of 619	ee[%] of 619
a	CH ₃	33	88
b	C ₂ H ₅	47	>99
c	n-C ₃ H ₇	35	>99
d	i-C ₃ H ₇	40	96
e	n-C ₄ H ₉	36	98
f	n-C ₅ H ₁₁	25	97
g	CH ₂ C ₆ H ₅	43	>99
h	C ₆ H ₁₁	27	>99
i	C(CH ₃) ₃	recov. 80% of 618i	

SCHEME 131



615a,b gave 616a,b with low or no ee at all. 617 was not isolated.^{434,435}

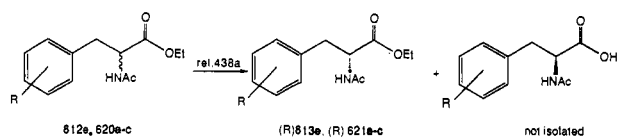
Variation of the alcohol part R of 618a-h (to yield products 619a-h whereas 614a was not isolated) (Scheme 130) showed little or no influence on the course of hydrolysis. No reaction, however, was obtained for *tert*-butyl ester 618i.

The course of hydrolysis has been investigated for 612a,c,e in more detail, and its time dependency is depicted in Scheme 131.

By use of the quadruple-mutant *ABYS1* (from the wild type *X2 180-1A*) deficient of four vacuolar peptidases, i.e., the nonspecific proteinases *yscA* and *yscB* and the nonspecific carboxypeptidases *yscY* and *yscS*, and due to the close analogy of the hydrolytic behavior with α -chymotrypsin, the active enzyme for these hydrolyses was supposed to be an nonspecific carboxylester hydrolase (E.C. 3.1.1.1, optimum pH 8) rather than a lipase, esterase, or phospholipase.⁴³⁴

As an alternative to the use of fresh baker's yeast, the use of acetone-dried powders^{323,436} has been suggested. It was shown that these powders remained active for several months. The presence of nicotinamide during the process of crushing the yeast for providing a cell-free preparation having high fermentative power appears necessary. Alternatively, lyophilized yeast has been prepared and found to offer several advantages as compared to the use of viable cells or acetone-dried powders. It is easily accessible and a ratio of educt to yeast (ca. 1:1.5, w/w) allows the reactions to be performed in a convenient manner. Since no metabolism is detected as long as no carbohydrates are supplied, both working up of the reaction mixture and easy monitoring (e.g., by use of a pH-stat) are facilitated. In addition, reductions of carbonyl groups are suppressed. The hydrolytic activity is fully maintained and was

SCHEME 132

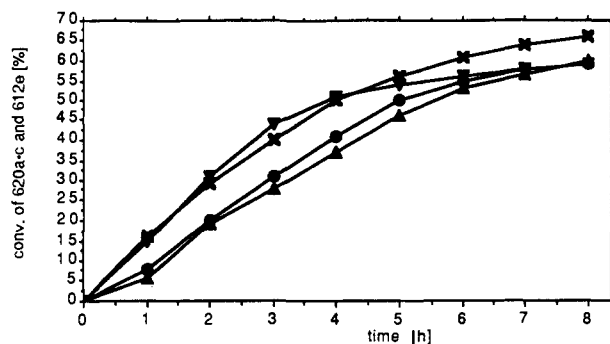


R	educt	y[%]	ee[%]	config.
H	612e	42	>96	R
o-F	620a	40	>96	R
m-F	620b	38	>96	R
p-F	620c	43	>96	R

SCHEME 133

hydrolysis of 612e and 620a-c

▼ conversion of 620a [%] ▲ conversion of 620b [%] ● conversion of 620c [%]
 ■ conversion of 612e [%]



shown to remain stable for several months when stored at 0–4 °C.⁴³⁷

As shown for the hydrolysis of 612e and the fluorinated analogues 620a–c (Scheme 132), the reaction stopped at about 50% conversion (Scheme 133) and thus allowed very easily the isolation of the D-configured esters (R)-613e⁴³⁴ and (R)-621a–c in about 40% yield.^{438a} Very recently, the enantioselective hydrolysis of methyl esters of racemic N-acetyl- α -amino acids by BY (*Saccharomyces cerevisiae* NCIM3044) in reverse micellar suspension has been reported.^{438b}

C. Other α -Substituted Carboxylic Esters

It seems noteworthy in this context that racemic α -substituted carboxylic esters other than amino acid esters gave in general bad results.

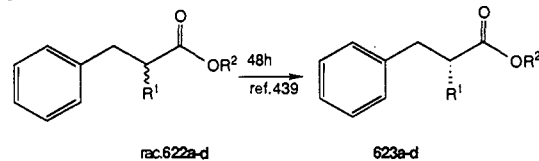
Thus, hydrolysis of 622a–c (Scheme 134) resulted in low yields and low ee's of products 623a–c. Hydrolysis of 622d afforded 40% of (R)-623d with low ee (15%) and 15% of (R)-622d (ee = 60%). The low ee may be due to racemization during the reaction (pH 4). An excellent ee of 99%, however, could be achieved for (R)-622d (31% yield) by use of the lipase of *Candida cylindracea* for the hydrolysis step.⁴³⁹

Racemic 624 was hydrolyzed under aerobic conditions with both moderate yield and ee to afford (S)-624 (40% yield, 35% ee). For racemic 625 the cleavage occurred also at the benzoate, and therefore (S)-625 was obtained although in moderate yield (27%) but fair ee (79%).⁴³⁹

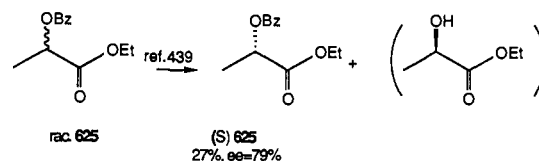
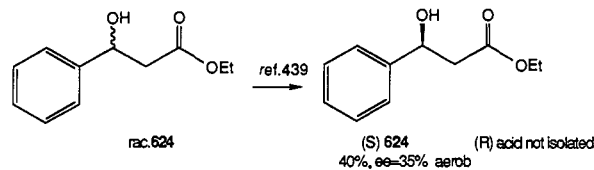
D. Acyloxy Esters and Lactones

