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Preparation of Optically Active α-Acetoxyacylophenones via Enzyme Mediated Hydrolysis

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Microorganisms that hydrolyzes α-acetoxyacylophenones were screened, and it was found that Pichia miso IAM 4682, a type culture belonging to yeast, gave the best results. The esterase of this microbe hydrolyzed (R)-acetates in a highly enantioselective manner.

It has become increasingly important to synthesize physiologically active compounds in their optically active forms. In these asymmetric synthesis, introduction or migration of chiral centers, and regulation of diastereoselectivities are the points to be overcome on the route for the target molecules. Recently, it has been demonstrated from our laboratory that activation of a sulfonyloxy group with organoaluminium compounds causes elimination of this group accompanied by concomitant stereospecific migration of carbon moieties from the adjacent position.[1)] This reaction has cleanly transformed a C-O chirality to a C-C chirality. The scope of this new methodology depends on the availability of optically active α-hydroxyketones as the starting materials. Naturally occurring α-hydroxycarboxylic acids and amino acids have been used as chiral sources so far. To develop the scope of this novel stereospecific rearrangement, it is inevitable to prepare optically active α-hydroxyketones which are difficult to be supplied from natural sources. In such a case, application of enzymatic reactions are often promising, as we have already reported the enantioselective reduction of α, β-diketones to afford chiral α-hydroxyketones.[2)]

Reported herein is our another trial for obtaining chiral α-hydroxyketone derivatives, i.e., utilization of esterases of microorganisms in distinguishing the chirality of α-acetoxyketones. 2-Acetoxy-1-phenyl-1-propanone (1a) was selected as the representative substrate, and microorganisms that enantioselectively hydrolyze the ester group were screened. Screening test was carried out based on the following supposition. Such microbes that hydrolyzes a half of 1a within 2-day cultivation and left the other half even after 4 days were considered to be ideal. In fact, Pichia miso IAM 4682, a kind of yeast, was selected as the best among our stock cultures.

The reaction was carried out in a phosphate buffer of pH 6.0 to avoid non-enzymatic hydrolysis of starting acetates 1. Ninety ml of sterilized nutrient medium of pH 7.2[3)] was inoculated with 10 ml of seed culture of P. miso and

incubated for 2 days at 30 °C. The grown cells were collected by centrifugation and washed with phosphate buffer of pH 6. These cells were suspended in 50 ml of the same buffer and shaken with 0.1 ml of a substrate. After a period cited in Table 1, extraction of the broth with ethyl acetate followed by ordinary after-treatment afforded in a nearly quantitative yield a mixture of optically active 1 and 1-phenyl-2-hydroxy-1-alkanone (2). In many cases the reaction proceeded more rapidly than general ones using intact cells.[4] When the R's are lower alkyl groups, the enzyme system cleanly distiguished the absolute configurations of the substrates. Thus, when the reaction was quenched at the time when the reaction exceeded over 50%, the optical purities of recovered acetates were more than 98%. The acetate with branched alkyl chain (1e) also resulted chiral acetate of high optical purity after incubation with *P. miso*.

Interesting is the effect of concentration of the substrate on the reactivity. As the representative acetate, the reaction of 1b was examines in various

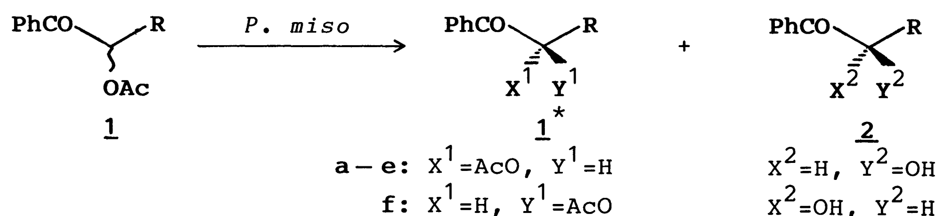


Table 1. Asymmetric Hydrolysis of α-Acetoxyacylophenones

Compd.	R	Cultivation/h	Recov. of 1/%	Opt. purity of 1/% e.e.	$[\alpha]_D$ in acetone/° (c)	Config.
a	Me	6	41	99	-25 (1.3)	S
b	Et	6	42	98	+5.2 (1.8)	S
c	n-Pr	6	33	99	+1.9 (0.8)	S
d	Bu	24	34	98	+1.0 (2.2)	S
e	i-Pr	12	47	99	+49 (1.3)	S
f	$PhCH_2$	72	37	34	-20 (0.8)	R

