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DIASTEREOSELECTIVE AND ENANTIOSELECTIVE MICROBIAL REDUCTION OF CYCLIC alpha-ALKYL beta-KETOESTERS

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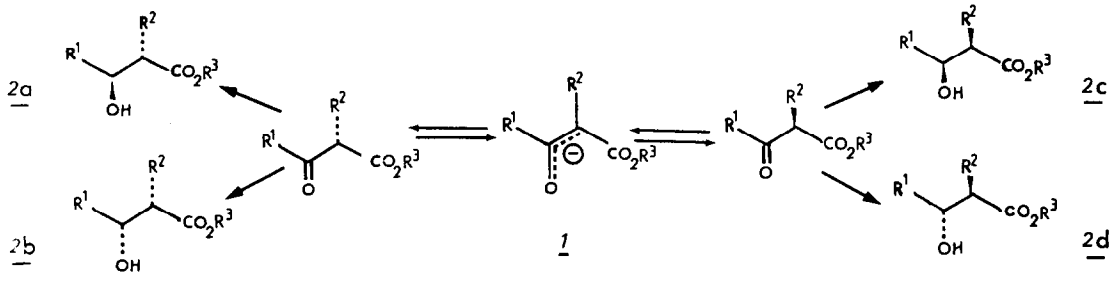
Abstract: The reduction of racemic cyclopenta- and cyclohexanone 2-carboxyesters by various yeast and mould strains was shown to produce different amounts of isomeric beta-hydroxyesters with predominant (1S) stereochemistry. With several strains, only one optically pure cis or trans stereoisomer was obtained in high yield, indicating a diastereoselective and enantioselective reduction.

The microbiological reduction of beta-keto esters has provided a useful method for the synthesis of highly optically active beta-hydroxyesters[1]. Several other versatile asymmetric synthons, such as the alpha-alkyl beta-hydroxyesters 2a-d have been obtained by yeast reduction of the corresponding alpha-alkyl beta-keto esters 1, with varying degrees of diastereo- and/or enantiomeric selectivity[2,3].



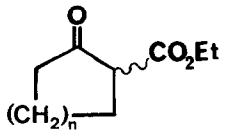
All these studies have pointed out that the keto-enol equilibrium of 1 during the reduction could be driven by the enantioselection of one of the ketoester enantiomers to produce one major stereoisomer 2, . Thus, it may be possible to obtain each one of the four possible pure reduced stereoisomers separately, by making the correct choice of the microorganism, and taking advantage of natural differences in diastereo- and enantioselectivities.

We have thus investigated the stereochemistry of the reduction of model substrates such as ethyl esters of 2-carboxy-cyclopentanone 3 and cyclohexanone 4 by a large number of mould strains. A typical reduction procedure is described as follows: to a yeast or mould culture (50 ml)[4] grown for 48-72 hours, 50 mg of ester in ethanol (0.5 ml) were added and the incubation was continued at 25°C. After the disappearance of the ketoester (2-3 days), the suspension was filtered with celite and the filtrate extracted repetitively with ethyl acetate. The crude reduction products, derivatized to their isopropyl-urethanes[5], were analyzed by GPC on a chiral column[6] , which separated all optical isomers of 6, but only the enantiomeric pairs of 5; in the latter case, a direct complementary analysis of the hydroxyesters on a standard column[7] afforded the diastereoisomer ratio.

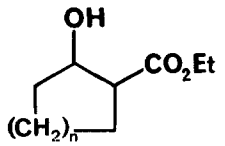
Table I: Stereochemistry of the reduction of the ethyl esters of 2-carboxy cyclopentanone 3 and 2-carboxy cyclohexanone 4 to hydroxy esters 5 and 6 by yeasts and moulds.