Studies were extended to acyloxy carboxylic acid esters 626a–k (Scheme 135). No dramatic differences in the ee values of the products could be observed with use of either viable cells or lyophilized yeast although the ee values were slightly better with lyophilized cells due to facile and appropriate monitoring of the reaction

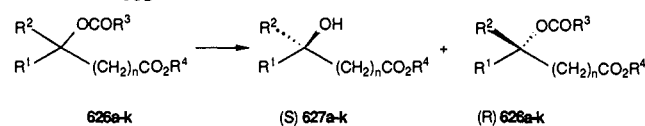
SCHEME 134



R ¹	R ²	y[%]	ee[%]	conf. of 623	cond.
a	OH C ₂ H ₅	25	21	R	an
b	OH n-C ₈ H ₁₇	12	37	R	an
c	CH ₃ C ₂ H ₅	10	40	R	an
d	OC(=O)CH ₃ C ₂ H ₅	40	15	R	ae



SCHEME 135

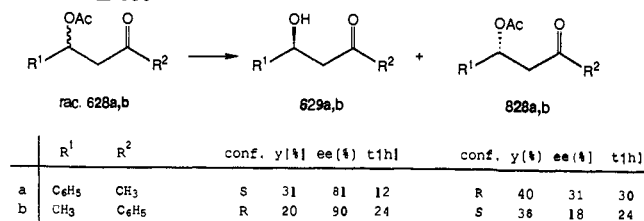


R ¹	R ²	R ³	R ⁴	n	y[%] of (R) 626	ee[%] of (R) 626	y[%] of (S) 627	ee[%] of (S) 627	
a	n-C ₈ H ₁₇	H	CH ₃	C ₂ H ₅	1		non substrate		
b	C ₆ H ₅	H	CH ₃	C ₂ H ₅	1		-.		
c	HC=O	H	CH ₃	C ₂ H ₅	1	>97	91		
d	C ₆ H ₅	H	CH ₃	C ₂ H ₅	1	25	79	21	85
e	CH ₂ -C ₆ H ₅	H	CH ₃	C ₂ H ₅	1	30	<10	17	97
f	(E) PhHC=CH	H	CH ₃	C ₂ H ₅	1	67	33	18	91
g	C ₆ H ₅	H	CH ₃	C ₂ H ₅	0	<10		<10	
h	C ₆ H ₅	H	CH ₃	C ₂ H ₅	2			non substrate	
i	C ₆ H ₅	CH ₃	CH ₃	C ₂ H ₅	1			-.	
j	C ₆ H ₅	H	n-C ₈ H ₁₇	C ₂ H ₅	1			-.	
k	C ₆ H ₅	H	CH ₃	n-C ₈ H ₁₇	1			-.	

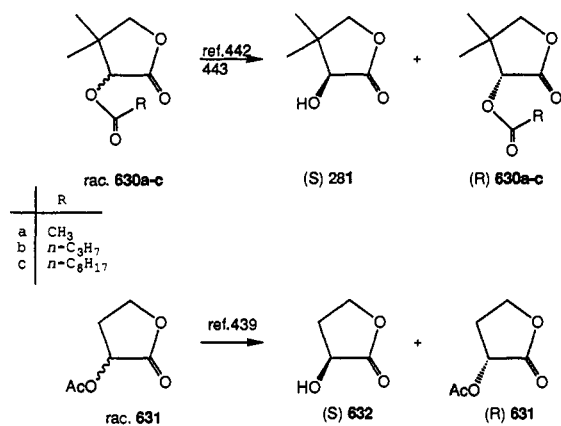
by means of a pH-stat. For example, racemic 626d afforded after 24 h under fermenting conditions 43% of (S)-627d (ee = 76%) whereas prolonged reaction time (48 h) allowed the isolation of (R)-626d (26% yield, 72% ee). Similar results were obtained with lyophilized yeast: (S)-627d was shown to exhibit an ee of 85%, and for (R)-626d an ee of 79% was reported. Enantiomerically pure (R)-627d was obtained by using the lipase from *Pseudomonas* sp. No hydrolysis was observed for butyrate 626j,⁴³⁴ a finding that is in good accord with the reported resistance of butyrates upon hydrolysis by yeasts.⁴⁰⁷ The recovered octyl ester 626k (11% after 48 h) showed no optical rotation; no reaction was observed for 626i either.⁴³⁹

Racemic 626e afforded 17% of 627e (97% ee after 20 h), and 30% of unreacted (nearly) racemic starting material was recovered. (E)-Ethyl 3-acetoxy-5-phenylpent-5-enoate (626f) afforded 18% of 627f with 91% ee after 24 h. Stopping the hydrolysis after 12 h

SCHEME 136



SCHEME 137



afforded 67% of starting material (33% ee). **627f** is a valuable starting material for the synthesis of an inhibitor of 3-hydroxy-3-methylglutaric acid CoA-reductase.⁴⁴⁰ The *n*-pentyl and cyclohexyl analogues **626a,b** were found to be nonsubstrates for yeast (*Saccharomyces cerevisiae* Hansen) mediated hydrolyses.⁴³⁹ These results show that enantioselective hydrolysis is achieved only when the acetoxy moiety is located in β -position to the carboxylate and that the asymmetric center has to bear an unsaturated substituent since simple aliphatic groups of similar size are not sufficient for an effective hydrolysis.⁴⁴¹

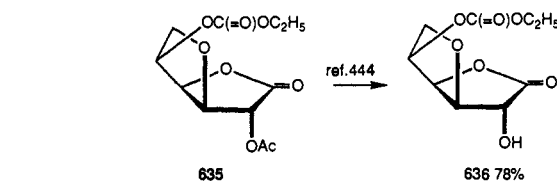
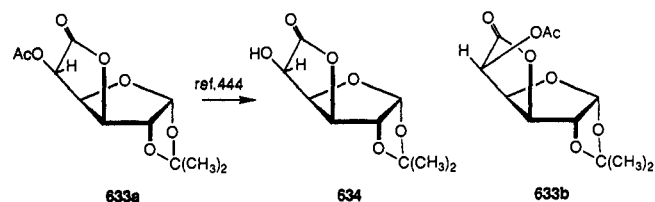
The structural analogues **628a,b** (Scheme 136) were hydrolyzed, and this resulted in the formation of (*S*)-**629a** and (*R*)-**629b** of 81% and 90% ee (isolated after 40% conversion) and of (*R*)-**628a** (31% ee) and (*S*)-**628b** (18% ee), respectively.

