Enzyme-Mediated Enantioface-Differentiating Hydrolysis of α-Substituted Cycloalkanone Enol Esters

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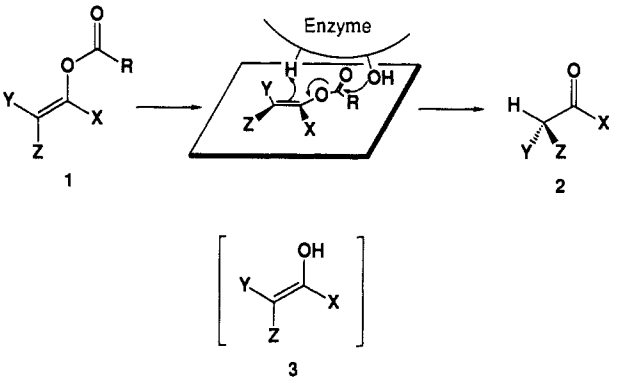
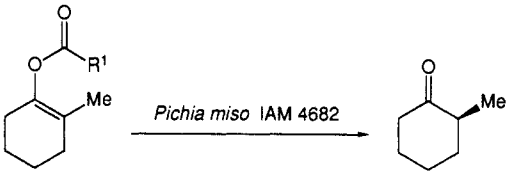
Abstract: A new type of enzymatic hydrolysis, enantioface-differentiating hydrolysis of enol esters, is disclosed. As a result of screening, *Pichia miso* IAM 4682, a type of yeast, was selected as the best strain to perform the enantioselective hydrolysis of enol esters to give α-chiral ketones. For example, incubation of 1-acetoxy-2-methylcyclohexene (**4a**) with *P. miso* afforded (*S*)-2-methylcyclohexanone (**5**) in high optical yield. This enzymatic hydrolysis is applicable to various α-substituted cycloalkanone enol esters, and thereby chiral six-, eight-, ten-, and twelve-membered-ring ketones of 70–96% enantiomeric excess (ee) are easily prepared.

In the synthesis of natural products, optically active α-substituted ketones play an important role as synthons, and hence a number of methods for stereoselective α-alkylation have been reported.[1-3] These methods, however, are not always satisfactory in terms of their yields, stereoselectivities, and simplicity of operation. On the other hand, it has been established recently that enzymatic transformation of organic compounds is a useful technique for the synthesis of chiral molecules.[4] Enzymatic hydrolysis is especially advantageous because it requires no cofactors (NAD^+, NADH, ATP, etc.). Conventional biochemical hydrolyses can be classified into three categories: (1) kinetic resolution of racemic compounds,[5] (2) asymmetrization of compounds with a prochiral center,[6] and (3) enantioselective reaction of meso compounds.[7] These hitherto known hydrolyses afford optically active esters, amides, acids, alcohols, amines, and other compounds as products but cannot directly result in chiral ketones.[8] In this paper, we present a simple and generally applicable method for obtaining optically active α-substituted ketones via a new type of enzymatic hydrolysis, *i.e.*, enantioface-differentiating hydrolysis of enol esters.[9]

Scheme I



Scheme II



Scheme I illustrates the guiding principle of this new reaction. Enzymatic hydrolysis is known to proceed generally via a two-step reaction: acylation of an enzyme followed by hydrolysis of the acylated enzyme. If protonation in the enzyme acylation step occurs on the oxygen atom to give intermediary enol **3**, the resulting ketone **2** must be racemic. However, optically active **2** would be obtained if a proton attacks at the C=C double bond from one side simultaneously with elimination of the acyl group. While the induction of chirality by conventional hydrolyses is based on the ability of enzymes to recognize the chiral or prochiral centers of the substrates, the present reaction requires the esterase to have the ability to differentiate enantiotopic faces. Although enzymatic transesterification with enol esters[10] such as ethenyl

(1) Review: (a) Tomioka, K.; Koga, K. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, 1983; Vol. 2, p 201. (b) Bergbreiter, D. E.; Newcomb, M. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, 1983; Vol. 2, p 243. (c) Enders, D. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, 1984; Vol. 3, p 275. (d) Seebach, D. In *Modern Synthesis Methods*; Scheffold, R., Ed.; Springer-Verlag: Berlin, Heidelberg, 1986; Vol. 4, p 125.

(2) Representative references: (a) Mêa-Jacheet, D.; Horeau, A. *Bull. Soc. Chim. Fr.* **1968**, 4571. (b) Kitamoto, M.; Hiroi, K.; Terashima, S.; Yamada, S. *Chem. Pharm. Bull.* **1974**, *22*, 459. (c) Enders, D.; Eichenauer, H. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 549. (d) Whitesell, J. K.; Felman, S. W. *J. Org. Chem.* **1977**, *42*, 1663. (e) Worster, P. M.; McArthur, C. R.; Leznoff, C. C. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 221. (f) Hashimoto, S.; Koga, K. *Chem. Pharm. Bull.* **1979**, *27*, 2760. (g) Meyers, A. I.; Williams, D. R.; Erickson, G. W.; White, S.; Druelinger, M. *J. Am. Chem. Soc.* **1981**, *103*, 3081. Meyers, A. I.; Williams, D. R.; White, S.; Erickson, G. W. *Ibid.* **1981**, *103*, 3088. (h) Ito, Y.; Sawamura, M.; Kominami, K.; Saegusa, T. *Tetrahedron Lett.* **1985**, *26*, 5303. (i) Stetin, C.; De Joso, B.; Pommier, J.-C. *J. Org. Chem.* **1985**, *50*, 3863. (j) Recent example of α-alkylation of cyclohexanone: Tomioka, K.; Shindo, M.; Koga, K. *Chem. Pharm. Bull.* **1989**, *37*, 1120.

(3) Asymmetric protonation by a nonenzymatic process has been reported: (a) Review: Duhamel, L.; Duhamel, P.; Launay, J.-C.; Plaquevent, J.-C. *Bull. Soc. Chim. Fr.* **1984**, II-421. (b) Recent reports: Duhamel, L.; Fouquay, S.; Plaquevent, J.-C. *Tetrahedron Lett.* **1986**, *27*, 4975. Fehr, C.; Galindo, J. *J. Am. Chem. Soc.* **1988**, *110*, 6909.

(4) Recent reviews: (a) Whitesides, G. M.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617. (b) *Enzymes as Catalysts in Organic Synthesis*, NATO ASI Series; Schneider, M., Ed.; D. Reidel Publishing: Dordrecht, The Netherlands, 1986; Series C., Vol. 178. (c) Jones, J. B. *Tetrahedron* **1986**, *42*, 3351. (d) Ohta, H. *J. Synth. Org. Chem. Jpn.* **1988**, *46*, 726. (e) Yamada, H.; Shimizu, S. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 622. (f) Wong, C.-H. *Science* **1989**, *244*, 1145. (g) Chen, C.-S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 695. (h) Crout, D. H. G.; Christen, M. In *Modern Synthetic Methods*; Scheffold, R., Ed.; Springer-Verlag: Berlin, Heidelberg, 1989; Vol. 5, p 1.

(5) Ohta, H.; Miyamae, Y.; Tsuchihashi, G. *Agric. Biol. Chem.* **1989**, *53*, 215.

(6) Lam, L. K. P.; Hui, R. A. H. F.; Jones, J. B. *J. Org. Chem.* **1986**, *51*, 2047.

(7) Laumen, K.; Schneider, M. *Tetrahedon Lett.* **1985**, *26*, 2073.

(8) Only those ketones that have hydrolyzable functional groups can be obtained in their chiral forms via enzymatic hydrolysis. For example: Ohta, H.; Ikemoto, M.; Ii, H.; Okamoto, Y.; Tsuchihashi, G. *Chem. Lett.* **1986**, 1169.

(9) (a) A preliminary report of this reaction: Ohta, H.; Matsumoto, K.; Tsutsumi, S.; Ihori, T. *J. Chem. Soc., Chem. Commun.* **1989**, 485. (b) Kinetic resolution of enol esters via enzyme-mediated hydrolysis has been also developed in our laboratory; see: Matsumoto, K.; Ohta, H. *Chem. Lett.* **1989**, 1109. Sugai, T.; Kakeya, H.; Morooka, M.; Ohba, S.; Ohta, H. *Tetrahedron* **1989**, *45*, 6135.

