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Journal of Molecular Catalysis B: Enzymatic 5 (1998) 255–259

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Microbial reduction of varying size cyclic β-ketoesters. Stereoselective synthesis of chiral lactones and epoxides

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Received 24 October 1997; accepted 19 November 1997

Abstract

The reduction of four- to eight-membered cyclic β-ketoesters to the corresponding β-hydroxyesters by various yeasts and fungi is described. The microbial reduction of ethyl cyclobutanone 2-carboxylate is reported for the first time. The different stereospecificities observed in these reductions involving a dynamic kinetic resolution are analysed. The enantiopure reduction products have been used in the synthesis of both enantiomers of functionalized lactones and epoxides.

Keywords: 1-Cyclobutanol-2-carboxylate; β-Hydroxyester; ω-Epoxyester; Enantiospecificity; Diastereoselectivity; Dynamic kinetic resolution

1. Introduction

The microbial reduction of (±) α-substituted β-ketoesters is now a well-established method for preparation of the enantiopure corresponding β-hydroxyesters, taking advantage of the dynamic kinetic resolution resulting from the enolization/racemisation of the β-ketoester and the different specificities of the reducing enzymes [1,2]. Excellent results are obtained for the reduction of cyclic substrates. For example, the homochiral (1*S*,2*R*)-*cis* hydroxyesters **3a** and **4a** have been obtained by baker's yeast reduction [1] of **1** and **2**, respectively, whereas the (1*S*,2*S*)-*trans*-hydroxyesters **3b** and **4b** were obtained by reduction with *Rhizopus arrhizus* [3] (Scheme 1).

Such cyclic hydroxyesters are highly versatile chiral synthons and have been currently used in the asymmetric synthesis of biologically active compounds [4–7]. We have recently described [8] the preparation of both enantiomers of the lactones **8** ($n = 1,2$) with $R =$ TBDPS. In order to extend these results to differently sized lactones, we have investigated the microbial reduction of the cyclic ketoesters **5**, **6** and **7**. We report here the first reduction of the cyclobutanone derivative **5** by baker's yeast. The stereospecificities of the reduction of **6** and **7** [9] by

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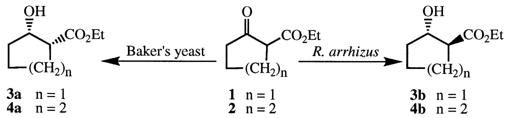
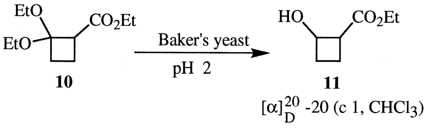
PII: S1381-1177(98)00045-9



Scheme 1.



Scheme 2.

some microorganisms have been determined and compared with the stereospecificities of the reduction of **1** and **2**. At last, some of the resulting β-hydroxyesters have been used for the synthesis of chiral lactones **8** with $R=$ TBDPS or $R=$ Ts, which have been then transformed into chiral epoxides **9** by applying stereoconvergent or stereodivergent methods.

2. Microbial reduction

2.1. Reduction of the cyclobutanone-2-carboxyester *5*

Ethyl cyclobutanone 2-carboxylate **5** is not easily obtained in a pure state. In one of the synthetic routes, the diethylacetal derivative **10** is deprotected in strongly acidic conditions [10], where the ketoester **5** is poorly stable. We have tried to obtain deprotection in conditions compatible with a sufficiently fast microbial reduction, in order to prevent the accumulation of the unstable ketoester.

For this purpose, we have investigated the baker's yeast reduction of ethyl cyclopentanone 2-carboxylate **1**, as a model compound, in citric acid buffered solutions (Table 1). The reduction is decreased at lower pH's but is still possible at pH 2. On the other hand, the diethylacetal derivative **10** is deprotected in a pH 2 solution.

When incubated with baker's yeast in a 0.1 M citric acid solution (pH 2), the slow deprotection and microbial reduction of **10** resulted in the formation of optically active ethyl cyclobutanol-2-carboxylate **11** with a presumed (1*S*,2*R*)-configuration (Scheme 2). A 30% yield was obtained without further optimisation. Enantiomeric excess, measured by GC of its (*S*)-*O*-acetyllactyl ester [11] was greater than 93%.

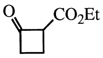
Table 1
Baker's yeast reduction of the β-ketoester **1** at different pH's

pH	Incubation time (h)	Ketoester **1** (%)	Hydroxyester **3a** (%)
4	1.5	19	81
3.2[a]	1.5	32	68
2.6[a]	1.5	44	56
2	2.5	24	76
	3	20	80
	6	10	90

[a] Total reduction was completed after 15 h.

2.2. Reduction of β-ketoesters *6* and *7*

About 50 strains of yeasts and fungi, which were previously found active in the reduction of **1** and **2**, were tested for the reduction of β-ketoesters **6** and **7**. Using selected microorganisms [9] it was possible to prepare one enantiomer of the *cis*-hydroxyesters **12**–**13** and the *trans*-hydroxyesters **14**–**15** (Scheme 3). Their respective (1*S*,2*R*) and (1*S*,2*S*) configurations were determined by transformation into the known corresponding methyl ketones.