Regio- and enantiodifferentiation of the enzymes in yeast cells can be used for the selective hydrolysis of 2-*O*-acyl lactones. Thus, 2-*O*-acetylpantoyllactone (**630a**) (Scheme 137) gave with fermenting BY under anaerobic conditions (48 h) or with lyophilized BY 28% of (*S*)-**281** (86% ee) and 35% of (*R*)-**630a**.⁴⁴² No dependency of the ee from the length of the acyl chain was observed (85% versus 81% from **630b** or **630c**), whereas the conversion rate decreased for the butyrate but increased for the octanoate.⁴⁴³ It is of interest to note that only the lipase from *Aspergillus sp.* exhibited the same enantioselectivity as compared to BY, thus allowing isolation of 32% of (*S*)-**281** (ee = 61%) and 32% of (*R*)-**630** (96% ee). No conversions were achieved with other commercially available enzymes (e.g., the lipases from *Candida cylindracea*, *Pseudomonas sp.*, porcine pancreas, or α -chymotrypsin).^{439,442}

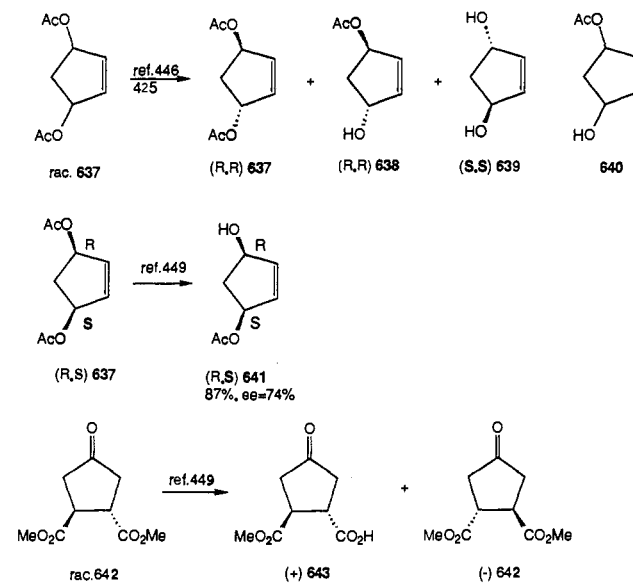
For comparison, the analogue *rac*-**631** however, gave upon hydrolysis with BY under anaerobic fermentative conditions only 14% of (*S*)-**632** and 25% of (*R*)-**631** (70% ee).⁴³⁹

Similarly, the deacetylation of carbohydrate-derived α -acetoxy lactone **633a** (Scheme 138) afforded **634**,

SCHEME 138



SCHEME 139

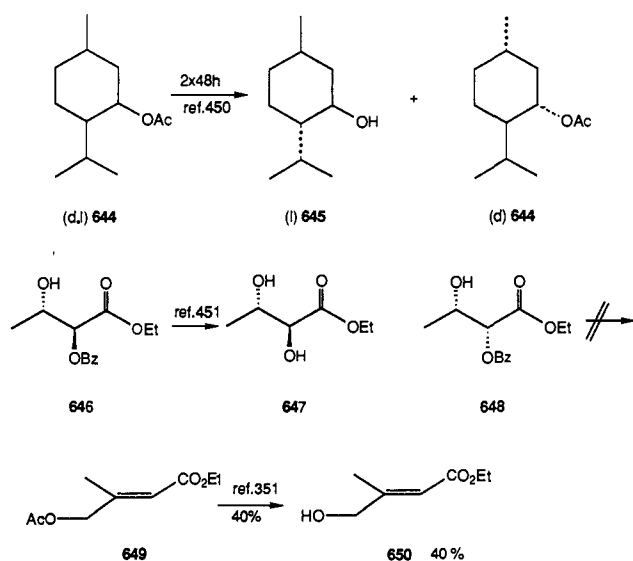


whereas **633b** was not affected by lyophilized BY but by several enzymes.⁴⁴⁴ By means of this educt enantioselectivity, mixtures of epimers **633a** and **633b** could be separated very easily. The anhydro sugar **635** was deacetylated by the same procedure within 15 h to yield **636**; unfortunately, this method was not extendible to the regioselective deacetylation of peracetylated carbohydrate-derived lactones⁴⁴⁴ or anhydro sugars.⁴⁴⁵

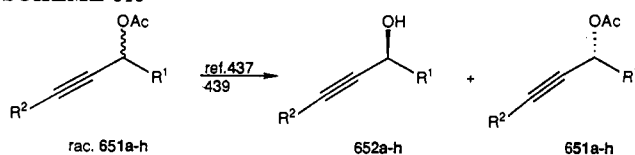
A further application for these regioselective hydrolyses was performed for the diacetylated cyclopentene derivative racemic **637** (Scheme 139), which gave a mixture of (*R,R*)-**637**, (*R,R*)-**638**, and (*S,S*)-**639**.⁴⁴⁶ It was found⁴²⁵ for a 1:1 mixture of *cis*- and *trans*-**637** that the meso compound *cis*-**637** was more rapidly hydrolyzed than *trans*-**637**. The highest ee values for (*R,R*)-**637** (93%) and (*R,R*)-**638** (90%) were obtained after 48 h, whereas the ee for (*S,S*)-**639** dropped with prolonged reaction time (32% after 17 h, 10% after 48 h).⁴²⁵ As a byproduct due to the action of (a) reductase(s) **640** was obtained. Since these compounds are valuable starting materials for the synthesis of optically active prostaglandins, alternative enzymic approaches have been reported.^{425,447} BY treatment of (*R,S*)-**637** afforded in 87% chemical yield (*R,S*)-**641** of 74% ee.⁴⁴⁸