(10) (a) Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1986**, *108*, 6421. (b) Degueil-Castaing, H.; De Jeso, B.; Drouillard, S.; Maillard, B. *Tetrahedron Lett.* **1987**, *28*, 953. (c) Wang, Y.-F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 7200. (d) Wang, Y.-F.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 3127. (e) Laumen, K.; Breitgoff, D.; Schneider, M. *J. Chem. Soc., Chem. Commun.* **1988**, 1459. (f) Wang, Y.-F.; Chen, S.-T.; Liu, K. K.-C.; Wong, C.-H. *Tetrahedron Lett.* **1989**, *30*, 1917.

0002-7863/90/1512-9614$02.50/0

Table I. Enantioselective Hydrolysis of 1-Acetoxy-2-methylcyclohexene (**4a**)[a]

entry	wt of wet cells, g	% yield of **5**[b]	% ee[c]	config[d]
1	4	77	72	*S*
2	7	78	83	*S*
3	11	72	88	*S*
4	14	79	90[e]	*S*

[a] Incubation was performed with 0.2% of substrate **4a** for 3 h in 0.2 M phosphate buffer (pH 6.5). [b] Determined by GLC analysis with 1-nonanol as internal standard. [c] Determined by the optical rotation of **5**. [d] Determined by comparing the sign of optical rotation. [e] $[\alpha]^{23}_D$ +12.6° (*c* 0.73, MeOH), (lit.[2g] $[\alpha]_D$ +12.2° (*c* 4, MeOH) (87% ee, *S* form)).

and isopropenyl acetates as acyl donors in organic solvent has been demonstrated, these reactions have never been utilized as an approach to the preparation of chiral carbonyl compounds.

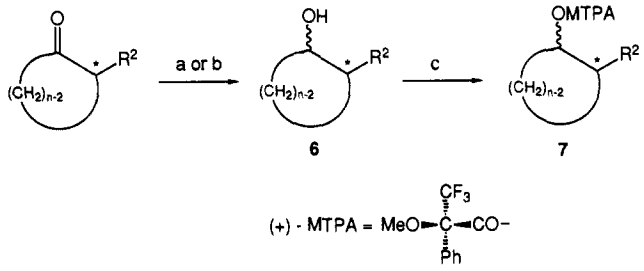
Results and Discussion

Screening of the Enzyme System. 1-Acetoxy-2-methylcyclohexene (**4a**) was chosen as the substrate for screening of the enzyme system because it can be readily prepared from racemic 2-methylcyclohexanone (**5**) by reaction with acetic anhydride and a catalytic amount of perchloric acid without contamination of the regio- and stereoisomers.[11] Since ketone **5** obtained via enzymatic transformation is highly volatile, the yield was determined by GLC analysis with 1-nonanol as the internal standard.

In the first screening, the selection of an enzyme system was carried out on the basis of hydrolytic ability without consideration of the stereoselectivity. Among 73 strains of stock cultures and 11 commercially available enzymes, 13 strains and pig liver esterase (PLE, Sigma) were selected for further screening because ketone **5** was produced in high yields, consuming the entire substrate **4a** within a few days. Then, in the second screening, the specific rotation of **5** was measured after purification by chromatography on silica gel following Kugelrohr distillation. Almost all the strains that had been selected via the first screening produced racemic **5**. PLE, which has frequently worked well in asymmetric hydrolyses,[6,12] also showed no stereoselectivity. Finally, *Pichia miso* IAM 4682,[8,13] a type of yeast, was found to give the best result, optically active 2-methylcyclohexanone (**5**) being obtained in 75% yield (R^1 = Me, Scheme II). The ketone was confirmed to have *S* configuration by its sign of optical rotation, $[\alpha]^{23}_D$ +2.3° (*c* 0.79, MeOH) [lit.[2g] $[\alpha]_D$ +12.2° (*c* 4, MeOH) (87% ee, *S* form)]. However, the enantiomeric excess (ee) was low (ca. 20%) when the hydrolysis was carried out by direct addition of the substrate to the broth containing grown cells of the microorganism.

Effort was next focused upon optimization of the incubation conditions. After several trials, it was found that the asymmetric hydrolysis could be performed successfully with use of a large amount of grown cells of *P. miso* with the substrate. The cells of *P. miso* were collected by centrifugation after a 2-day incubation in a nutrient medium. Addition of 80 μL (78 mg, 0.2% to the medium) of enol acetate **4a** to 40 mL of a suspension of *P. miso* in 0.2 M phosphate buffer (pH 6.5) followed by incubation at 30 °C afforded the desired ketone **5**. Table I shows that increasing the amount of cells used improved the ee. When 14 g of the wet cells was used (entry 4), the reaction was finished in only 3 h, resulting in (*S*)-**5**, $[\alpha]^{23}_D$ +12.6° (*c* 0.73, MeOH), in a high chemical (79%) and optical (90%) yield. This effect can be explained by assuming the presence of plural hydrolytic enzymes in this microorganism. Because the Michaelis constant of each enzyme should be different, it is reasonable that the change in the amount of cells, *i.e.*, the ratio of enzymes to substrate, will affect the rates of reactions catalyzed by different enzymes in a different manner.

Scheme III[a]



[a] Key: (a) $LiAlH_4$/THF, 0 °C (in the case of **17**, **19**, **25**, and **29**); (b) DIBAL/THF, 0 °C (in the case of **12** and **27**); (c) (+)-(MTPA)-Cl, catalytic DMAP/Py.

Table II. Enantioselective Hydrolysis of 2-Methylcyclohexanone Enol Esters **4**[a]

entry	substrate	R^1	time, h	% yield of **5**[b]	% ee[c]	config[d]
1	**4a**	Me	3	79	90	*S*
2	**4b**	Et	3	78	92[e]	*S*
3	**4c**	Pr	3	73	87	*S*
4	**4d**	Ph	24	71	71	*S*

[a] Incubation was performed with 0.2% of substrate in 0.2 M phosphate buffer (pH 6.5). [b] Determined by GLC analysis. [c] Determined by the optical rotation of **5**. [d] Determined by the sign of optical rotation. [e] $[\alpha]^{24}_D$ +12.9° (*c* 0.68, MeOH).

The optical purities of the resulting ketones were evaluated from their optical rotations. In cases where the values are reported for ketones of low optical purity, are too small, or are not reported, the ee's of the products were determined as follows. The ketones were reduced with $LiAlH_4$, and the resulting cycloalkanols **6** were converted to (+)-MTPA esters **7**[14] (Scheme III). It was confirmed that the alcohol **6** was completely consumed in the esterification process (TLC). The four stereoisomers of **7** were separated, and the ee's were calculated from the ratio of (1*S*,2*S* + 1*R*,2*S*)/(1*R*,2*R* + 1*S*,2*R*). These values were almost equal to the ratio of 1*R*,2*S*/1*S*,2*R* in all cases. In cases where the four peaks were not completely separated, the ketone was reduced with $(i\text{-Bu})_2AlH$ (DIBAL) and converted to the MTPA esters as above. The ratio of the two diastereomers originating from the cis (or trans) isomer was measured to determine the ee of the original ketone. The ee's of the products strictly correspond to the enantioface selectivity of the enzyme, since spontaneous hydrolysis was confirmed to be negligible under the reaction conditions.

As a minor product of the reaction, 2-methylcyclohexanol (**8**) was formed (in ca. 10% yield)[15] and must originate from the primary product **5** via the action of alcohol dehydrogenase in the cell. Thus, a large amount of cells (17 g of wet cells to 78 mg of **4a**) accelerated the reduction, resulting in a decrease of the yield of **5** to 64%.

Application of the Enzymatic Hydrolysis to Various Substrates. As summarized in Table II, changes in the structure of the acyl group of enol esters affect the stereoselectivity of the reaction.

(11) House, H. O.; Gall, M.; Olmstead, H. D. *J. Org. Chem.* **1971**, *36*, 2361.

