Enantioselective Reduction of 2-Methyl-3-oxopropionate by Bakers' Yeast[1)]

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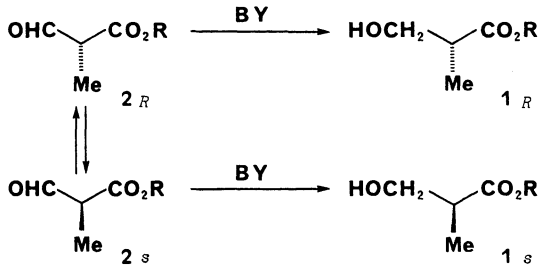
Various esters of 2-methyl-3-oxopropionic acid were subjected to the reduction with bakers' yeast. Effects of size and bulkiness as well as hydrophobicity of alcohol moieties of the substrates on the enantioselectivity of the reduction were investigated. The substrates having bulky ester groups gave the (*R*)-hydroxy esters with high enantioselectivities. A mechanism to alter the stereoselectivity of the reduction is proposed.

Optically active 3-hydroxy-2-methylpropionic acid derivatives (**1**) have been used widely as versatile chiral building blocks.[2)] Although the use of a microbe in hydroxylation of isobutyric acid to obtain **1** of high optical purity has been reported,[3,4)] the handling of special microbes such as those reported is not so easy in laboratories of organic chemistry. However, bakers' yeast (BY), which is also a microbe, has advantages as a reagent in organic chemistry. BY is easily available and cheap. Special caution, such as sterilization or careful operation, is not necessary. Because of its simplicity of handling, BY has been used as a convenient pack of various enzymes to convert achiral organic compounds into chiral products.[5)] In this paper, we would like to report an enantioselective reduction of 2-methyl-3-oxopropionate (**2**) with BY to obtain chiral **1**. Although the reduction of **2** with BY was attempted by Seebach et al., the enantiomer excess (e.e.) observed was not satisfactory for practical use (54%).[6)] It should be noted that in the process of reduction, chirality is introduced at the position different from the reaction center. That is, the chirality arises on the C-2 position, whereas the reduction takes place at the C-3 position. Therefore, there is no doubt that the introduced chirality stems from difference in the rate of reduction of both enantiomers of the starting material (*R*-2 and *S*-2) by an enzyme or a set of enzymes that are operating simultaneously in bakers' yeast. In other words, the present reaction is a reductive resolution of racemic material. Since the starting material **2** is composed of a mixture of an aldehyde and its enol form, which was confirmed by ^{1}H NMR spectroscopy, interconversion between the two enantiomers (of the aldehyde form) is expected to be fast enough to keep the starting material as a racemic mixture during the whole reduction period even though one enantiomer is reduced faster than the other. Therefore, in principle, a particular enantiomer of the product can be obtained quantitatively, although the process itself is a kinetic resolution.



Scheme 1.

It seems that relatively low selectivity reported by Seebach et al. was due to a small difference in the reaction rates of both enantiomers of the aldehyde. One may therefore be able to obtain chiral **1** in high e.e. if one can modify the substrate so that the enantiomers exhibit a large difference in the reaction rate. According to the concept mentioned above, we investigated the reduction of **2** by BY.

Usually, the microbial reduction of a carbonyl compound does not necessarily afford the alcohol of desired configuration with satisfied e.e., and several methods to control the stereochemistry of reduction have been developed. Screening of microbes[7)] has been the method employed most widely but this is troublesome for organic chemists. Immobilization of microbes can also control the stereochemistry of the product.[8)] Modification of substrates has been used for stereochemical control.[9-11)] Since the latter two methods are familiar techniques for organic chemists, we have investigated the application of these two techniques for the reduction of **2**. The present report will discuss the results obtained from the modification of a substrate.

Results

In the ^{1}H NMR spectrum of **2a** ($CDCl_3$-TMS), the aldehydic proton appears at δ 9.77 and the olefinic proton appears at δ 6.98, which indicates that **2a** exists as a mixture of the aldehyde and its enol form.

