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Enzyme-Mediated Enantioface-Differentiating Hydrolysis of α-Substituted Sulfur-Containing Cyclic Ketone Enol Esters

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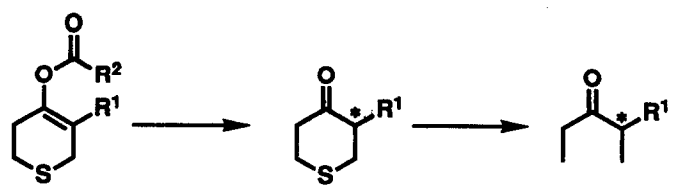
Abstract: An enzymatic method for obtaining optically active α-substituted acyclic ketones is described. *Pichia farinosa* IAM 4682, a kind of yeast, hydrolyzed enol esters of sulfur-containing cyclic ketone with differentiation of the enantiotopic face. The resulting ketone could be easily desulfurized to afford the corresponding acyclic ketone without loss of its optical purity.

Optically active α-substituted ketones are important intermediates in asymmetric organic synthesis. Generally, optically active compounds are obtained via resolution of racemates or asymmetrization of prochiral molecules. In the case of α-substituted ketones, construction of a chiral center via enantioface-differentiating reactions of corresponding enolates is particularly fascinating, because prochiral enolates are easily prepared from racemic precursors. Some examples of such reactions using metal enolates have been demonstrated to be successful.[1] On the other hand, enzymatic transformation has been recently recognized as a useful technique for the synthesis of chiral molecules.[2] In the course of our efforts to apply microorganisms and enzymes in organic synthesis, we have reported an enantioface-differentiating hydrolysis of the enol esters catalyzed by a microorganism, *Pichia farinosa* IAM 4682.[3] The enol esters of 2-substituted cyclohexanone have been chosen as the substrates, because they can be easily obtained from the corresponding ketones without contamination of the regio- and stereoisomers. To extend this methodology to the acyclic substrates is accompanied with some difficulties, because acyclic enol esters are usually obtained as a mixture of stereoisomers.[4] To overcome this difficulty, we employed the enol esters of α-substituted sulfur-containing

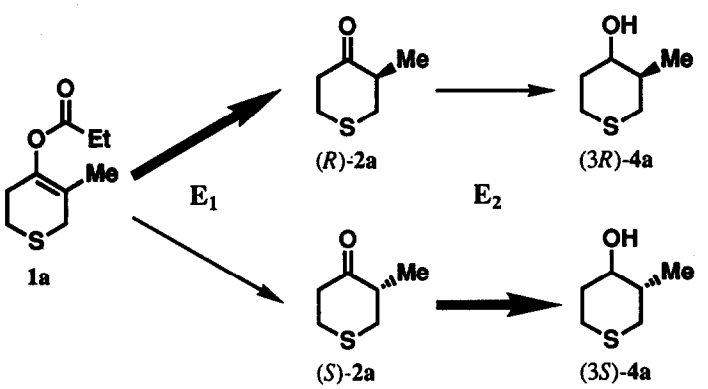


Scheme 1

cyclic ketones **1**. A sulfur atom can be easily removed under mild conditions[5] to afford the corresponding acyclic alkanones **3** (Scheme 1). Moreover, introduction of a sulfur atom in the substrate of an enzymatic reaction might bring about the enhancement of reactivities and selectivities.[5, 6]

The substrates were readily available by acylation of racemic 3-substituted tetrahydrothiopyran-4-one (±)-**2**[7] with acid anhydrides in the presence of a catalytic amount of perchloric acid. The representative procedure of microbial conversion is as follows. Four 500-mL Erlenmeyer flasks each containing 100 mL of sterilized nutrient medium (glucose 1.0%, peptone 0.7%, yeast extract 0.5%, K_2HPO_4 0.5%, pH 7.2) were inoculated with *P. farinosa* and shaken for 2 days at 30 °C. The grown cells were harvested by centrifugation and washed with 0.2 M phosphate buffer (pH 6.5) to give *ca.* 15 g of wet cells. The cells and 80 mg of **1a** were added to 40 mL of 0.2 M phosphate buffer (pH 6.5) in a 500-mL Sakaguchi flask, and the suspension was incubated for 10 minutes at 30 °C. Extraction of the broth with ether followed by ordinary aftertreatment gave optically active ketone **2a** and alcohol **4a** in 54% and 36% yield, respectively. The yields were determined by GLC analysis with octanoylbenzene as the internal standard. The absolute configuration of ketone **2a** was confirmed to be (*R*) by its sign of optical rotation $[\alpha]_D^{22}$ +26.8° (c 0.88, $CHCl_3$), [lit.[7] $[\alpha]_D^{20}$ -22.4° (c 1, $CHCl_3$), 66%e.e., *S* form]. The enantiomeric excess (e.e.) of **2a** was determined to be 98% by capillary GLC analysis[8] of α-methoxy-α-trifluoromethylphenylacetate (MTPA) derived by reduction of **2a** followed by esterification of resulting alcohol.