(12) Representative references: (a) Huang, F.-C.; Lee, L. F. H.; Mittal, R. S. D.; Ravikumar, P. R.; Chan, J. A.; Sih, C. J.; Caspi, E.; Eck, C. R. *J. Am. Chem. Soc.* **1975**, *97*, 4144. (b) Ohno, M.; Kobayashi, S.; Iimori, T.; Wang, Y.-F.; Izawa, T. *Ibid.* **1981**, *103*, 2405. (c) Iriuchijima, S.; Hasegawa, K.; Tsuchihashi, G. *Agric. Biol. Chem.* **1982**, *46*, 1907. (d) Mohr, P.; Waespe-Sărčević, N.; Tamm, C.; Gawronska, K.; Gawronski, J. K. *Helv. Chim. Acta* **1983**, *66*, 2501. (e) Wilson, W. K.; Baca, S. B.; Barber, Y. J.; Scallen, T. J.; Morrow, C. J. *J. Org. Chem.* **1983**, *48*, 3960. (f) Laumen, K.; Schneider, M. *Tetrahedron Lett.* **1984**, *25*, 5875. (g) Bjorkling, F.; Boutelje, J.; Gatenbeck, S.; Hult, K.; Norin, T.; Szmulik, P. *Tetrahedron* **1985**, *41*, 1347. (h) Gais, H.-J.; Lukas, K. L.; Ball, W. A.; Braun, S.; Lindner, H. J. *Liebigs Ann. Chem.* **1986**, 687. (i) Luyten, M.; Muller, S.; Herzog, B.; Keese, R. *Helv. Chim. Acta* **1987**, *70*, 1250. (j) Sabbioni, G.; Jones, J. B. *J. Org. Chem.* **1987**, *52*, 4565. (k) Eberle, M.; Egli, M.; Seebach, D. *Helv. Chim. Acta* **1988**, *71*, 1.

(13) Kinetic resolution of cyanohydrin acetates catalyzed by *P. miso* has been reported; see: Ohta, H.; Kimura, Y.; Sugano, Y.; Sugai, T. *Tetrahedron* **1989**, *45*, 5469.

(14) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.

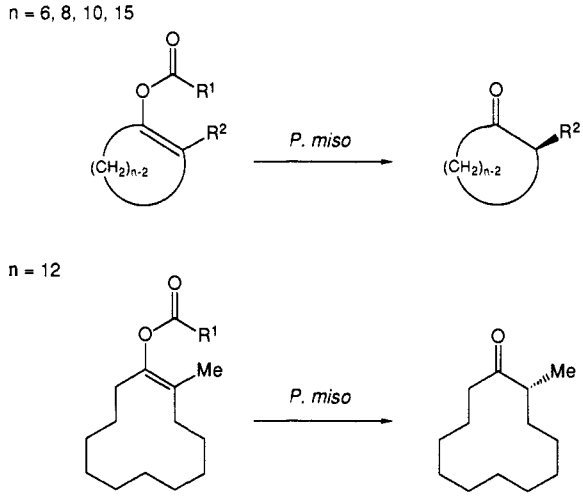
(15) The diastereoselectivity of reduction was not so high. The cis/trans ratio was 1/1–1/3.

Table III. Enantioselective Hydrolysis of α-Substituted Cyclohexanone Enol Esters (n = 6)[a]

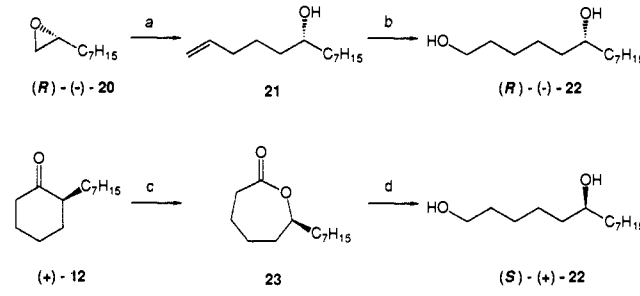
entry	substrate	R^1	R^2	% sub conc	time, h	product	% yield[b]	% ee	config[c]
1	**4a**	Me	Me	1.0	24	**5**	86	41[d]	*S*
2	**4b**	Et	Me	1.0	24	**5**	71	58[d]	*S*
3	**4c**	Pr	Me	1.0	24	**5**	71	43[d]	*S*
4	**9a**	Me	Pr	0.2	3	**10**	80	82[d]	*S*
5	**9a**	Me	Pr	1.0	24	**10**	84	67[d]	*S*
6	**9b**	Et	Pr	0.2	3	**10**	78	86[d,e]	*S*
7	**9b**	Et	Pr	1.0	24	**10**	92	74[d]	*S*
8	**11a**	Me	C_7H_{15}	0.2	3	**12**	77[f]	87[g,h]	*S*[i]
9	**11a**	Me	C_7H_{15}	1.0	24	**12**	82[f]	85[g]	*S*[i]
10	**11b**	Et	C_7H_{15}	0.2	3	**12**	75[f]	87[g]	*S*[i]
11	**11b**	Et	C_7H_{15}	1.0	24	**12**	80[f]	85[g]	*S*[i]
12	**14a**	Me	$C_{11}H_{23}$	0.2	24	**15**	trace		
13	**16a**	Me	CH_2=$CHCH_2$	0.2	3	**17**	49	70[j]	*R*
14	**16a**	Me	CH_2=$CHCH_2$	1.0	24	**17**	86	61[j]	*R*
15	**16b**	Et	CH_2=$CHCH_2$	0.2	3	**17**	57	78[j,k]	*R*
16	**16b**	Et	CH_2=$CHCH_2$	1.0	24	**17**	92	77[j]	*R*
17	**18a**	Me	$PhCH_2$	0.2	3	**19**	71[f]	84[g,l]	*R*
18	**18a**	Me	$PhCH_2$	1.0	24	**19**	63[f]	82[g]	*R*
19	**18b**	Et	$PhCH_2$	0.2	3	**19**	75[f]	84[g]	*R*
20	**18b**	Et	$PhCH_2$	1.0	24	**19**	68[f]	79[g]	*R*

[a] Incubation was performed in 0.2 M phosphate buffer (pH 6.5). [b] Determined by GLC analysis unless otherwise noted. [c] Determined by the sign of optical rotation unless otherwise noted. [d] Determined by the optical rotation of ketone. [e] $[\alpha]^{24}_D$ +24.0° (*c* 1.15, MeOH) (lit.[2g] $[\alpha]_D$ +27.9° (*c* 4, MeOH) (99% ee, *S* form)). [f] Isolated yield. [g] Determined by HPLC analysis of the corresponding MTPA ester (Scheme III). [h] $[\alpha]^{23}_D$ +24.1° (*c* 0.90, $CHCl_3$). [i] Determined by the method in Scheme V. [j] Determined by GLC analysis of the corresponding MTPA ester (Scheme III). [k] $[\alpha]^{24}_D$ +10.6° (*c* 1.06, MeOH) (lit.[2g] $[\alpha]_D$ +15.8° (*c* 3, MeOH) (99% ee, *R* form)). [l] $[\alpha]^{26}_D$ +40.0° (*c* 1.01, MeOH) (lit.[2g] $[\alpha]_D$ +41.4° (*c* 5, MeOH) (88% ee, *R* form)).

Scheme IV



Scheme V[a]



[a] Key: (a) CH_2=$CHCH_2CH_2MgBr$, Cu_2Br_2/THF, −10 °C; (b) BH_3·THF/THF, 0 °C; (c) *m*-CPBA/CH_2Cl_2, room temperature; (d) $LiAlH_4$/Et_2O, 0 °C.

When R^1 is ethyl (entry 2), the hydrolysis proceeds with higher selectivity than in the case of enol acetate (R^1 = Me, **4a**) to afford **5** (92% ee) in 78% yield. On the other hand, displacement of methyl with propyl (entry 3) leads to a slight lowering of the enantioselectivity. When the benzoyl ester **4d** was employed, it was not entirely consumed in 3 h and the stereoselectivity also decreased (entry 4). In summary, the enzyme is shown to be sensitive to the structure of the acyl moiety of the substrate and thus the enantioselectivity of the reaction can be controlled by selecting the appropriate acyl group.

