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Kinetic Resolution of Enol Esters via Enzyme-Mediated Hydrolysis

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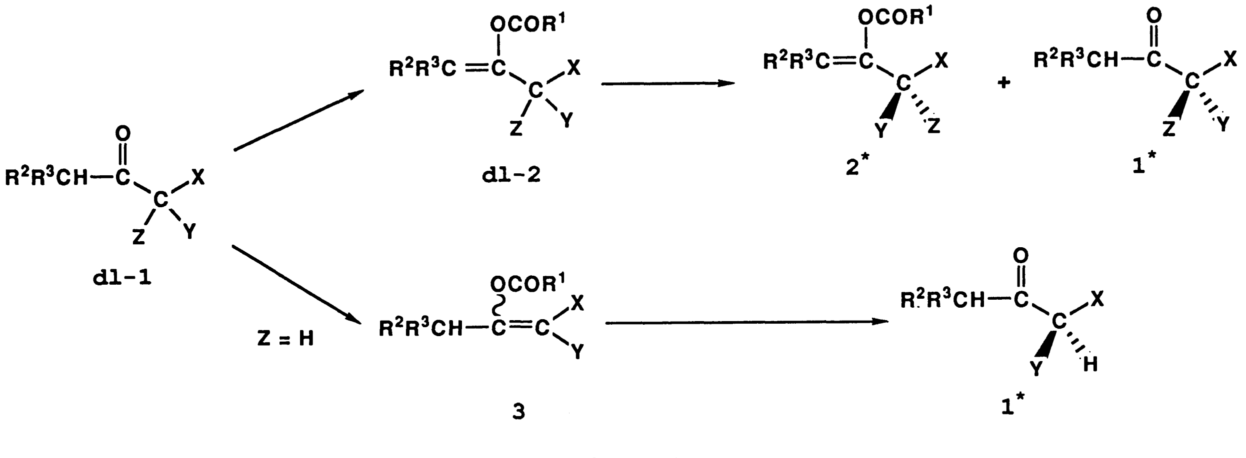
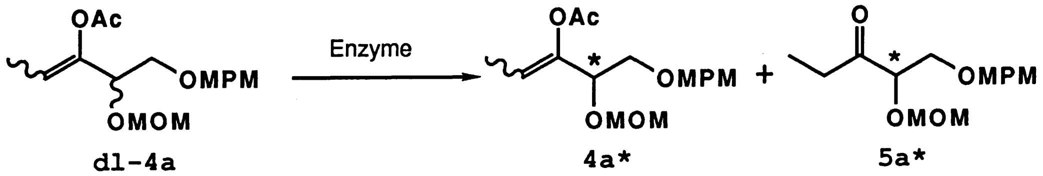
A new type of enzyme-mediated kinetic resolution has been developed. Enol esters, such as dl-3-acetoxy-1-p-methoxy-benzyloxy-2-methoxymethoxy-3-pentene, were hydrolyzed by incubation with *Bacillus coagulans* to afford optically pure enol esters, and the corresponding α-substituted ketones.

In the synthesis of natural products, optically active α-substituted ketones are considered to be available as chiral synthones.[1)] For example, dihydroxy ketone of type **1** (Scheme 1, X = CH_2OMPM, Y = OMOM, Z = H), has been demonstrated to be the key intermediate in the total synthesis of mycinolide IV.[2)] In spite of their usefulness, such chiral glycerol derivatives have been obtained only from naturally occurring chiral pools, such as D-mannitol[3)] and L-ascorbic acid.[4)]

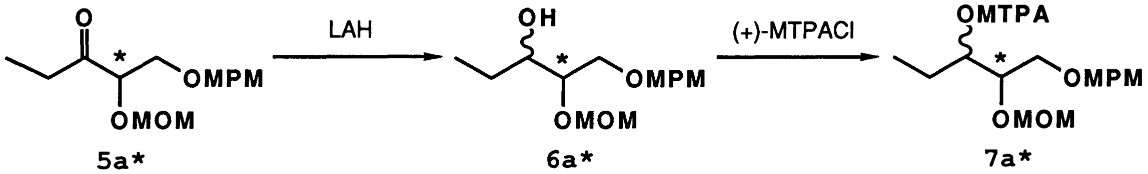
On the other hand, it has been established recently that application of enzymatic reactions to organic compounds is effective in introducing chiralities to synthetic substrates.[5)] Hydrolysis is especially advantageous because it requires no cofactors (NAD^+, NADH, ATP etc.). Thus we tried to design a generally applicable reaction system for obtaining optically active α-substituted ketones via asymmetric hydrolysis. The essential drawback to be overcome is that the α-substituted ketone is, as it is, no primary product obtainable via hydrolysis. The only device to transform a ketone to hydrolyzable derivative is the acylation of its enol form. Enzymatic hydrolysis of enol esters would be expected to afford chiral ketones if the enzyme system distinguishes the



Scheme 1.