Similarly, (\pm)-*trans*-3,4-bis(methoxycarbonyl)cyclopentanone (**642**) was hydrolyzed to yield (+)-**643** and (-)-**642** although with low chemical yield (25%) and low ee (30%); better results could be achieved with *Candida*

SCHEME 140



SCHEME 141



R ¹	R ²	652			651		
		y[%]	ee[%]	t[h]	y[%]	ee[%]	t[h]
a	CH ₂ CH(CH ₃) ₂	22	91	24	19	72	35
b	CH ₂ CH(CH ₃) ₂	10	94	24	56	<10	24
c	n-C ₆ H ₁₁	29	>97	26	21	74	48
d	n-C ₇ H ₁₅	7	91	24	36	18	18
e	CH ₂ CH=CH ₂	10	95	9	33	35	18
f	CH ₂ CO ₂ Et	37	91	18	40	>97	15
g	C ₆ H ₅	14	86	9	45	64	17
h	(CH ₃) ₂ C=CHC ₂ H ₅	66	87	23	42	37	35

humicola CCY29-11-1 (ee > 99%).⁴⁴⁹

Although with low rate of hydrolysis (14% with *Saccharomyces cerevisiae* var. *ellipsoideus*, 41.8% with *Rhodotorula mucilaginosa*, ee = 99.2%), *dl*-menthyl acetate (644) (Scheme 140) was hydrolyzed; it was found that *l*-menthylacetate was preferentially hydrolyzed to form *l*-menthol (645).⁴⁵⁰ The rate of hydrolysis and the ee values dropped on increasing of the acyl moiety. No hydrolysis occurred with isomenthylacetate by yeasts (but by bacteria and other fungi); citronellol isolated from microbial hydrolysis of *dl*-citronellyl acetate was optically inactive.⁴⁵⁰

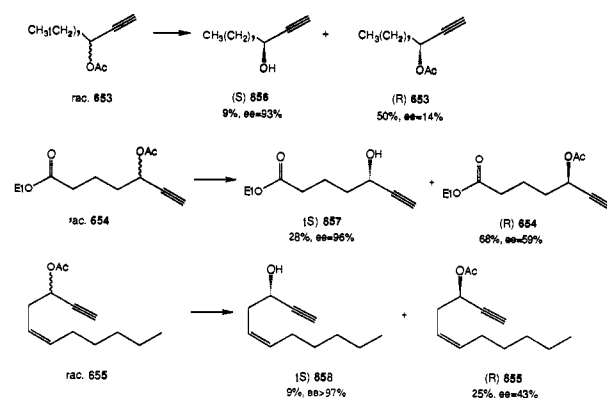
Hydrolysis⁴⁵¹ of *anti*-646 yielded 647 (but no saponification of *syn*-648 occurred); the hydrolysis of 649 gave 40% of 650.³⁵¹

E. Alkynol Acetates

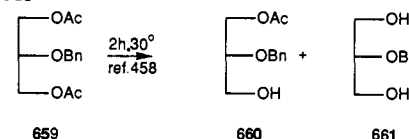
Chiral propargylic alcohols have gained importance in the synthesis of natural products. Both enantiomers of optically active 1-alkyn-3-ols of high optical purity can be obtained by resolution of their corresponding racemic acetates by use of lyophilized BY.⁴³⁷

Previously, enantioselective hydrolyses of such compounds have been performed with selected microorganisms that may not be cultivated without sterile fermentation equipment, e.g., *Bacillus subtilis*,⁴⁵²⁻⁴⁵⁵ *Brevibacterium ammoniagenes*,⁴⁵⁶ and *Rhizopus nigricans*.⁴⁵⁷ Thus, *rac*-651a-h (Scheme 141) afforded on

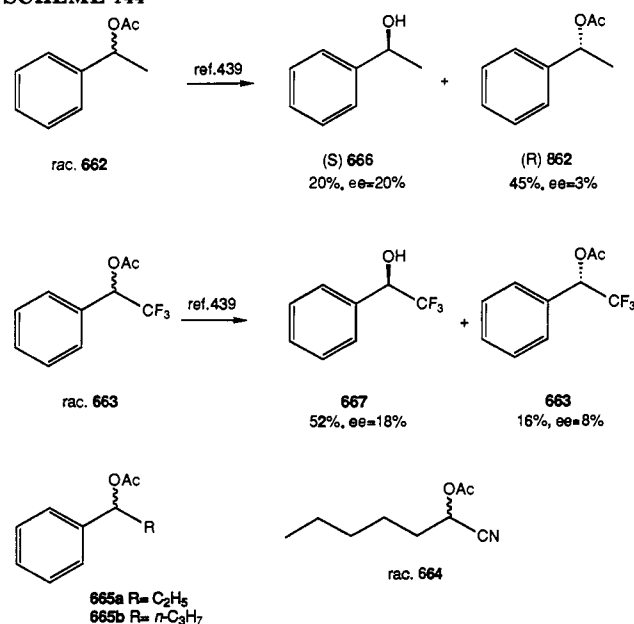
SCHEME 142



SCHEME 143



SCHEME 144



treatment with lyophilized BY^{437,439} 652a-h in high optical purity.