Next, the substrate specificity of the enzymatic hydrolysis was examined (Scheme IV). First, a number of substrates with various substituents on the α-position were subjected to the microbial reaction. As summarized in Table III, the asymmetric hydrolysis of compounds having propyl (**9a** and **9b**) and heptyl (**11a** and **11b**) as the α-substituent proceeded to give the corresponding chiral ketones **10** and **12**, respectively. The substrates with a side chain having a terminal double bond (**16a** and **16b**) were also hydrolyzed selectively to afford chiral ketone **17**. The significance of the carbon number of the side chain is shown by the drastic decrease in reactivity observed for enol acetate **14a**. In the case of R^2 = undecyl (entry 12), very little ketone **15** was produced after incubation for 24 h. Introduction of an aromatic group into the ring (entries 17 and 19) had no detrimental effect, in contrast to the substrate with a phenyl group in the acyl moiety (**4d**). The absolute configurations of the resulting ketones can be determined simply by comparing their signs of specific rotation with those reported.[2g] The only exception is the case of (+)-2-heptylcyclohexanone (**12**), for which the specific rotation has not been reported. To determine the stereochemistry of **12**, an authentic sample of chiral diol **22** was prepared via ring opening of optically active epoxide (*R*)-**20**[16] with Grignard reagent in the presence of cuprous bromide, followed by hydroboration and oxidation (Scheme V). For comparison, ketone **12** (ca. 85% ee) was transformed to diol **22** via Baeyer–Villiger oxidation, followed by reduction with $LiAlH_4$. Diol **22** of different origins exhibited optical rotations of opposite sign: $[\alpha]^{24}_D$ −1.6° (*c* 2.31, $CHCl_3$) for that from (*R*)-epoxide **20** and $[\alpha]^{25}_D$ +1.3° (*c* 2.13, $CHCl_3$) for that from ketone **12**. Thus, the absolute configuration of **22** derived from ketone **12** was unambiguously confirmed to be *S*, and hence that of the original ketone **12** was also *S*. The absolute configurations of the ketones in Table III indicate that attack of the proton always occurs from the α-side enantioface of the enol esters.[17]

The hydrolysis can also be performed at high substrate concentration (1.0% to the medium). In almost all cases, the yields of ketones were higher than those obtained in the reactions carried out at a concentration of 0.2%. This can be accounted for by postulating that the presence of a large amount of substrate inhibits

(16) Furuhashi, K. *J. Synth. Org. Chem., Jpn.* **1987**, *45*, 162.
(17) Matsumoto, K.; Ohta, H. *Chem. Lett.* **1989**, 1589.

Table IV. Enantioselective Hydrolysis of 2-Methylcycloalkanone Enol Esters (R^2 = Me)[a]

entry	substrate	n	R^1	time, h	product	% yield[b]	% ee	config[c]
1	**24a**	8	Me	3	**25**	71	67[d,e]	*S*
2	**24d**	8	Ph	24	**25**	68	35[d]	*S*
3	**26b**	10	Et	3	**27**	83	89[f,g]	*S*
4	**26d**	10	Ph	24	**27**	84	86[f]	*S*
5	**28a**	12	Me	24	**29**	67	95[f,h]	*R*
6	**28d**	12	Ph	24	**29**	59	96[f]	*R*
7	**30a**	15	Me	24	**31**	38	<2[i]	*S*
8	**30d**	15	Ph	24	**31**	25	11[i,j]	*S*

[a] Incubation was performed with 0.2% of substrate in 0.2 M phosphate buffer (pH 6.5). [b] Isolated yield. [c] Determined by comparing the sign of optical rotation. [d] Determined by capillary GLC analysis of the corresponding MTPA ester (Scheme III). [e] $[\alpha]^{27}_D$ +20.9° (*c* 1.07, $CHCl_3$) (lit.[28] $[\alpha]_D$ +8.07° (*c* 7, $CHCl_3$) (20% ee, *S* form)). [f] Determined by ^{19}F NMR analysis of the corresponding MTPA ester (Scheme III). [g] $[\alpha]^{23}_D$ −13.4° (*c* 1.19, $CHCl_3$) (lit.[28] $[\alpha]^{25}_D$ −3.16° (*c* 4.5, $CHCl_3$) (30% ee, *S* form). [h] $[\alpha]^{23}_D$ +13.9° (*c* 1.09, $CHCl_3$) (lit.[28] $[\alpha]^{25}_D$ +10.30° (*c* 4.7, $CHCl_3$) (81% ee, *R* form). [i] Determined by the optical rotation of **31**. [j] $[\alpha]^{27}_D$ −1.1° (*c* 0.91, $CHCl_3$) (lit.[28] $[\alpha]^{25}_D$ +7.90° (*c* 6.1, $CHCl_3$) (81% ee, *R* form).

dehydrogenase activity in this system, which is responsible for the degradation of the resulting ketones. However, the enantiomeric excess of the products was generally lower, probably because of the effect of the decrease of enzyme/substrate ratio as mentioned above. In the case of the reactions of **11a**, **11b**, and **16b**, the yield of ketone increased with increasing concentration of the substrate and virtually no lowering in the ee was observed. The different results observed among these substrates may be interpreted as a different degree of matching of each substrate with the enzyme.

Finally, we extended this new reaction to the preparation of optically active medium-ring cyclic ketones. As seen from Table IV, chiral 2-methylcycloalkanones of eight-, ten-, and twelve-membered rings (entries 1–6) were smoothly obtained from the corresponding enol esters whereas larger ring compounds, such as the fifteen-membered-ring **30a** and **30d**, showed only poor reactivity and selectivity. Whereas changing the acyl group of the eight-membered ring from acetyl (**24a**) to benzoyl (**24d**) decreased the enantioselectivity similar to the six-membered-ring compound, the hydrolysis with enol benzoate **26d** (C_{10} ring) and **28d** (C_{12} ring) proceeded to give the corresponding ketones in high optical yields. It is noteworthy that 2-methylcyclododecanone (**29**) was obtained in extremely high optical yield and had the opposite absolute configuration (*R*) to that of other ketones (*S*). Two explanations are possible for the inversion of configuration: The configuration of the starting material could be reversed, or it could interact in a different manner with the enzyme. An NOE experiment (400-MHz ^1H NMR) indicated that the stereochemistry of the substrate was *Z*, consistent with the others (see Experimental Section). Thus, it is believed that the interaction between the enzyme and the twelve-membered-ring substrate is different from the other cases, although the details are not yet clear.

In conclusion, a new type of enzyme-mediated hydrolysis of enol esters has been achieved with differentiation of the enantiotopic faces of the C=C double bond by incubation with *P. miso*. Various carbonyl compounds with chirality at the α-position can be easily obtained from their racemates via enol ester formation by this process. The present method is applicable not only to cyclic compounds but also to acyclic systems[17] and is a potentially useful tool for organic synthesis.

Experimental Section

^1H NMR spectra were measured on a Varian EM-390, JEOL FX90A, or JEOL JNMGX-400 with tetramethylsilane (Me_4Si) as the internal standard. ^{13}C NMR spectra were recorded on a JEOL FX90A with Me_4Si as the internal standard. ^{19}F NMR spectra were measured on a JEOL FX90A with trifluoroacetic acid as the external standard. IR spectra were recorded with a Jasco A-202 spectrometer. Mass spectra were obtained with a Hitachi M-80 instrument. Optical rotations were measured with a Jasco DIP-360 polarimeter. HPLC data were obtained on a Jasco 880-PU and 875-UV. GLC data were taken on a Hitachi 163 or Hitachi C-3000. Kieselgel 60 F_{254} Art. 5715 was used for analytical TLC. Preparative TLC was performed on glass sheets coated with a 0.75-mm thickness of silica gel (Wakogel B-5F, Wako Pure Chemical Co.). Flash column chromatography[18] was performed with Kieselgel 60 K070W (70–230 mesh, Katayama Chemical). Kugelrohr distillation was performed with GTO-250RS (Shibata). Melting points and boiling points were not corrected. Lipases were provided from Amano Pharmaceutical Co., Ltd. PLE (EC 3.1.1.1) in $(NH_4)_2SO_4$ suspension was purchased from Sigma Chemical Co.

Preparation of Racemic α-Substituted Ketones. 2-Propylcyclohexanone (10). To a solution of 2-(methoxycarbonyl)cyclohexanone (**32**) (10.0 g, 64.2 mmol) and propyl iodide (21.4 g, 125 mmol) in acetone (100 mL) was added K_2CO_3 (26.6 g, 193 mmol) at 0 °C, and the mixture was refluxed overnight. After filtration through a Celite pad and evaporation, the residue was purified by flash column chromatography (hexane/AcOEt = 5/1) to give 2-(methoxycarbonyl)-2-propylcyclohexanone (**33**) as a colorless oil (11.8 g, 93%).