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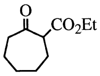

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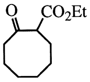

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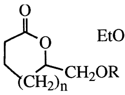

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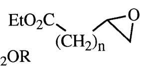

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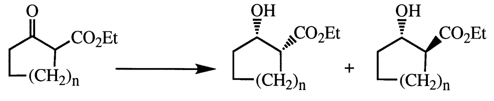
However, several distinct features have been noted compared to the reduction of **1** or **2**. A number of microorganisms are inactive, about



6 n = 3
7 n = 4
12 n=3
13 n=4
14 n=3
15 n=4

Scheme 3.

1/3 of them for **6**, 2/3 for **7**. Baker's yeast, for example, is not able to reduce **7** and the rate of reduction of **6** is much slower than the rate of reduction of **1** or **2**. Longer reaction times induce the formation of cycloalkanones as a consequence of the hydrolysis of the ketoesters and subsequent decarboxylation. Generally, a mixture of diastereomeric *cis*-hydroxyesters **12**–**13** and *trans*-hydroxyesters **14**–**15** is observed (Table 2), with a major *S*-stereogenic specificity ($> 93\%$). In some examples, the *cis* to *trans* ratio observed with the same strain varies with the ring size. To analyse this variation, we have reported in Table 2 the calculated enantiospecificity for the *R*-enantiomer of the ketoester associated with the reduction with a *S*-configuration [$= R_{(S)}$], and the enantiospecificity for the *S*-enantiomer of the ketoester associated with the reduction with a *R*-configuration [$= S_{(R)}$], obtained by the following equations:

$$R_{(S)} = \frac{(1S,2R) - (1S,2S)}{(1S,2R) + (1S,2S)},$$

$$S_{(R)} = \frac{(1R,2S) - (1R,2R)}{(1R,2S) + (1R,2R)}.$$

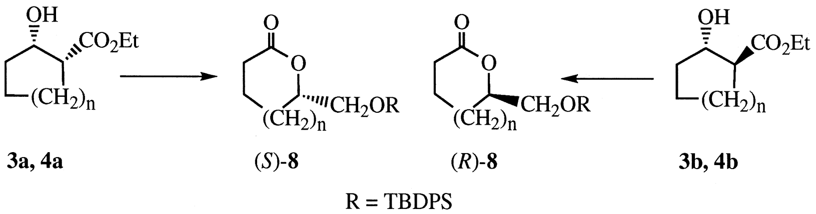
$R_{(S)}$ and $S_{(R)}$ values may vary from -1 to $+1$ depending on the relative enantiospecificities of *S* and *R* reductions: in both cases, a positive value indicates a major *cis*-hydroxyester formation whereas a negative value is indicative of a major *trans*-hydroxyester formation.

An examination of $R_{(S)}$ and $S_{(R)}$ values in the case of the reduction by baker's yeast, when effective, shows very similar positive values for the same ring size, corresponding to the fact that a unique and identical positioning of the cycloalkanone ring is realized, independently of the orientation of the hydride donor and independently of the stereochemistry of the ketoester substrate, affording the same major *cis* product. Such a similarity may be indicative of the involvement of a single reducing enzyme, and is in agreement with the previously proposed model for α-substituted β-ketoester reduction by baker's yeast [2]. A similar result is observed with another yeast, *R. mucilaginosa*, which shows more extended reductive capabilities.

Table 2
Cis / trans ratios and selectivities in the microbial reduction of cyclic β-ketoesters

	β-Ketoester			
	1	**2**	**6**	**7**
Baker's yeast				
cis / trans	86/14	88/12	88/12	–[a]
S %	97.2	98.2	99.5	–
$R_{(S)}$	0.72	0.76	0.36	–
$S_{(R)}$	0.85	0.93	0.36	–
Rhodotorula mucilaginosa				
cis / trans	21/79	77/23	74/26	60/40
S %	–[b]	98.4	98.2	97.5
$R_{(S)}$	–	0.53	0.48	0.21
$S_{(R)}$	–	0.86	0.26	0.28
Kloekera magna				
cis / trans	85/15	100/0	100/0	–[a]
S %	–[b]	99.5	99.5	–
$R_{(S)}$	–	1	1	–
$S_{(R)}$	–	–	–	–
Cunninghamella echinulata				
cis / trans	95/5	85/15	97/3	–[a]
S %	97	92.8	98	–
$R_{(S)}$	0.95	0.82	0.97	–
$S_{(R)}$	−0.68	−0.88	−0.57	–
Mucor racemosus				
cis / trans	100/0	98/2	45/55	11/89
S %	99.5	97.4	95.3	98.2
$R_{(S)}$	1	0.98	−0.11	−0.78
$S_{(R)}$	–	0.32	0.06	−0.94
Mucor circinelloides				
cis / trans	100/0	80/20	27/73	3/97
S %	97	91.8	93.4	95.9
$R_{(S)}$	1	0.59	−0.52	−0.94
$S_{(R)}$	1	0.76	0.34	−0.88
R. arrhizus				
cis / trans	13/87	21/79	31/69	44/56
S %	99.5	98.7	97.3	97.8
$R_{(S)}$	−0.74	−0.59	−0.4	−0.1
$S_{(R)}$	−0.74	0.41	0.24	−0.8

[a] No reduction.
[b] Not determined.