Racemic alkynol acetates 653-655 (Scheme 142), which are precursors for the synthesis of leukotrienes, were hydrolyzed in an enantioselective manner by means of lyophilized BY. Thus, *rac*-653 afforded (S)-656 (93% ee) and (R)-653 (14% ee), 654 gave (S)-657 (96% ee), and 655 was hydrolyzed to yield (S)-658 (>97% ee).⁴³⁹

From these results it becomes clear that replacement of the acetylenic hydrogen by a methyl group shows a dramatic decrease in the speed of hydrolysis, making a conversion of 60% inaccomplishable. An unsubstituted CH₂ unit adjacent to the asymmetric center is necessary for a high degree of enantioselection.⁴³⁷

F. Miscellaneous Hydrolyses

1,3-Di-O-acetyl-2-O-benzylglycerol (659) (Scheme 143) gave upon hydrolysis with BY⁴⁵⁸ 29% of 660 and 1% of completely deacetylated 661. The ee of 660 was low

(32.7%) but could be improved on use of enzymes.⁴⁵⁸⁻⁴⁶⁰

Similar low ee values have been obtained for the hydrolyses of racemic **662-664** and **665a,b** (Scheme 144).⁴³⁹

Contrary to bacteria and several yeast strains,^{461,462} *Saccharomyces cerevisiae* neither had the ability to utilize lactams nor was able to hydrolyze them.⁴⁶² On the other hand, extracts from disintegrated BY cells found an application in the hydrolysis of several amino acid naphthylamides.⁴⁶³ Very recently *dl*-3-acetylazetidinone derivatives have been reduced by BY without affecting the lactam moiety.⁴⁶⁴

VIII. Immobilized Baker's Yeast

A. General Remarks

Biotransformations with immobilized yeast cells are attractive due to several reasons although the catalytic activity of the cells is generally reduced when compared to the same amount of cells in solution. This loss of activity is caused by an additional permeability barrier²⁶ introduced by the carrier material and due to some cell damage occurring during the immobilization. Four major categories for immobilization of microorganisms in general and of baker's yeast in particular can be recognized in analogy to the immobilization of enzymes.⁴⁶⁵

(i) *Immobilization by physical or chemical adsorption*: surface adsorption to a water-insoluble, solid support, e.g., a metal oxide, DEAE-cellulose, or an ion-exchange resin.

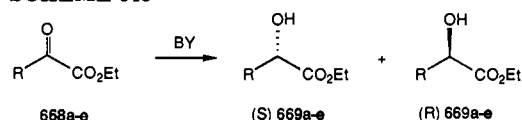
(ii) *Cell aggregation of the microorganism*: physical or chemical (e.g., glutaraldehyde) cross-linking.

(iii) *Covalent attachment to a carrier material*: e.g., carboxymethyl cellulose.

(iv) *Microorganism entrapment in a gel or a membrane or within microcapsules*: applicable in industrial and laboratory use (urethane, cellulose, agar, alginate, collagen, chitosan, κ -carrageenan, and polyacrylamide have been used as polymeric porous networks for entrapment).

Beside enhancing the operational stability of yeast by immobilization, easier isolation of the products is provided. In addition, reuse of the catalyst is often possible. Due not only to minimal inhibitory influences but also to high cell population, product formation rates are usually high.⁴⁶⁶ Continuous operation is performed easily since the immobilized cells are easily removed from the reaction medium and can repeatedly be used although with decreasing activity of the immobilized cells. In contrast to enzyme immobilization, a required coenzyme is supplied and regenerated within the intact cell.¹⁴ Comparative studies between "free" yeast and immobilized yeast cells have only scarcely been performed. Some differences in stereoselectivity and yield, however, have been observed depending on the kind of immobilization. This observation seems reasonable since immobilized yeast cells (either gel entrapped or adsorbed on various carriers) exhibit altered physiological, morphological, and metabolic properties.⁴⁶⁷ Significant differences in kinetics, growths, and DNA/RNA and protein content of *Saccharomyces cerevisiae* adsorbed on gelatin-coated glass beads were reported.^{468,469} It seems therefore that immobilization affects the cells in several aspects simultaneously and

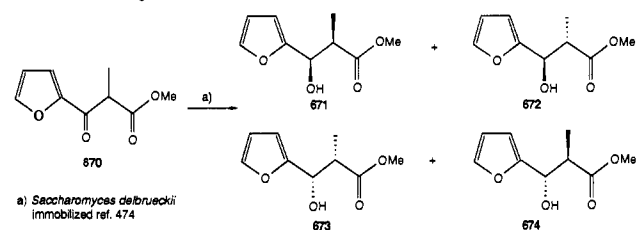
SCHEME 145



n	educt ^a	free BY product ee / y (%)/[%]	imm. BY (water) product ee / y (%)/[%]	imm. BY (hexane) product ee / y (%)/[%]
0	668a	(S)-669a 91/47	(S)-669a 87/43	(S)-669a 94/33
1	668b	(S)-669b 77/42	(S)-669b 66/42	(S)-669b 36/28
2	668c	(S)-669c 31/36	(S)-669c 39/36	(S)-669c 32/27
3	668d	(S)-669d 50/29	(S)-669d 78/20	(R)-669d 47/41
4	668e	(S)-669e 30/23	(S)-669e 63/31	(R)-669e 54/36
mean time of reaction (h)		5	6	24-72

^a taken from ref. 473

SCHEME 146



a) *Saccharomyces delbrueckii* immobilized ref. 474

Course of reduction of 670 in dependence of prepolymer used for immobilization (*Saccharomyces delbrueckii*).⁴⁷⁴

prepolymer	yield ^a [%]	671 ee[%]	672 ee[%]	673 ee[%]	674 ee[%]	ratio syn/anti
none	43	90	91	--	--	39/61
PU-6b	33	56	74	--	--	41/59
ENT-4000 ^c	64	--	--	81	57	50/50

^a total yield; ^b immobilization with urethane; ^c immobilization with a photon crosslinkable prepolymer.