concentrations (Fig. 1). As the initial concentration of 1b became higher, the recovery of 1b approached indefinitely at 50%. This fact will be best accountd for by assuming the presence of two enzymes that concern to this reaction. One enzyme hydrolyzes (R)-1b and the other hydrolyzes (S)-enantiomer. Suppose resulting ketol (R)-2b (*vide infra*) inhibits Enz-2 specifically when it reaches to a certain level of concentration, then the reaction will stop at the time when (R)-1b is completely hydrolyzed (Scheme 1). It is natural that the concentration of 2b will soon reach its critical point, when the initial concentration of the substrate is high. Accordingly hydrolysis

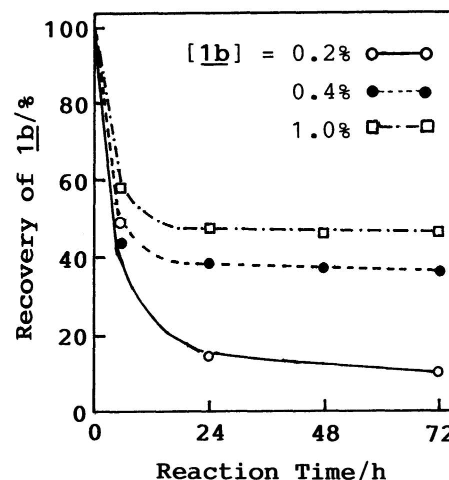
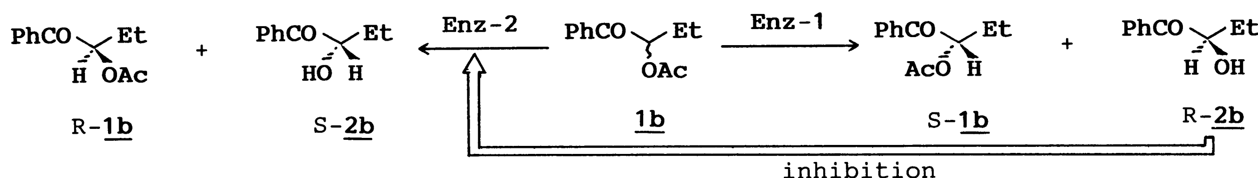


Fig. 1. Effect of concentration of **1b** on its conversion.

of 1b is considered to stop at slightly over 50%. This assumption is also supported by the following control experiment. When the incubation was carried out in a medium initially containing dl-ketol 2b, it clearly inhibited the reaction after a half of 1b was consumed (Table 2). The difference in enantioselectivity of treated cells shown in Table 3 would be also interpreted by the difference in stability of two enzymes.



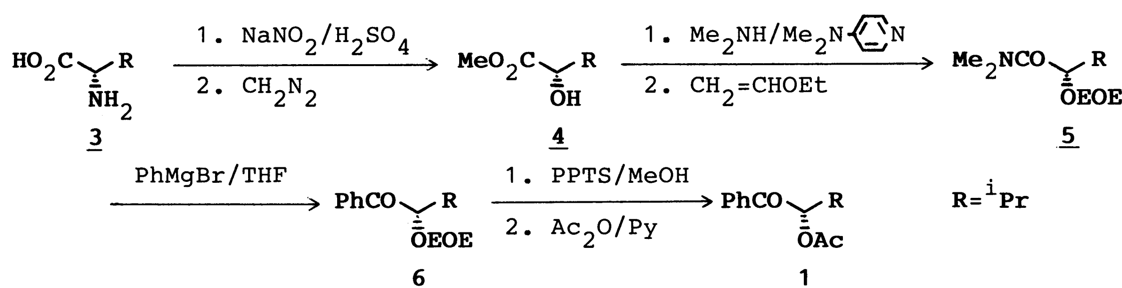
Scheme 1.