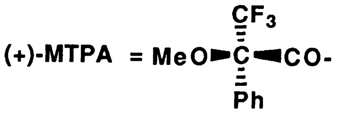
Scheme 2.



Scheme 3.

MOM = $MeOCH_2$-

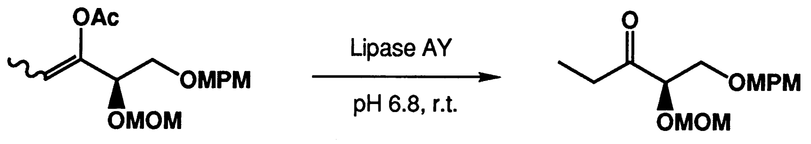
MPM = p-MeO-$C_6H_4CH_2$-



$$(+)\text{-MTPA} = MeO\!-\!\overset{CF_3}{\underset{Ph}{C}}\!-\!CO\text{-}$$

configulation of α-position. We have already reported the enzyme-mediated enantioface differentiating hydrolysis of enol esters of type **3** to afford chiral carbonyl compounds.[6)] This type of reaction is feasible only when Z in dl-**1** is hydrogen. In the present study, we tried another approach for obtaining optically active α-substituted ketones, i.e., kinetic resolution of enol esters of type **2** by microbial transformation.

As the substrate for screening test, dl-3-acetoxy-1-p-methoxybenzyloxy-2-methoxymethoxy-3-pentene (**4a**) was employed (Scheme 2).[7)] Among our stock cultures and commercially available enzymes, *Bacillus coagulans* KU 5185 (FERM P-9237),[8)] a kind of bacterium, was selected as the best strain. Representative experimental procedure is as follows. Forty ml of sterilized nutrient medium of pH 6.8[9)] was inoculated with *B. coagulans* and incubated at 30 °C. After 2 days, 60 μl of dl-**4a** was added to the suspension of grown cells and the incubation was continued for additional 2 days. Extraction of the broth with ethyl acetate followed by ordinary aftertreatment gave optically active enol acetate (R)-**4a**[10)] and ketone (S)-**5a**[11)] in 33% and 64% yield, respectively (Table 1). The reaction also proceeded smoothly when the substrate was employed twice in concentration. The absolute configuration of the products were determined by comparing the specific rotation with that of an authentic sample[12)] derived from D-mannitol. The enantiomeric excess of recovered (R)-**4a** was confirmed to be over 95%e.e., based on 400 MHz ^{1}H-NMR spectrum obtained in the presence of $Eu(tfc)_3$. On the other hand, the enantiomeric excess of ketone (S)-**5a** was rather low (42%e.e.) because the reaction was quenched at the time when the reaction exceeded over 50%. It was determined by HPLC analysis[13)] of the MTPA ester **7a**, which was derived from **5a** via reduction and esterification (Scheme 3).



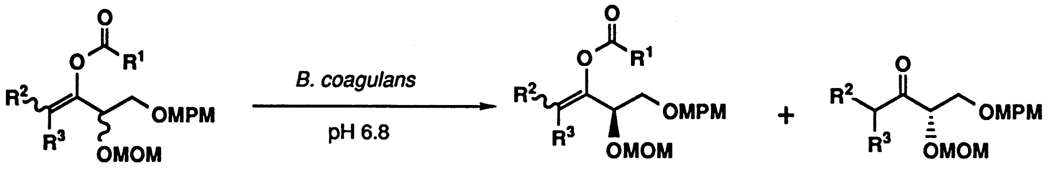
(R)-4a (R)-5a

Scheme 4. 96%, 96%e.e.

The resulting chiral enol acetate (R)-**4a** must be hydrolyzed to generate chiral ketone (R)-**5a** for further synthetic development. Hydrolysis under weakly basic conditions resulted complex by-products presumably via further reactions of the desired ketone. In this transformation, an enzyme again worked well. As shown in Scheme 4, incubation of (R)-**4a** with Lipase AY14) in buffer (pH 6.8) at 25 °C afforded (R)-5 of 96%e.e. in 96% yield without any racemization.15)

The results for other compounds are summarized in Table 1. While hydrolysis of propionyl ester dl-**4b** proceeded to afford optically pure (R)-**4b** similarly to **4a**, the recovery of **4c** was very small. Although the details are not clear at present, the low material balance in the reaction of **4c** is supposed to be due to side reactions. While steric bulkiness inhibited the reaction (dl-**4e**), the substrate without an alkyl group on the terminus of the double bond (dl-**4d**) hydrolyzed without regard to the configuration of α-position.