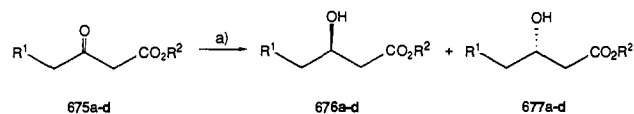
that the combination of these effects imposes stresses on the metabolic behavior.⁴⁷⁰ Among these effects are found diffusional limitations (leading to partitioning of hydrophobic material, reduced oxygen concentration, and oxygen-transfer rates⁴⁷¹) as well as the creation of microenvironmental effects such as reduced water activity and high cell concentrations. As shown for polyacrylamide-hydrazide-entrapped cells of *Saccharomyces cerevisiae*, even the tolerance to ethanol and ethylene glycol was increased.⁴⁷²

B. Examples for the Use of Immobilized Baker's Yeast

Ethyl 2-oxoalkanoates **668a-e** (Scheme 145) have been reduced by free BY in water and by immobilized yeast (using a polyurethane prepolymer) either in water or in hexane.⁴⁷³ Some marked differences in the stereochemical control of these reactions have been elaborated obviously due to the effects of changes in the reaction conditions at the surface of the yeast (as evidenced by scanning electron microscopy the yeast cells are tightly surrounded by the polymer) or due to a change in the concentrations of the respective substrates.

In some cases, as for the reduction of **670** (Scheme 146) with *Saccharomyces delbrueckii*, the stereochemistry is affected by the used prepolymer. Thus, differing amounts of products **671-674** are obtained depending

SCHEME 147



	R ¹	R ²
a	Cl	C ₂ H ₅
b	Cl	CH ₃
c	CH ₃	CH ₃
d	H	C ₂ H ₅

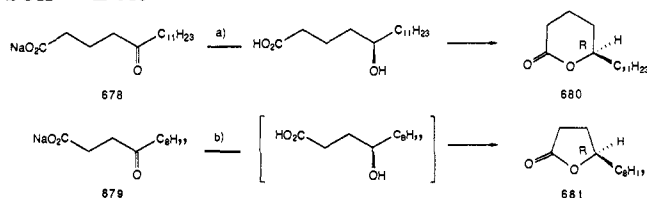
a) BY, 60-80%, rel.225

Entrapment dependent reduction of β-keto-esters.²²⁵

educt	entrapment	product	ee (%)	conc. of educt [mmol]
a	free BY	677a	42	10
a	free BY	677a	27	20
a	free BY	677a	15	50
a	ALG ^a	677a	16	20
a	CAR ^b	677a	11	20
a	PAA ^c	677a	3	10
a	PU ^d	676a	82	10
a	PU	676a	82	50
b	free BY	677b	31	10
b	free BY	677b	12	20
b	ALG	677b	10	20
b	PU	676b	90	20
c	free BY	676c	5	20
c	ALG	676c	17	20
c	PU	676c	86	20
d	free BY	677d	>98	20
d	ALG	677d	92	20
d	PU	677d	60	20

a) entrapment in calcium alginate; b) entrapment in carrageenan⁴⁷⁷; c) entrapment in polyacrylamide⁴⁸⁰; d) entrapment in polyurethane.⁴⁸¹

SCHEME 148



a) 5g κ-carrageenan/4g BY, 30°C, 48h, 32%; b) 30°C, 48h, 26%, ref.482

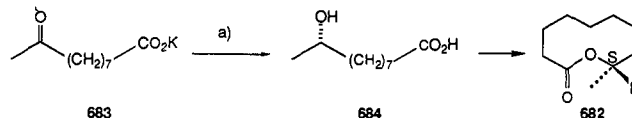
on the used prepolymer.⁴⁷⁷⁻⁴⁸¹ The use of a water-miscible organic solvent (DMSO, THF, or 1,4-dioxane) or nonpolar organic solvents saturated with water was shown to deactivate the immobilized cells.⁴⁷⁴ No reaction was observed with *Saccharomyces cerevisiae*.^{475,476}

The use of calcium alginate⁴⁷⁷ or κ-carrageenan⁴⁷⁸ has been shown to be unsuitable for the reduction of 668a-e since a certain amount of water oozed from such gels during the reaction. For the reduction of 675a-d, this seems to be insignificant with respect to the optical purity.²²⁵ For 676a-d and 677a-d (Scheme 147), the ee of each alcohol was unaffected by the substrate concentration in the reduction by polyurethane-entrapped BY whereas the ee value was susceptible to the concentration in the reduction by free BY.

κ-Carrageenan-immobilized BY was taken for the reductive lactonization of 678 and 679 (Scheme 148) used for the synthesis⁴⁸² of (R)-5-hexadecanolide ((R)-680), the pheromone component isolated from the heads of the queens of the oriental hornet, *Vespa orientalis*,⁴⁸³ and of (R)-4-dodecanolide ((R)-681), the defensive secretion from pygidial glands of rove beetles, *Bledus mandibularis* and *Bledus spectabilis*, respectively.⁴⁸⁴ These reductions employing immobilized BY gave lower yields as compared with the analogous reduction with "free" BY⁴⁸⁵ (Scheme 148).

It is of interest to note that in the synthesis⁴⁸⁶ of phorancolide I (682) (Scheme 149) the reduction of 683 by κ-carrageenan-immobilized BY with the first use of

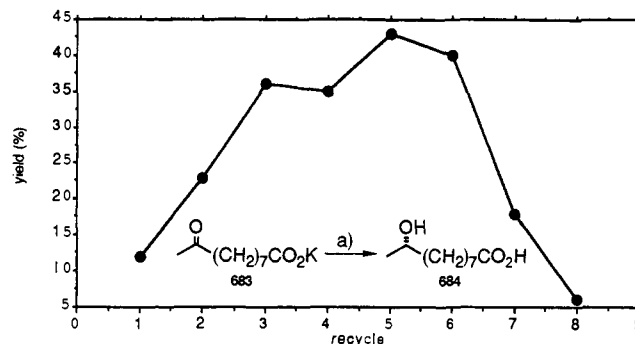
SCHEME 149



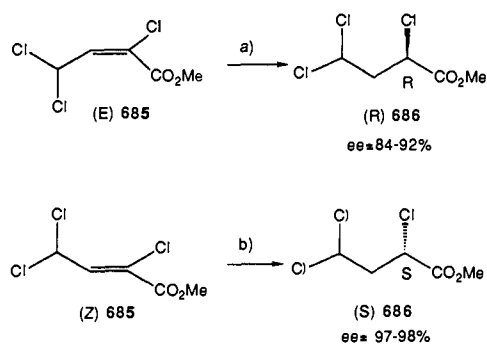
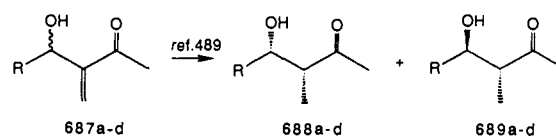
a) immobilized BY, 48 h, 35°C, ref.486