To a solution of the obtained **33** (10.1 g, 50.9 mmol) in acetic acid (45 mL) and H_2O (5 mL) was added concentrated H_2SO_4 (5 mL) at room temperature, and the mixture was refluxed for 6 h. Fifty milliliters of H_2O was added to the solution, and the products were extracted with Et_2O. The organic layer was washed with H_2O (1×), saturated $NaHCO_3$ aqueous solution (5×), and brine (1×) and dried over anhydrous Na_2SO_4. Evaporation and purification by flash column chromatography (hexane/Et_2O = 40/1) gave **10** as a colorless oil: 4.27 g, 60%; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3 H, *J* = 6.0 Hz), 0.9–2.7 (m, 13 H); IR (neat) 2900, 2850, 1700, 1440, 1370, 1300, 1220, 1190, 1110, 1000 cm^{-1}; MS, *m/z* (rel intens) 140 (14, M^+), 111 (15), 98 (100), 83 (20), 70 (11), 55 (5.8), 41 (1.9).

The other substrates were prepared by the same procedure. Spectral data are given below.

2-Heptylcyclohexanone (12): ^1H NMR (CCl_4) δ 0.87 (t, 3 H, *J* = 5.5 Hz), 1.0–1.5 (m, 12 H), 1.4–2.4 (m, 10 H); IR (neat) 2910, 2850, 1710, 1450, 1370, 1310, 1220, 1120, 1060 cm^{-1}; MS, *m/z* (rel intens) 196 (6.8, M^+), 111 (24), 98 (100), 83 (21), 70 (12), 55 (33), 41 (26).

2-Undecylcyclohexanone (15): ^1H NMR (CCl_4) δ 0.87 (t, 3 H, *J* = 6.0 Hz), 1.1–1.4 (m, 19 H), 1.4–2.4 (m, 10 H); IR (neat) 2920, 2850, 1710, 1460, 1450, 1380, 1310, 1220, 1120 cm^{-1}; MS, *m/z* (rel intens) 252 (2.9, M^+), 112 (8.7), 98 (100), 83 (5.8), 70 (5.0), 55 (11), 41 (12).

2-Allylcyclohexanone (17): ^1H NMR (CCl_4) δ 1.0–2.7 (m, 11 H), 4.8–5.1 (m, 2 H), 5.4–6.0 (m, 1 H); IR (neat) 3100, 2950, 2870, 1710, 1640, 1450, 1310, 1230, 1200, 1120, 990, 910 cm^{-1}; MS, *m/z* (rel intens) 138 (80, M^+), 123 (25), 109 (63), 94 (100), 79 (59), 67 (85), 55 (42), 41 (74).

2-Benzylcyclohexanone (19): ^1H NMR (CCl_4) δ 1.0–2.2 (m, 7 H), 2.2–2.7 (m, 3 H), 2.9–3.4 (m, 1 H), 6.8–7.3 (m, 5 H); IR (neat) 3050, 2950, 2880, 1710, 1600, 1500, 1450, 1320, 1220, 1130, 730, 700 cm^{-1}; MS, *m/z* (rel intens) 188 (22, M^+), 149 (100), 97 (17), 91 (12), 71 (16), 57 (23), 55 (13), 43 (20), 41 (9.6).

2-Methylcyclooctanone (25): ^1H NMR (CCl_4) δ 0.98 (d, 3 H, *J* = 6.5 Hz), 1.1–2.1 (m, 11 H), 2.31 (t, 2 H, *J* = 7.0 Hz); IR (neat) 2950, 2900, 1710, 1460, 1380, 1340, 1200, 1160, 1050 cm^{-1}; MS, *m/z* (rel intens) 140 (30, M^+), 112 (23), 98 (100), 83 (29), 70 (25), 69 (30), 55 (62), 41 (42).

2-Methylcyclodecanone (27): ^1H NMR (CCl_4) δ 0.98 (d, 3 H, *J* = 7.0 Hz), 1.1–2.1 (m, 14 H), 2.1–3.0 (m, 3 H); IR (neat) 2920, 2880, 1700, 1450, 1370, 1260, 1180, 1090, 1050 cm^{-1}; MS, *m/z* (rel intens) 168 (37, M^+), 125 (48), 111 (60), 98 (77), 85 (72), 71 (97), 69 (100), 55 (95), 43 (91).

2-Methylcyclododecanone (29): ^1H NMR (CCl_4) δ 1.01 (d, 3 H, *J* = 7.0 Hz), 1.1–1.4 (m, 14 H), 1.4–2.0 (m, 4 H), 2.0–2.9 (m, 3 H); IR (neat) 2940, 2860, 1710, 1460, 1370, 1280, 1240, 1200, 1130, 1040, 1000 cm^{-1}; MS, *m/z* (rel intens) 196 (100, M^+), 167 (23), 139 (32), 98 (46), 97 (28), 69 (32), 55 (52).

2-Methylcyclopentadecanone (31): ^1H NMR (CCl_4) δ 1.00 (d, 3 H, *J* = 7.0 Hz), 1.1–2.1 (m, 24 H), 2.1–2.8 (m, 3 H); IR (neat) 2950, 2860, 1710, 1460, 1370, 1280, 1200, 1130, 1060, 1010 cm^{-1}; MS, *m/z* (rel intens) 238 (100, M^+), 209 (36), 181 (18), 139 (31), 125 (21), 111 (25), 98 (43), 85 (50), 72 (70), 69 (52), 55 (59), 41 (41).

Preparation of the Enol Esters. 1-Acetoxy-2-methylcyclohexene (4a). A previously reported procedure was employed.[11] To a solution of 2-methylcyclohexanone (**5**; 6.17 g, 55.1 mmol) in CCl_4 (30 mL) were added acetic anhydride (11.2 g, 110 mmol) and a catalytic amount of 60% $HClO_4$ aqueous solution at 0 °C, and the mixture was stirred overnight at room temperature. The solution was diluted with Et_2O (300 mL), washed with saturated $NaHCO_3$ aqueous solution (4×), and dried over anhydrous Na_2SO_4. After evaporation, the residue was purified by flash column chromatography (hexane/Et_2O = 30/1) to give **4a** as a colorless oil (7.22 g, 85%). This was further purified with Kugelrohr distillation

(18) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(bath temperature, 150 °C (18 mmHg)): ^{1}H NMR (CCl_4) δ 1.45 (s, 3 H), 1.5–1.9 (m, 4 H), 1.9–2.2 (m, 4 H), 2.02, (s, 3 H); IR (neat) 2920, 2870, 1750, 1710, 1440, 1370, 1210, 1100 cm^{-1}; MS, m/z (rel intens) 154 (3.9, M^+), 126 (11), 112 (100), 97 (61), 84 (43), 69 (25), 55 (22), 43 (42); HRMS, m/z 154.1015 (154.0993 calcd for $C_9H_{14}O_2$, M^+).

The other substrates were prepared by the same procedure from the corresponding ketones without contamination of the regio- and stereoisomers.

1-(Propionyloxy)-2-methylcyclohexene (4b): ^{1}H NMR (CCl_4) δ 1.13 (t, 3 H, J = 7.5 Hz), 1.45 (s, 3 H), 1.5–1.9 (m, 4 H), 1.9–2.2 (m, 4 H), 2.31 (q, 2 H, J = 7.5 Hz); IR (neat) 2950, 2860, 1750, 1710, 1450, 1340, 1260, 1160, 1110 cm^{-1}; MS, m/z (rel intens) 168 (15, M^+), 112 (100), 97 (39), 84 (21), 69 (10), 57 (40), 55 (19), 41 (17); HRMS, m/z 168.1124 (168.1148 calcd for $C_{10}H_{16}O_2$, M^+).

1-(Butyryloxy)-2-methylcyclohexene (4c): ^{1}H NMR (CCl_4) δ 0.96 (t, 3 H, J = 7.5 Hz), 1.46 (s, 3 H), 1.4–1.9 (m, 6 H), 1.9–2.2 (m, 4 H), 2.29 (t, 2 H, J = 7.5 Hz); IR (neat) 2950, 2880, 1750, 1710, 1450, 1350, 1250, 1160, 1110 cm^{-1}; MS, m/z (rel intens) 182 (33, M^+), 112 (100), 97 (69), 84 (32), 71 (43), 69 (11), 55 (17), 43 (45); HRMS, m/z 196.1489 (196.1462 calcd for $C_{12}H_{20}O_2$, M^+).