Scheme 4.

On the contrary, opposite results with frequent reversal of enantiospecificity are generally observed with most fungi (*C. echinulata*, *Mucor* sp. and *R. arrhizus*), emphasizing the possible occurrence of multiple reducing activities. A strong dependence of enantiospecificity on the ring size increase is generally observed, probably as a result of increased ring mobility.

However, from a practical point of view, as previously described, it is possible to find in each case a preferred strain for obtaining enantiopure β-hydroxyesters with a 1*S*-configuration.

3. Preparation of chiral lactonic and epoxide synthons

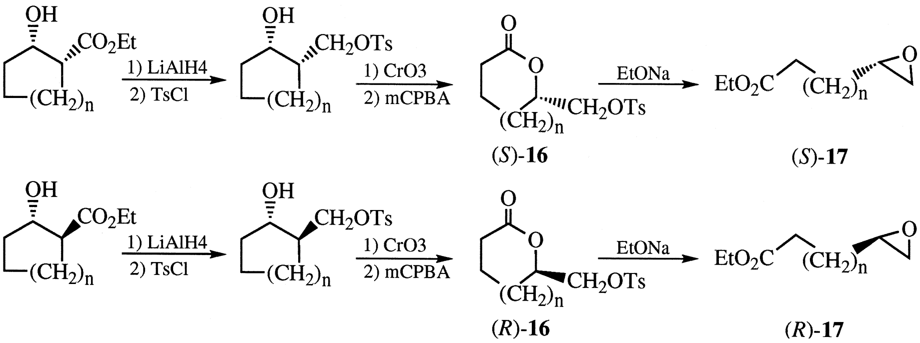
3.1. Lactones

We have already shown [8] that hydroxyesters **3** and **4** can be transformed into the protected hydroxymethyl lactones **8** ($n = 1,2$) in four steps (reduction, protection with TBDPS, oxidation, Baeyer–Villiger reaction) in 45–63% chemical yield. (*S*)-Enantiomers were obtained from **3a** and **4a**, (*R*)-enantiomers from **3b** and **4b** (Scheme 4). The same sequence of reactions from the hydroxyester **12** afforded the lactone (*S*)-**8** ($n = 3$).

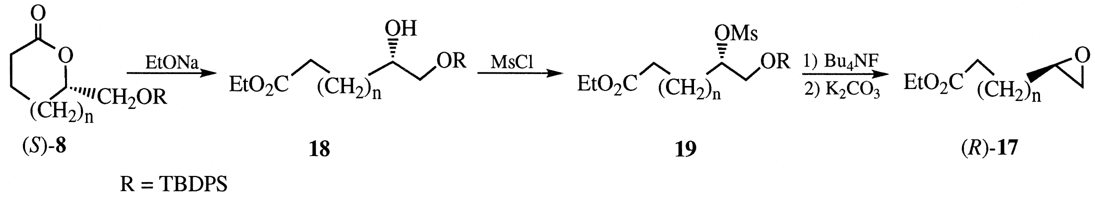
3.2. Epoxides

3.2.1. Retention of configuration

In the cyclohexane series, both enantiomers of lactones **16** and epoxides **17** could be obtained from the *cis* or the *trans* hydroxyesters resulting from the microbial reduction, using tosylate as a protecting and a leaving group (Scheme 5). However, in the cyclopentane series, this method could not be applied because the tosyl ester derivative of the hydroxymethyl ketone was unstable. The corresponding epox-



Scheme 5.



Scheme 6.

ide can be obtained after deprotection of the lactone **8** ($n = 1$) and tosylation.

3.2.2. *Inversion of configuration*

After opening of the lactone **8** ($n = 1,2$) to obtain the monoprotected diol **18**, the use of the mesylate as a leaving group promotes the formation of the epoxyester **17** with an inversion of configuration (Scheme 6).

4. Conclusion

The microbial reduction of β-ketoesters gives enantiopure *cis*- and *trans*- hydroxyesters including one of the stereoisomers of ethyl cyclobutanol 2-carboxylate, the absolute configuration of which remains to be determined. Hydroxyesters can be used in the synthesis of various functionalized epoxides. These enantiomeric epoxides can be obtained either from each diastereomeric microbial reduction product with retention, or from a single diastereomer using both routes (retention and inversion).

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