SCHEME 150

Dependence of the yield from the recycle number

a) BY: ee=96%, ref.486
fresh BY: 36%, ee=92%

SCHEME 151

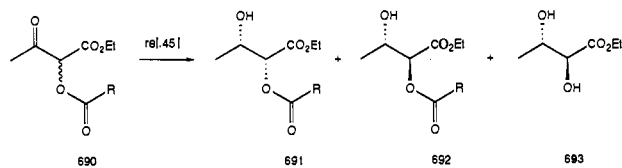
a) i) Ca-alginate immobilized BY, 32°C, 30h, pH 7 to 5, 65% ii) CH₂N₂ b) 60%, ref.487.

R	y[%]	ratio 688:689	ee[%] of 688	ee[%] of 689	
a	C ₂ H ₅	61	53:47	98	98
b	n-C ₃ H ₇	64	42:58	98	72
c	n-C ₄ H ₉	56	33:67	98	67
d	n-C ₅ H ₁₁	72	36:64	98	69

the catalyst gave a lower yield of (S)-684 (12%), whereas a maximum yield of 43% was obtained after the fifth use (Scheme 150). In addition, the immobilized BY could be stored in an aqueous solution of KCl for 6 months at 0-5 °C. The optical purity of 684 was constant within experimental error throughout the use of the catalyst but only slightly superior than that obtained by the use of free BY.

Sodium alginate/CaCl₂ immobilized BY⁴⁷⁷ has been applied for the highly stereocontrolled synthesis of D- and L-armentomycin.⁴⁸⁷ L-Armentomycin, (S)-2-amino-4,4-dichlorobutanoic acid, is known as a naturally occurring antibiotic from the culture broth of *Strep-*

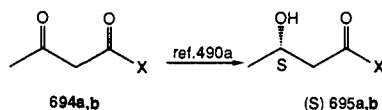
SCHEME 152

690a: R= CH₃; 690b: R= C₂H₅

yeast	[η]	γ[%]	691: 692: 693 [%]
a	free BY	48	23:18:54
a	imm. BY	12	19:58:23
a	imm. 8Y	43	18:26:56
a	imm. BY	53	18:19:63
b	free BY	23	14:86:0
b	imm BY	21	6:54:0
b	immBY	21	51:49:0

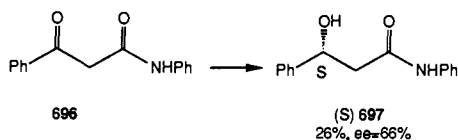
[at pH=7]

SCHEME 153



X	condition *	γ[%]	ee[%]
a	NHET	BY (dry / fresh)	0
		dry BY (alginate)	9
		BY (fresh)	11
b	NHPh	SC H194	48
		dry BY (alginate)	36
		fresh	21
		dry BY (polyurethane)	28

* 7d, 30°



tomyces armentosus var. *armentosus*.⁴⁸⁸ The remarkable feature of this reduction is the highly effective stereochemical control for each geometrical isomer ((*Z*)- or (*E*)-685) to produce precursors of L-armentomycin ((*S*)-686) or its D enantiomer ((*R*)-686) (Scheme 151).

The lower stereoselectivity for the reduction of the *E* isomer ((*E*)-685) to *R*-configured 686 as compared to the reduction of (*Z*)-685 to (*S*)-686 reflects the enantioselectivity of the involved yeast reductase(s), since both (*E*)- and (*Z*)-685 were found to be stable under reaction conditions.⁴⁸⁷ Although immobilization of BY in calcium alginate gels represents an extremely economical method, the half-lifetime of such a preparation seems to be limited to several weeks.⁴⁷⁷

Examples for the reduction of α -methylene-branched carbonyl compounds by alginate-immobilized BY have been provided. Thus, 687a-d afforded *syn*-(3*R*)-688a-d and *anti*-(3*R*)-689a-d. The methylene group was reduced in all cases whereas no reduction of the carbonyl group took place.⁴⁸⁹

The use of calcium alginate gel immobilized BY was shown to improve the anti selectivity as well as the total

yield of the reduction products for some β -keto esters. Thus, 690 (Scheme 152) gave on treatment with BY or immobilized BY *syn*-configured 691 and the *anti*-configured compounds 692 and 693; the ee of each of the products was higher than 95%.⁴⁵¹ From the data provided in Scheme 152 it can be seen that only the *anti*-configured product 692 is hydrolyzed by BY to yield 693.⁴⁵¹

The reductions of 694 and 696 (Scheme 153) were achieved to yield the corresponding (*S*)-hydroxy compounds 695 and 697, respectively.^{490a} While in the case of substrate 694a no reduction occurred using dry or fresh BY, alginate-immobilized BY afforded 9% conversion and 695a was shown to possess an ee of 90–95%. As for 694b, *Saccharomyces cerevisiae* (H-194) gave a yield of 48% of 695b showing an ee of >90%, treatment with fresh BY afforded only 11% of the product, but alginate-immobilized BY again gave 21–36% yield (ee 95–98%).^{186,490a}