1-(Benzoyloxy)-2-methylcyclohexene (4d): ^{1}H NMR (CCl_4) δ 1.46 (s, 3 H), 1.3–1.9 (m, 4 H), 1.9–2.3 (m, 4 H), 7.2–7.6 (m, 3 H), 7.9–8.1 (m, 2 H); IR (neat) 3090, 2950, 2850, 1730, 1600, 1580, 1450, 1260, 1140, 1110, 710 cm^{-1}; MS, m/z (rel intens) 216 (82, M^+), 105 (100), 77 (79), 55 (11), 51 (13); HRMS, m/z 216.1125 (216.1149 calcd for $C_{14}H_{16}O_2$, M^+).

1-Acetoxy-2-propylcyclohexene (9a): ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3 H, J = 7.5 Hz), 1.1–2.7 (m, 12 H), 2.09 (s, 3 H); IR (neat) 2940, 2860, 1750, 1700, 1450, 1370, 1220, 1200, 1110 cm^{-1}; MS, m/z (rel intens) 182 (34, M^+), 140 (100), 111 (100), 97 (47), 83 (16), 55 (20), 43 (20), 41 (13); HRMS, m/z 182.1285 (182.1305 calcd for $C_{11}H_{18}O_2$, M^+).

1-(Propionyloxy)-2-propylcyclohexene (9b): ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3 H, J = 7.5 Hz), 1.0–2.8 (m, 12 H), 1.16 (t, 3 H, J = 7.5 Hz), 2.35 (q, 2 H, J = 7.5 Hz); IR (neat) 2940, 2910, 1750, 1700, 1460, 1350, 1260, 1160, 1110 cm^{-1}; MS, m/z (rel intens) 196 (19, M^+), 140 (100), 111 (100), 97 (25), 83 (8.0), 57 (31), 41 (12); HRMS, m/z 139.1084 (139.1121 calcd for $C_9H_{15}O$, $M^+ - C_2H_5CO$).

1-Acetoxy-2-heptylcyclohexene (11a): ^{1}H NMR (CCl_4) δ 0.87 (t, 3 H, J = 5.5 Hz), 1.0–1.5 (m, 10 H), 1.5–2.5 (m, 10 H), 2.01 (s, 3 H); IR (neat) 2940, 2860, 1750, 1700, 1450, 1370, 1220, 1110 cm^{-1}; MS, m/z (rel intens) 238 (5.9, M^+), 196 (100), 111 (81), 98 (29), 83 (4.9), 55 (13), 43 (24); HRMS, m/z 238.1901 (238.1931 calcd for $C_{15}H_{26}O_2$, M^+).

1-(Propionyloxy)-2-heptylcyclohexene (11b): ^{1}H NMR (CCl_4) δ 0.85 (t, 3 H, J = 7.5 Hz), 1.0–1.5 (m, 11 H), 1.11 (t, 3 H, J = 8.0 Hz), 1.5–2.1 (m, 9 H), 2.31 (q, 2 H, J = 8.0 Hz); IR (neat) 2930, 2850, 1750, 1700, 1460, 1340, 1260, 1150, 1110 cm^{-1}; MS, m/z (rel intens) 252 (2.9, M^+), 196 (100), 111 (69), 98 (50), 83 (4.0), 57 (31), 55 (8.9), 41 (12); HRMS, m/z 252.2080 (252.2087 calcd for $C_{16}H_{28}O_2$, M^+).

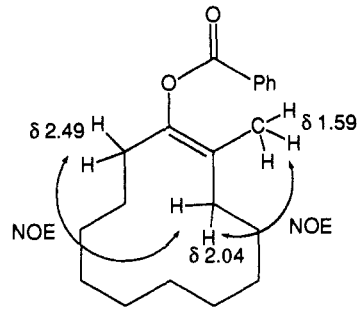
1-Acetoxy-2-undecylcyclohexene (14a): ^{1}H NMR (CCl_4) δ 0.87 (t, 3 H, J = 6.0 Hz), 1.0–1.4 (m, 18 H), 1.4–2.2 (m, 10 H), 2.03 (s, 3 H); IR (neat) 2930, 2850, 1760, 1700, 1460, 1370, 1220, 1120 cm^{-1}; MS, m/z (rel intens) 294 (2.8, M^+), 252 (100), 111 (80), 98 (37), 83 (3.9), 55 (14), 43 (24), 41 (11); HRMS, m/z 294.2566 (294.2557 calcd for $C_{19}H_{34}O_2$, M^+).

1-Acetoxy-2-allylcyclohexene (16a): ^{1}H NMR ($CDCl_3$) δ 1.4–1.9 (m, 4 H), 1.9–2.4 (m, 4 H), 2.11 (s, 3 H), 2.66 (d, 2 H, J = 7.0 Hz), 4.8–5.2 (m, 2 H), 5.4–6.0 (m, 1 H); IR (neat) 3090, 2940, 2870, 1750, 1700, 1640, 1440, 1370, 1220, 1180, 1130, 1010, 910 cm^{-1}; MS, m/z (rel intens) 180 (5.5, M^+), 138 (100), 123 (6.9), 110 (27), 97 (47), 79 (20), 67 (25), 55 (17), 43 (57); HRMS, m/z 180.1131 (180.1148 calcd for $C_{11}H_{16}O_2$, M^+).

1-(Propionyloxy)-2-allylcyclohexene (16b): ^{1}H NMR (CCl_4) δ 1.13 (t, 3 H, J = 8.5 Hz), 1.4–1.8 (m, 4 H), 1.8–2.3 (m, 4 H), 2.32 (q, 2 H, J = 8.5 Hz), 2.60 (d, 2 H, J = 7.0 Hz), 4.7–5.1 (m, 2 H), 5.3–5.9 (m, 1 H); IR (neat) 3090, 2940, 2850, 1750, 1700, 1640, 1460, 1350, 1270, 1150, 950, 910 cm^{-1}; MS, m/z (rel intens) 194 (7.3, M^+), 153 (1.7), 138 (100), 110 (33), 97 (64), 79 (13), 67 (16), 57 (73), 55 (16), 41 (19); HRMS, m/z 137.0999 (137.0966 calcd for $C_9H_{13}O$, $M^+ - C_2H_5CO$).

1-Acetoxy-2-benzylcyclohexene (18a): ^{1}H NMR (CCl_4) δ 1.4–2.0 (m, 6 H), 2.0–2.4 (m, 2 H), 2.05 (s, 3 H), 3.20 (s, 2 H), 6.9–7.9 (m, 5 H); IR (neat) 3020, 2930, 2850, 1750, 1700, 1600, 1490, 1450, 1370, 1220, 1080, 700 cm^{-1}; MS, m/z (rel intens) 230 (5.9, M^+), 188 (100), 170 (7.9), 159 (8.3), 145 (5.9), 115 (7.3), 97 (33), 91 (21), 55 (1.0), 43 (7.3); HRMS, m/z 230.1299 (230.1305 calcd for $C_{15}H_{18}O_2$, M^+).

1-(Propionyloxy)-2-benzylcyclohexene (18b): ^{1}H NMR (CCl_4) δ 1.13 (t, 3 H, J = 8.5 Hz), 1.3–2.0 (m, 6 H), 2.0–2.3 (m, 2 H), 2.35 (q, 2 H, J = 8.5 Hz), 3.19 (s, 2 H), 6.9–7.3 (m, 5 H); IR (neat) 3050, 2950, 2850, 1750, 1700, 1600, 1500, 1450, 1350, 1150, 1090, 700 cm^{-1}; MS, m/z (rel intens) 244 (17, M^+), 188 (100), 170 (10), 159 (5.3), 148 (4.1), 115 (6.9), 97 (19), 91 (14), 57 (9.7); HRMS, m/z 188.1187 (188.1199 calcd for $C_{13}H_{16}O$, $M^+ - C_2H_5CO + H$).



28d (n = 12)

Figure 1. NOE measurement of enol ester **28d**.