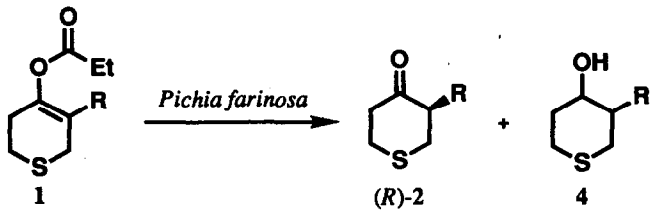
Effort was focussed upon optimization of the incubation conditions for obtaining **2a** in a higher yield. It was shown that the yield of **2a** increased according to the reduction of amount of the cell, at the sacrifice of optical purity. Furthermore, the same tendency was observed on addition of several compounds such as halo esters which were known to be potent inhibitors of the reductase of ketones.[9] This effect can be explained by assuming that stereoselectivity of hydrolysis of the enol ester **1a** is only moderate, and minor enantiomer of resulting ketone (*S*)-**2a** is preferentially converted to alcohol **4a** by the alcohol dehydrogenase contained in the microorganism (Scheme 2).



(E_1 : Esterase , E_2 : Alcohol dehydrogenase)

Scheme 2

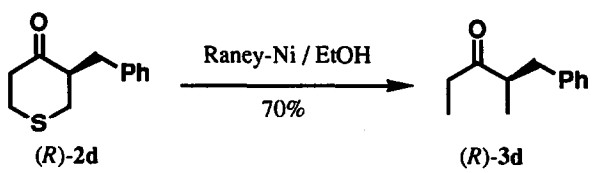
Table 1. Microbial Hydrolysis of Enol Esters



Entry	Substrate	R	Time (min)	(*R*)-2		4
				Yield (%)[a]	% e.e.[c]	Yield (%)[a]
1	**1a**	Me	10	54	98	36
2	**1b**	CH_2=$CHCH_2$	30	74	55	23
3	**1b**	CH_2=$CHCH_2$	120	53	66	40
4	**1c**	*i*-Bu	30	67	25	5
5	**1c**	*i*-Bu	120	52	65	27
6	**1d**	$PhCH_2$	30	76[b]	52[d]	trace
7	**1d**	$PhCH_2$	120	48[b]	67[d]	41

[a]Determined by GLC analysis. [b]Isolated yield. [c]Determined by GLC or ^{19}F NMR analysis of the corresponding MTPA ester of the reduced alcohol. [d]Determined by HPLC analysis with CHIRALCEL OJ (Daicel Chemical Industries,Ltd.).

Next, the substrate specificity was examined. Four substrates having substituents at the α-position were subjected to the microbial reaction. As summarized in Table 1, the expected ketones were obtained in 67-76% yields, at the time when all of the starting materials was consumed. Unfortunately, the e.e.s of the products were not satisfactory (Entry 2, 4, 6). Therefore, the incubation period was extended to two hours to promote the enzymatic reduction of the (*S*)-ketone on the basis of the above assumption. As expected, the e.e.s of the ketones were appreciably improved (Entry 3, 5, 7). The absolute configurations were assigned by octant rule analysis of the Cotton effects observed in CD spectra. The ketones **2b-d** were confirmed to have (*R*) configurations since positive Cotton effects were observed.[7, 10]



Scheme 3

Desulfurization was performed with Raney-Ni in refluxing ethanol by using optically active ketone **2d** as a representative substrate to afford the corresponding acyclic ketone 2-phenylmethyl-3-pentanone **3d** without loss of the e.e. (Scheme 3)

In conclusion, enantioselective hydrolysis of sulfur-containing cyclic ketone enol esters have been achieved, although the stereoselectivity was not so high as the case of the carbocyclic compounds. The e.e. of the ketones could be increased via subsequent enzymatic reduction, which was probably stimulated by the presence of a sulfur atom. Desulfurization was easily achieved to result in the formation of acyclic optically active ketones without loss of the e.e.

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8. Conditions for capillary GLC analysis: column, PEG-20M, 0.25 mm× 50 m, Gasukuro Kogyo Inc.; carrier gas, He; head pressure, 2.4 kg/cm^2; retention times, cis(*R*) 91 min, cis(*S*) 92 min, trans 97 min.
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10. The data of CD spectra (degree•cm^2 / dmol, measured in methanol) : (+)-(*R*)-**2b** (c 0.003), $[\theta]_{330}$ 0, $[\theta]_{290}$ 110, $[\theta]_{267}^{*}$ 69, $[\theta]_{240}$ 270; (+)-(*R*)-**2c** (c 0.001), $[\theta]_{330}$ 0, $[\theta]_{294}$ 92, $[\theta]_{266}^{*}$ 42, $[\theta]_{240}$ 190; (+)-(*R*)-**2d** (c 0.002), $[\theta]_{330}$ 0, $[\theta]_{290}$ 100, $[\theta]_{268}^{*}$ 63, $[\theta]_{239}$ 300.

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