Recently, the reduction of β -keto esters with BY immobilized by magnesium alginate has been introduced as exemplified for the reduction of methyl 3-oxopentanoates to yield predominantly (*S*)-hydroxy esters (i.e., *L*-configured), whereas under "normal" reaction conditions the (*R*)-hydroxy ester is obtained.^{490b}

IX. Miscellaneous Reactions

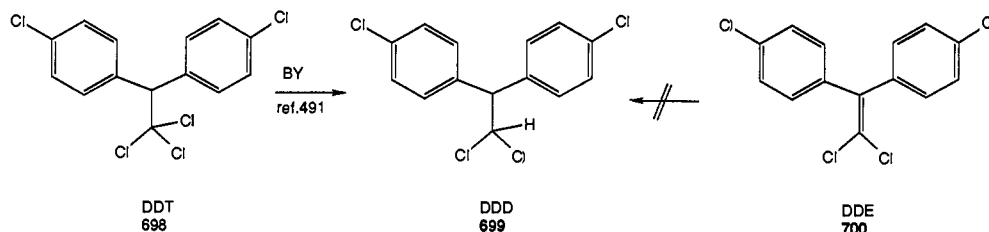
Of importance in environmental chemistry are attempts to probe the yeast-mediated abilities of degradation of pesticides as exemplified in the transformation of DDT (698) (Scheme 154) into (1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethane) (DDD, 699) by reductive dechlorination of the former. DDE (700) gave upon treatment with BY no 699.⁴⁹¹

Contrary, reduction of (*Z*)-3-chloro-3-alken-2-ones 701a-c (Scheme 155) with fermenting BY proceeded well and afforded optically active α -chloro ketones 702a-c, which were reduced on further treatment with BY to optically pure chlorohydrins 703a-c and 704a-c. It was shown that the reduction of the double bond was fast—independent of the length of the carbon chain—while the reduction of the C=O bond is retarded as the carbon chain length increases.⁴⁹²

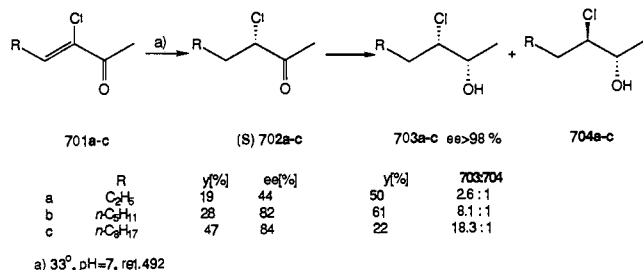
Phosphorylations^{16,53a,54} by means of BY have been reported very early. Thus, nucleoside 5'-phosphates,⁴⁹³⁻⁵⁰⁰ galactose 1-phosphate,⁵⁰¹ glucose and fructose 1,6-diphosphate⁵⁰² as well as 2-deoxyglucose 1,6-diphosphate⁵⁰³ and 2- or 3-phosphoglycerates⁵⁰⁴⁻⁵⁰⁶ have been prepared.

The reduction of galactose to dulcitol⁵⁰⁷ as well as the cleavage of glycoside aesculin (705) (Scheme 156) to yield the phenolic aglycon aesculetin (706)⁵⁴ have been reported; on treatment of amygdalin (707) with BY, both glycosidic bonds were cleaved.⁵⁴

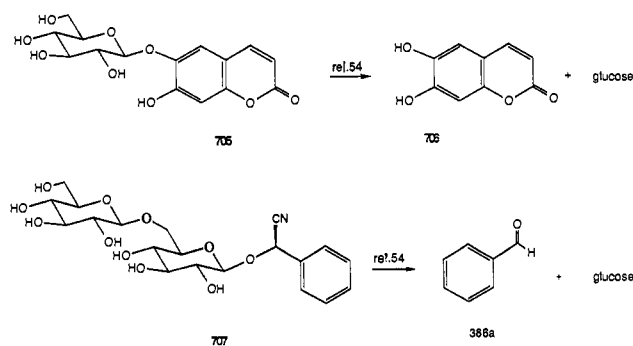
SCHEME 154



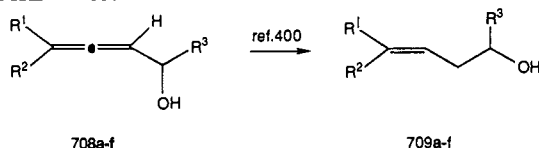
SCHEME 155



SCHEME 156



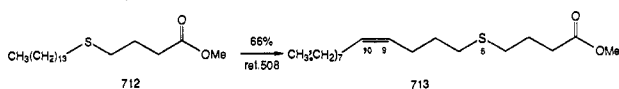
SCHEME 157



	R ¹	R ²	R ³	[α] _D	y[%] 708:709
a	CH ₃	C ₂ H ₅	H	2	2 80
b	CH ₃	CH ₃	H	2	25 65
c	CH ₃	H	H	2	20 70
d		C ₂ H ₅	H	2	30 55
e	CH ₃	C ₂ H ₅	CH ₃	3	20 55
f	CH ₃	CH ₃	CH ₃	5.5	35 50
g	CH ₃	H	CH ₃		no reaction



SCHEME 158



Of special interest is the reduction of α -allynic alcohols **708a-f** (Scheme 157), which gave the corresponding β -ethylenic alcohols **709a-f** whereas β -allynic alcohol **710** afforded 35% of γ -acetylenic alcohol **711**.⁴⁰⁰

There are many examples of BY-mediated hydrogenations of alkenes to yield the corresponding alkanes, but there seems to be only one example of the reverse process. Thus, methyl 5-thioesterate (**712**) (Scheme 158) afforded upon BY (SC NRC2335) treatment methyl 5-thiooleate (**713**) in 66% yield. In addition, it was shown that addition of **712** changed the fatty acid profile of the cell extracts dramatically although no

observable effect on the growth of the yeast cells could be detected.⁵⁰⁸

Abbreviations Used

BY, baker's yeast; IBY, immobilized baker's yeast; ee, enantiomeric excess; de, diastereomeric excess.

X. References

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