	5						6					
	cis			trans			cis			trans		
	%	absolute configur.	ee%	%	absolute configur.	ee%	%	absolute configur.	ee%	%	absolute configur.	ee%
Saccharomyces cerevisiae[a]	80	1S,2R	-	20	1S,2S	-	88	1S,2R	96	16	1S,2S	>99
Saccharomyces montanus[b]	81	1S,2R	-	19	1S,2S	-	81	1S,2R	95	19	1S,2S	96
Saccharomyces uvarum[c]	54	1S,2R	-	46	1S,2S	-	57	1S,2R	93	43	1S,2S	34
Kluyveromyces fragilis[d]	87	1S,2R	-	13	1S,2S	-	83	1S,2R	81	17	rac.	0
Kloeckera magna[e]	85	1S,2R	-	15	1S,2S	-	100	1S,2R	>99	-	-	-
Rhodotorula mucilaginosa[a]	21	1S,2R	-	79	1S,2S	-	77	1S,2R	96	23	1S,2S	>99
Absidia glauca[a]	20	1S,2R	>99	80	1S,2S	>99	78	1S,2R	89	22	1S,2S	>99
Aspergillus niger[a]	84	1S,2R	-	16	1S,2S	-	84	1S,2R	98	16	1S,2S	98
Curvularia lunata[f]	67	1S,2R	-	33	1S,2S	-	92	1S,2R	95	8	1S,2S	86
Geotrichum candidum[a]	100	1S,2R	>99	-	-	-	72	1S,2R	95	28	1S,2S	>99
Mucor racemosus[a]	100	1S,2R	>99	-	-	-	98	1S,2R	96	2	-	-
M. circinelloides[g]	100	1S,2R	>99	-	-	-	95	1S,2R	97	5	1S,2S	63
Cunninghamella echinulata[h]	95	1S,2R	>99	5	-	-	85	1S,2R	>99	15	1S,2S	10
Colletotrichum gloeosporoides[i]	45	-	-	55	-	-	5	1S,2R	54	95	1S,2S	>99
Rhizopus arrhizus[j]	13	1S,2R	>99	87	1S,2S	>99	21	1S,2R	91	79	1S,2S	>99
Penicillium chrysogenum[a]	13	-	-	87	1S,2S	97						
Sporotrichum exile[k]			no reduction				100	1R,2S	35	-	-	-

[a] local strain; [b] CBS 67-72; [c] NRRL Y-969; [d] NRRL Y-610; [e] NRRL Y-1611; [f] NRRL 2380; [g] MMP 1609; [h] MMP 2203; [i] MMP 3233; [j] ATCC 11-1145; [k] QM 1250.



3 : n=1
4 : n=2



5 : n=1
6 : n=2

The most significant results are reported in Table I[8] . In contrast to yeasts[2,3], which generally gave a mixture of optically pure diastereoisomeric hydroxy-esters, several mould strains exhibited a very high diastereo- and enantioselective behaviour. For example, the 1S,2R (cis) isomers of 5 and 6 were both obtained in very high chemical and optical yields with Mucor racemosus, and Mucor circinelloides. On the contrary, Rhizopus arrhizus afforded high amounts of optically pure 1S,2S (trans) isomers, which were easily separated from the contaminating 2R esters by silicagel chromatography[9]. A higher diastereo- and enantioselectivity was generally found with the cyclopentane derived ester but the diastereofacial selectivity was essentially directed to the formation of the 1S isomers. An exception was observed however with Sporotrichum exile, which reduced the cyclohexane derived ester 4 with the 1R configuration slightly predominant.

Several reports have shown that a modification in the hydrophobicity of the alcohol radical of unsubstituted beta-ketoesters could produce impressive changes in the enantioselectivity of the reduction, either by modifying the bonding mode of the substrate in the dehydrogenase affinity site, or by changing the relative affinity for several enzymes of opposite enantioselectivities[1,10]. No such correlations have been established about the diastereoselectivity of the reducing enzymes. In the reduction with two different strains (R. arrhizus and C. lunata), when the ethyl radical in 4 was replaced by a methyl or a n-butyl radical, no change in the stereochemistry of the carbinol group was observed. Whith these strains, the diastereoselectivity of the reduction was only slightly modified, but in an unfavorable way: for example, the cis/trans ratios for the products of reduction of the 2-carboxycyclohexanone esters by R. arrhizus were 13:87 with the methyl and the ethyl ester,and 35:65 with the n-butyl ester.