1-Acetoxy-2-methylcyclooctene (24a): ^{1}H NMR (CCl_4) δ 1.4–1.8 (m, 8 H), 1.47 (s, 3 H), 1.9–2.4 (m, 4 H), 2.05 (s, 3 H); IR (neat) 2950, 2880, 1760, 1700, 1450, 1370, 1220, 1160, 1120 cm^{-1}; MS, m/z (rel intens) 182 (27, M^+), 140 (100), 112 (73), 97 (89), 84 (29), 69 (19), 55 (14), 43 (29), 41 (18); HRMS, m/z 182.1314 (182.1306 calcd for $C_{11}H_{18}O_2$, M^+).

1-(Benzoyloxy)-2-methylcyclooctene (24d): ^{1}H NMR ($CDCl_3$) δ 1.3–1.9 (m, 11 H), 2.0–2.6 (m, 4 H), 7.3–7.7 (m, 3 H), 8.0–8.3 (m, 2 H); IR (neat) 3060, 2920, 2850, 1730, 1700, 1600, 1580, 1450, 1280, 1240, 1150, 1110, 1090, 700 cm^{-1}; MS, m/z (rel intens) 244 (8.8, M^+), 122 (15), 105 (100), 77 (58), 69 (6.8), 55 (12), 41 (19); HRMS, m/z 244.1441 (244.1462 calcd for $C_{16}H_{20}O_2$, M^+).

(*Z*)-1-(Propionyloxy)-2-methylcyclododecene (26b): ^{1}H NMR ($CDCl_3$) δ 0.7–1.7 (m, 15 H), 1.20 (t, 3 H, J = 7.5 Hz), 1.49 (s, 3 H), 2.0–2.6 (m, 4 H), 2.43 (q, 2 H, J = 7.5 Hz); IR (neat) 2940, 2860, 1750, 1690, 1470, 1450, 1350, 1270, 1150, 1110 cm^{-1}; MS, m/z (rel intens) 224 (3.6, M^+), 168 (100), 150 (16), 125 (23), 111 (46), 97 (26), 84 (14), 69 (10), 57 (21), 55 (10); HRMS, m/z 225.1849 (225.1853 calcd for $C_{14}H_{25}O_2$, M^+).

(*Z*)-1-(Benzoyloxy)-2-methylcyclododecene (26d): ^{1}H NMR (C_6D_6) δ 1.28–1.70 (m, 12 H), 1.55 (s, 3 H), 2.04–2.20 (m, 2 H), 2.57 (t, 2 H, J = 6.1 Hz), 7.03–7.14 (m, 3 H), 8.18–8.27 (m, 2 H); IR (neat) 3060, 2910, 2850, 1730, 1690, 1600, 1580, 1470, 1450, 1270, 1230, 1120, 1110, 1090, 700 cm^{-1}; MS, m/z (rel intens) 272 (8.0, M^+), 167 (13), 150 (25), 105 (100), 77 (4.0), 55 (0.9); HRMS, m/z 272.1781 (272.1775 calcd for $C_{18}H_{24}O_2$, M^+).

(*Z*)-1-Acetoxy-2-methylcyclododecene (28a): ^{1}H NMR ($CDCl_3$) δ 1.18–1.33 (m, 5 H), 1.33–1.43 (m, 8 H), 1.43–1.58 (m, 3 H), 1.52 (s, 3 H), 2.11 (t, 2 H, J = 7.1 Hz), 2.14 (s, 3 H), 2.32 (t, 2 H, J = 6.8 Hz); IR (neat) 2930, 2850, 1750, 1710, 1460, 1440, 1370, 1250, 1210, 1120, 1020 cm^{-1}; MS, m/z (rel intens) 238 (2.4, M^+), 196 (100), 167 (10), 139 (31), 125 (22), 11 (29), 98 (45), 97 (60), 69 (50), 55 (46), 43 (69); HRMS, m/z 238.1913 (238.1931 calcd for $C_{15}H_{26}O_2$, M^+).

(*Z*)-1-(Benzoyloxy)-2-methylcyclododecene (28d): ^{1}H NMR (C_6D_6) δ 1.04–1.49 (m, 6 H), 1.49–1.67 (m, 10 H), 1.59 (s, 3 H), 2.04 (t, 2 H, J = 7.1 Hz), 2.49 (t, 2 H, J = 6.6 Hz), 7.02–7.09 (m, 2 H), 7.09–7.17 (m, 1 H), 8.20–8.33 (m, 2 H); IR (neat) 3080, 2940, 2870, 1730, 1690, 1600, 1580, 1470, 1450, 1280, 1240, 1130, 1090, 1070, 1040, 710 cm^{-1}; MS, m/z (rel intens) 300 (2.0, M^+), 178 (12), 105 (100), 77 (49), 55 (12); HRMS, m/z 300.2078 (300.2087 calcd for $C_{20}H_{28}O_2$, M^+).

(*Z*)-1-Acetoxy-2-methylcyclopentadecene (30a): ^{1}H NMR (CCl_4) δ 1.0–1.6 (m, 22 H), 1.47 (s, 3 H), 1.67 (t, 2 H, J = 8.5 Hz), 1.9–2.4 (m, 2 H), 2.05 (s, 3 H); IR (neat) 2930, 2850, 1760, 1690, 1460, 1370, 1250, 1210, 1140, 1020 cm^{-1}; MS, m/z (rel intens) 280 (2.3, M^+), 238 (100), 209 (4.9), 181 (3.1), 139 (5.0), 125 (4.9), 111 (2.9), 97 (11), 85 (5.9), 72 (8.8), 69 (7.8), 55 (7.8), 43 (46); HRMS, m/z 237.2186 (237.2216 calcd for $C_{16}H_{29}O$, $M^+ - CH_3CO$).

(*Z*)-1-(Benzoyloxy)-2-methylcyclopentadecene (30d): ^{1}H NMR (C_6D_6) δ 1.20–1.70 (m, 22 H), 1.61 (s, 3 H), 1.99 (t, 2 H, J = 7.8 Hz), 2.44 (t, 2 H, J = 7.8 Hz), 7.03–7.15 (m, 3 H), 8.21–8.29 (m, 2 H); IR (neat) 2940, 2860, 1730, 1690, 1600, 1580, 1450, 1270, 1240, 1130, 1090, 1060, 1020, 710 cm^{-1}; MS, m/z (rel intens) 342 (5.9, M^+), 220 (8.0), 117 (100), 105 (87), 77 (19), 55 (8.6); HRMS, m/z 342.2566 (342.2557 calcd for $C_{23}H_{34}O_2$, M^+).

NOE Measurement of Enol Ester 28d. The configurations of **26d**, **28d**, and **30d** were assigned to be *Z* by NOE experiment of 400-MHz ^{1}H NMR (Figure 1). Enol benzoate **28d** was dissolved in C_6D_6. Irradiation at δ_H 2.04 (2 H, allylic CH_2 adjacent to methyl) caused enhancement at δ_H 1.59 (3 H, Me, 6.8%) and δ_H 2.49 (2 H, allylic CH_2 adjacent to benzoyl, 7.3%). Irradiation at δ_H 1.59 (3 H), however, did not cause enhancement at δ_H 2.49. In addition, irradiation at δ_H 2.49 did not

enhance the signal at δ_H 2.04 (8.7%), but no enhancement was observed at δ_H 1.59. Thus, the configuration of **28d** is concluded to be *Z*. Similar studies on **26d** and **30d** resulted in the same conclusion. The configurations of acetates **26a** and **30a** and propionate **28b** were analogously estimated.

Basal Medium for Enzyme-Mediated Transformation. The basal medium for screening of microorganisms consists of glucose (10 g), polypeptone (7 g), and yeast extract (5 g) in 1000 mL of 0.2 M phosphate buffer (pH 6.8).

The composition of the basal medium for hydrolysis with *P. miso* IAM 4682 was glucose (10 g), polypeptone (7 g), yeast extract (5 g), and K_2HPO_4 (5 g) in 1000 mL of distilled water. The initial pH of the medium was adjusted to 7.2 with 2 N HCl.

Screening Test. Case of Microorganisms. In the first screening test, a loopful of each microorganism from a nutrient slant was transferred to a test tube containing 5 mL of the sterilized basal medium. After cultivation for 2 days at 30 °C, 10 μL (9.7 mg) of 1-acetoxy-2-methylcyclohexene (**4a**) was added to the suspension of grown cells and the incubation was continued for an additional 2 days. The broth was extracted with Et_2O, and the products were analyzed by GLC. The conditions of GLC analysis were as follows: column, butanediol succinate (15%); injection, 110 °C; oven, 95 °C; carrier gas, N_2; head pressure, 0.2 kg/cm^2; internal standard, 1-nonanol (8.8 min), **4a** (4.6 min), **5** (2.3 min), 2-methylcyclohexanol (**8**, 2.9 min).