Table 1. Hydrolysis of Enol Esters dl-**4**a)



dl-4 (R)-4 (S)-5

					Enol ester			Ketone		
	R^1	R^2	R^3	Time/h	Yield/%b)	$[\alpha]_D$/°c)	ee/%d)	Yield/%b)	$[\alpha]_D$/°c)	ee/%e)
a	Me	Me	H	48	33f)	+70.4	>95	64f)	- 7.6	42
				6	51f)	+41.7	59	43f)	-12.1	67
b	Et	Me	H	168	38	+51.9	>95	43	- 8.6	53
c	n-Pr	Me	H	168	42	+ 2.0	<3	8	- 5.5	32
d	Me	H	H	3	6	+ 4.9	26	84	- 0.4	<3
e	Me	Me	Me	48	81	——	——	0	——	——

a) The concentration of substrate was made up to 0.15% to the medium.
b) Isolated yields, unless otherwise noted.
c) Measured in $CHCl_3$ (c 0.4 - 1.5 for **4** and 0.9 - 1.9 for **5**).
d) Determined by 400 MHz ^{1}H-NMR in the presence of $Eu(tfc)_3$.
e) Determined by HPLC analysis of the corresponding MTPA esters (**7**).
f) Determined by GLC.

In conclusion, a new type of kinetic resolution of enol esters has been achieved by incubation with *B. coagulans*, resulting in the formation of optically active glycerol derivatives. Thus, the present method is expected to be potentially useful in preparing chiral carbonyl compounds.

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6) H. Ohta, K. Matsumoto, S. Tsutsumi, and T. Ihori, J. Chem. Soc., Chem. Commun., accepted for publication.
7) Enol acetate dl-**4** was used as a mixture of E and Z isomers.
8) H. Ohta, Y. Miyamae, and G. Tsuchihashi, Agric. Biol. Chem., 53, 215 (1989).
9) The medium consists of glucose 10 g, polypeptone 7 g, and yeast extract 5 g in 1000 ml of 0.2 M phosphate buffer. Under these conditions, no spontaneous hydrolysis of substrates was observed.
10) The resulting (R)-**4a** was a single isomer concerning E and Z configuration, but the structure was not determined. Spectral data of (R)-**4a**: $[\alpha]_D^{29}$ +70.4° (c 1.48, $CHCl_3$); ^{1}H-NMR (CCl_4) δ 1.49 (d, J=6.9 Hz, 3H), 2.04 (s, 3H), 3.25 (s, 3H), 3.40 (d, J=6.0 Hz, 2H), 3.73 (s, 3H), 4.12 (t, J=6.0 Hz, 1H), 4.33 (d, J=6.3 Hz, 1H), 4.65 (d, J=6.3 Hz, 1H), 4.37 (s, 2H), 5.33 (q, J=6.9 Hz, 1H), 6.73 (d, J=8.7 Hz, 2H), 7.13 (d, J=8.7 Hz, 2H); IR (neat) 2900, 1760, 1610, 1580, 1510, 1450, 1360, 1300, 1240, 1205, 1150, 1090, 1020, 980, 910, 810, 760 cm^{-1}; MS m/z (rel. intensity) 324 (0.4, M$^+$), 279 (4.8), 203 (1.0), 173 (7.3), 137 (5.8), 121 (100).
11) Spectral data of (S)-**5a**: $[\alpha]_D^{30}$ -7.6° (c 1.91, $CHCl_3$); ^{1}H-NMR (CCl_4) δ 0.97 (t, J=7.2 Hz, 3H), 2.54 (q, J=7.2 Hz, 2H), 3.30 (s, 3H), 3.60(d, J=4.5 Hz, 2H), 3.75 (s, 3H), 4.02 (t, J=4.5 Hz, 1H), 4.37 (s, 2H), 4.5 - 4.8 (m, 2H), 6.75 (d, J=8.4 Hz, 2H), 7.12 (d, J=8.4 Hz, 2H); IR (neat) 2900, 1720, 1600, 1580, 1510, 1450, 1400, 1350, 1300, 1240, 1170, 1150, 1100, 1030, 910, 820, 750 cm^{-1}; MS m/z (rel. intensity) 282 (0.5, M$^+$), 264 (2.8), 237 (0.8), 181 (8.9), 137 (16), 121 (100).
12) $[\alpha]_D^{25}$ -18.1° (c 0.98, $CHCl_3$).
13) Conditions for HPLC analysis: Column, Develosil ODS-5 (25 cm); Solvent, MeOH/H_2O (70/30), 0.5 ml/min; Retention time, 154, 161, 170, and 183 min corresponding to 4 diastereomers.
14) Lipase "Amano" AY from *Candida cylindracea* was kindly provided by Amano Phamaceutical Co., Ltd., Nagoya, Japan.
15) $[\alpha]_D^{24}$ +16.0° (c 0.79, $CHCl_3$).

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