We are currently exploring the application of these results to the stereospecific reduction of acyclic functionalized alpha-substituted beta-ketoesters.

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4- A culture medium containing glucose (30 g), KH_2PO_4 (1 g), K_2HPO_4 (2 g), corn steep liquor (10 g), $MgSO_4,7H_2O$ (0.5 g), $NaNO_3$ (2 g), $FeSO_4,7H_2O$ (0.02 g) and KCl (0.5 g/liter) was inoculated with a spore suspension and grown with rotatory shaking at 25°C.

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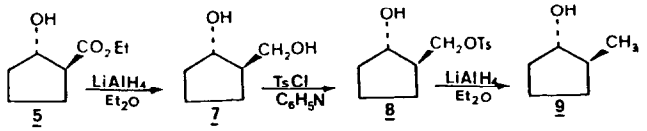
6- Chrompack capillary fused silica column (50 m x 0.25 mm) containing XE-60 S-valine S- -phenylethylamide; carrier gas: helium (1.5 bar);temp.:160°C. Retention times in min. (isopropyl-urethane derivatives): 1**R**,2**R** and 1**R**,2**S**-5, 33.7; 1**S**,2**S** and 1**S**,2**R**-5, 34.3; 1**R**,2**S**-6, 46.3; 1**S**,2**R**-6, 47.7; 1**R**,2**R**-6, 50.9; 1**S**,2**S**-6, 51.5.

7- OV-1701 capillary column (25 m x 0.2 mm); carrier gas: helium (1 bar); temp.: 110-140°C (5°C/min.).

8- The absolute configurations of the stereoisomers of 6 were determined by comparison of the $[\alpha]_D$ of isolated products (corrected for their enantiomeric purity determined by GPC [6,7]) with data from the literature: 1**S**,2**R**-6, $[\alpha]_D^{20}$= +18.8° (c 0.69, $CHCl_3$) (lit.[2]:$[\alpha]_D^{20}$ +20°); 1**S**,2**S**-6, $[\alpha]_D^{20}$= +58° (c 0.5, Et_2O) (lit.[11]: +47.7°). As no data was available for the cyclopentane derived hydroxy-esters, a correlation was established for 1**S**,2**S**-5, $[\alpha]_D^{20}$ = +66° (c 1.13, MeOH),>99% ee by GPC, obtained from the R.arrhizus reduction of 3: reduction by $LiAlH_4$ gave the hydroxymethyl-alcohol 7, $[\alpha]_D^{20}$= +42.2° (c 0.37, MeOH) which was converted to the tosyl derivative 8, $[\alpha]_D^{20}$= +29.9° (c 1.0, MeOH). $LiAlH_4$ reduction of 8 in ether afforded the known 1**S**,2**S**-trans-2-methyl cyclopentanol 13, $[\alpha]_D^{20}$= +44° (c 0.54, MeOH) (lit.[12]: +43.9°). The same sequence of reactions, applied to the optically pure 1**S**,2**R**-cis-isomer, $[\alpha]_D^{20}$= +23.5° (c 0.83, MeOH) (lit.: +13.9°[2], +14.1°[13] in $CHCl_3$), led to the unknown 1**S**,2**R**-cis-2-methyl cyclopentanol, $[\alpha]_D^{20}$= +19.8° (c 0.76, MeOH). All of these products were fully characterized by spectrometric data.



9- Medium pressure chromatography on Silicagel H_{60} (Merck); solvent: CH_2Cl_2-EtOAc (95:5).

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