In the second screening test, a loopful of a microorganism was transferred from a nutrient slant to 50 mL of the sterilized medium in a 500-mL Sakaguchi flask and was incubated for 2 days. To the broth was added 100 μL (97 mg) of **4a**, and the cultivation was continued for an additional 2 days. The products were extracted with Et_2O and dried over anhydrous Na_2SO_4. After careful evaporation of the solvent under reduced pressure at 0 °C, the yield of **5** was measured by GLC. Purification by flash column chromatography on silica gel (hexane/Et_2O = 15/1) and Kugelrohr distillation (bath temperature, 150 °C (20 mmHg)) gave pure **5** of which optical rotation was compared with the reference data.[2g]

Screening of Enzymes. In the first screening test, 10 μL (9.7 mg) of **4a** and 50 mg of commercially available enzyme were incubated in 5 mL of 0.2 M phosphate buffer (pH 6.5) for 2 days at 30 °C. After usual workup, the products were analyzed by GLC.

In the case of PLE, 100 μL (97 mg) of **4a** and 0.3 mL of PLE suspension were incubated in 50 mL of 0.2 M phosphate buffer (pH 6.8) for 2 days at 30 °C. Purification and analysis of products were performed by the same procedure as described above.

Asymmetric Hydrolysis with *P. miso*. Synthesis of (*S*)-2-Methylcyclohexanone (5) from 1-Acetoxy-2-methylcyclohexene (4a). Four 500-mL Erlenmeyer flasks each containing 100 mL of sterilized basal medium were inoculated with a loopful of *P. miso* IAM 4682, and the mixture was shaken for 2 days at 30 °C. The combined grown cells were collected by centrifugation and washed with 0.2 M phosphate buffer (pH 6.5) to give ca. 14 g of wet cells of *P. miso*. These cells and 80 μL (78 mg) of **4a** 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 3 h at 30 °C. The broth was extracted with Et_2O and dried over anhydrous Na_2SO_4. Evaporation and purification afforded **5** as a colorless oil (79% yield).

Asymmetric hydrolyses of the other substrates were carried out by the same procedure. All the spectral data (^{1}H NMR, IR, and MS) were in full agreement with those of the racemates. Optical rotations of the obtained ketones were shown in Tables I–IV. The absolute configurations of the ketones (except for **12**) were determined by comparing their signs of optical rotations with reported ones.[2g] The optical purities were determined by the optical rotation or analysis of the corresponding alcohols prepared analogously to Scheme III. The analysis was carried out with capillary GLC (PEG-20M, 0.25 mm × 50 m, Gasukuro Kogyo Inc.; carrier gas, He; head pressure, 2.4 kg/cm^2), HPLC (Zorbax SIL, 0.46 mm × 25 cm, Du Pont Instruments), or ^{19}F NMR (84.25 MHz, ppm relative to $CFCl_3$, CF_3CO_2H as external standard).

(*S*)-**12**: HPLC (MTPA ester of the reduced alcohol; eluent, hexane/AcOEt = 1000/1; flow rate, 1.0 mL/min) trans (*S*) 34 min, cis 37 min, trans (*R*) 40 min.

(*R*)-**17**: GLC (MTPA ester of the reduced alcohol; oven, 150 °C) cis (*R*) 143 min, cis (*S*) 145 min, trans (*R*) 159 min, trans (*S*) 161 min.

(*R*)-**19**: HPLC (MTPA ester of the reduced alcohol; eluent, hexane/AcOEt = 200/1; flow rate, 0.5 mL/min) trans (*R*) 29 min, trans (*S*) 32 min, cis (*S*) 35 min, cis (*R*) 39 min.

(*S*)-**25**: GLC (MTPA ester of the reduced alcohol; oven, 150 °C) trans (*S*) 212 min, trans (*R*) 214 min, cis (*R*) 224 min, cis (*S*) 227 min.

(*S*)-**27**: ^{19}F NMR ($CDCl_3$) (MTPA ester of the reduced alcohol) Φ 71.8 (S_1), 71.9 (R_1), 72.1 (S_2 and R_2).

(*S*)-**29**: ^{19}F NMR ($CDCl_3$) (MTPA ester of the reduced alcohol) Φ 71.9 (R_1), 72.0 (S_1), 72.1 (R_2), 72.2 (S_2).

Determination of the Absolute Configuration of (+)-12. Preparation of Authentic (*R*)-Tridecane-1,6-diol (22). To a mixture of 3-butenylmagnesium bromide (1.0 M THF, 6.0 mL, 6 mmol) and cuprous bromide (76 mg, 0.53 mmol) in THF (6 mL) was added (*R*)-1,2-epoxynonane[16] (**20**; 500 mg, 3.52 mmol) in THF (4 mL) with stirring at −10 °C under an atmosphere of argon, and the stirring was continued for 4 h. The reaction mixture was quenched with saturated NH_4Cl aqueous solution, and the products were extracted with Et_2O, washed with brine, and dried over anhydrous Na_2SO_4. After evaporation and purification with flash column chromatography (hexane/AcOEt = 15/1), (*R*)-1-tridecen-6-ol (**21**) was obtained as a colorless oil (626 mg, 90%). To a solution of (*R*)-**21** (101 mg, 0.466 mmol) in THF (4 mL) was added BH_3 in THF (1.0 M THF, 1.8 mL, 1.8 mmol) with stirring at 0 °C under an argon atmosphere. The reaction mixture was treated with a sequence of addition of water, 15% NaOH aqueous solution (2 mL), and 35% H_2O_2 aqueous solution (2 mL) and stirred for 1 h at room temperature. Extractive workup with Et_2O and purification with flash column chromatography (hexane/AcOEt = 1/1) afforded (*R*)-(−)-diol **22** as a colorless oil (98 mg, 98%), which crystallized on standing: mp 49–50 °C (recrystallized from hexane); $[\alpha]^{24}_D$ −1.6° (*c* 2.13, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3 H, *J* = 5.0 Hz), 1.1–1.8 (m, 20 H), 1.6–2.2 (m, 2 H), 3.4–3.8 (m, 3 H); IR ($CDCl_3$ solution) 3400, 2925, 2850, 2250, 1730, 1640, 1375, 1020 cm^{-1}; MS, *m/z* (rel intens) 199 (3.9, $(M + 1)^+ - H_2O$), 129 (24), 117 (38), 99 (69), 81 (100), 69 (59), 55 (80); Anal. Calcd for $C_{13}H_{28}O_2$: C, 72.17; H, 13.04. Found: C, 72.30; H, 12.50.

Conversion of (+)-12 to Diol 22. To a solution of (+)-**12** (70 mg, 0.36 mmol) in CH_2Cl_2 (3.5 mL) was added *m*-chloroperbenzoic acid (154 mg, 0.893 mmol) with stirring at 0 °C, and the mixture was stirred overnight at room temperature. The reaction was quenched with water, and the products were extracted with CH_2Cl_2, washed with saturated Na_2CO_3 aqueous solution (2×) and brine (1×), and dried over anhydrous Na_2SO_4. After evaporation, the residue was purified with flash column chromatography (hexane/AcOEt = 7/1) to give lactone **23** as a colorless oil (72.2 mg, 96%). To a suspension of $LiAlH_4$ (85 mg, 2.24 mmol) in Et_2O (1 mL) was added a solution of **23** (58.1 mg, 0.274 mmol) in Et_2O (13 mL) with stirring at 0 °C. The stirring was continued for 1 h, and the reaction was quenched with addition of Na_2SO_4 (10 H_2O). After filtration through a Celite pad and evaporation, the residue was recrystallized from hexane to give (+)-diol **22** as white needles: 52.3 mg, 89%; $[\alpha]^{25}_D$ +1.3° (*c* 2.13, $CHCl_3$). Other spectral data were in complete agreement with those of the authentic (*R*)-(−)-**22**. Thus, the absolute configuration of (+)-**12** was determined to be *S*.

Acknowledgment. We are grateful to Nippon Mining Co., Ltd. for the gift of chiral epoxide. Financial support is also acknowledged from Sapporo Bioscience Foundation and the Petroleum Energy Center.