Absolute configurations of the resulting chiral acetates 1 and 2 were determined as follows. First, acetylation of resulting 2 all afforded acetates with specific rotations reverse to those of the ones recovered after incubation,[5] indicating that the recovered 1 and 2 were of the opposite configurations with each other. Then, an authentic 2a was derived from (S)-ethyl lactate by a sequence of protection of hydroxy group with ethoxyethyl, reaction with morpholine, phenylmagnesium bromide followed by deprotection.[6] Chiral isopropyl derivative 1e was also prepared from naturally occurring valine by converting to hydroxy acid via van Slyke diazotization[7] followed by above series of reactons (In this case, dimethylamine was used instead of morpholine; Scheme 2).[8] Comparison of the specific rotation of authentic 2a and 1e with that of the one obtained by enzymatic reaction gave a conclusion that the enzyme system of P. miso hydrolyzed (R)-1a and 1e, leaving behind the (S)-acetates. The absolute configurations of recovered 2b-2d were tentatively estimated to be as those as cited in Table 1 from their specific rotations and the order of retention time in HPLC using a chiral column.[9] The configuration and optical rotation of 1f have already been reported.[10]

Table 2. Effect of 2b on the Conversion of 1b[a)]

Concn. of 2b/%	Recov. of 1b/%[b)]	Opt. y./%
0	14	
0.3	45	99
0.5	73	

a) Incubation was carried out at 30 °C for 24 h.
b) Concentration of 1b was 0.2% in each case.



Scheme 2.

It will be worthy to mention that acetone dried cells and lyophilized cells also kept their esterase activity. As shown in Table 3, the lyophilized cells smoothly hydrolyzed 1b after storage of several weeks in a refrigerator. The slight lowering of the enantioselectivity after a long period of storage will be accounted for by considering the presence of dual enzymes, i.e., by the difference of stabilities of two enzymes.

Table 3. Hydrolysis of α-Acetoxybutyrophenone 1b with Dry Cells[a)]

Entry	Method[b)]	Storage/day	Cultivation/h	Recov. of 1b/%	Opt. y./%
1	A	9	6	48	57
2	L	9	6	47	99
3	L	58	6	46	93

a) Cultivation was carried out at 30 °C in a phosphate buffer of pH 6, using 0.2% of substrate.

b) A, centrifuged cells were washed with water, followed by acetone; L, cells were lyophilized at -15 °C, after centrifugation.

It was confirmed that the deprotection of acetyl group with K_2CO_3/methanol system is accompanied by no racemization. Thus, the present method is expected to serve as convenient method of preparing chiral synthons which are difficult to obtain in other ways.

References

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2) J. Konishi, H. Ohta, and G. Tsuchihashi, Chem. Lett., 1985, 1111.

3) The medium consists of glucose 10 g, polypepton 7 g, yeast extract 5 g, and K_2HPO_4 5 g in 1000 ml of distilled water.

4) For, example, H. Ohta and H. Tetsukawa, Agric. Biol. Chem., 44, 863 (1980); M. Kasai, K. Kawai, M. Imuta, and H. Ziffer, J. Org. Chem., 49, 675 (1984).

5) Specific rotation of acetates derived from resulting 2 were as follows (compounds, $[\alpha]_D$ in acetone, c, % e.e.): a, +19°, 0.81, 80; b, -4.1°, 0.77, 75; c, -2.3°, 0.50, 50; d, -2.5°, 1.3, 37; e, -35°, 1.3, 65; f, +15°, 0.70, 28.

6) Specific rotaion of 2a in methanol: Sample from 1a, +35° (c 0.37); sample from (S)-ethyl lactate, -48° (c 1.0).

7) D. D. van Slyke, Ber., 43, 3170 (1910).

8) Specific rotation of 1e from (S)-valine is +48° (c 0.98, acetone).

9) Conditions for HPLC analysis. Column: Pirkle column,[11)] 25 cm. Solvent: hexane/isopropanol (30/1), 0.15 ml/min. Retention time corresponding to two enantiomers (min): 1a, 24.2, 26.3; 1b, 16.5, 18.5; 1c, 16.4, 18.0; 1d, 17.2, 19.2; 1e, 18.0, 20.3; 1f, 22.6, 25.5.

10) B. Marsman and H. Wynberg, J. Org. Chem., 44, 2812 (1979).

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