Various esters of 2-methyl-3-oxopropionic acid were reduced with BY in water in the presence of glucose. Results are summarized in Table 1. The reduction of **2a** (ethyl ester) was resulted in low enantioselectivity (e.e.=45%). The e.e. of the reduction was slightly improved in the reduction of octyl ester (**2c**, e.e.=60%). Better results were obtained, however, in the reduction of substrates with bulky alcohol moieties such as 3-methylbutyl (**2f**, e.e.=84%), cyclohexylethyl (**2p**, e.e.=74%), and 2-(trimethylsilyl)ethyl (**2n**, e.e.=79%) groups.

Table 1. Reduction of **2** by Bakers' Yeast

Substrate	R	$[\alpha]_D^{20}$	e.e./%	Isolated yield/%
2a	$-C_2H_5$	−8.459	45	94
2b	$-(CH_2)_3CH_3$	−4.167	53	73
2c	$-(CH_2)_7CH_3$	−6.103	60	41
2d	$-CH(CH_3)_2$	−8.736	19	49
2e	$-CH_2CH(CH_3)_2$	−7.195	64	84
2f	$-(CH_2)_2CH(CH_3)_2$	−8.000	84	66
2g	$-(CH_2)_3CH(CH_3)_2$	−8.889	81	83
2h	$-CH_2CH(CH_2CH_3)_2$	−8.210	71	76
2i	$-CH_2CH{=}CH_2$	−8.587	48	58
2j	$-(CH_2)_2OCH_2CH_3$	−4.789	29	73
2k	$-(CH_2)_2OCH_3$	−10.047	47	59
2l	$-CH_2C(CH_3)_3$	−13.471	90	78
2m	$-(CH_2)_2C(CH_3)_3$	−9.923	81	66
2n	$-(CH_2)_2Si(CH_3)_3$	−6.344	79	70
2o	$-CH_2$-*c*-C_6H_{11}	−7.778	74	70
2p	$-(CH_2)_2$-*c*-C_6H_{11}	− 6.799	74	71

The substrate having the 2,2-dimethylpropyl group **2l** gave the highest value (e.e.=90%).

3-Hydroxy-2-methylpropionates thus obtained by the reduction with BY were converted into the corresponding ethyl ester and the absolute configuration of this ethyl ester was determined by comparing the sign of optical rotation with that reported.[6]

Discussion

Recently two reports described the effect of length in alcohol moeity on yeast reduction. The reduction of 3-oxo-6-heptenoic acid and its esters afforded (*R*)-3-hydroxy-6-heptenoic acid and its esters, respectively. The e.e. increased as the length in the alcohol moiety decreased and the best result was observed with the free acid.[9] The reduction of 4-chloro-3-oxobutanoate gave the corresponding (*R*)-hydroxy ester of higher e.e. with the substrate which had the alcohol moiety of longer alkyl chain.[10] Since the configuration of (*R*)-4-chloro-3-hydroxybutanoic acid is opposite to that of (*R*)-3-hydroxy-6-heptenoic acid,[11] these two reports show that the length of alkyl chain in alcohol moiety exerts the same stereochemical effect on different substrate.

We reported that the length of alkyl chain in the alcohol moiety affects the diastereoselectivity of reduction of 2-methyl-3-oxobutanoate (**3**), an analog of **2**.[12] Thus although methyl, ethyl, and other short alkyl esters did not give satisfactory results, the octyl ester yielded (2*R*, 3*S*)-3-hydroxy-2-methylbutanoate in excellent diastereoselectivity. Since 2-substituted 3-oxobutanoates are known to afford the (3*S*)-hydroxy esters in higher e.e. than the corresponding unsubstituted substrates,[13] the high diastereoselectivity in the reduction of **3** directly means that the reduction is highly enantioselective with respect to the 2-position, or the reduction affords only one stereoisomer almost exclusively out of four possible isomers (2*R*,3*S*: 2*R*, 3*R*: 2*S*,3*R*: 2*S*,3*S*=95 : < 1 : < 1 : 5).

Since **2** and **3** have quite similar molecular structures (aldehyde vs. ketone) each other, it is reasonably expected that the BY reduction of the octyl or higher alkyl esters of **2** might exert satisfactory enantioselectivity.

However, contrary to the expectation, the reduction of **2c** (the octyl ester) did not give the hydroxy ester in a high e.e. Thus, although the e.e. increased in the order; ethyl (45%), butyl (53%), and octyl (60%), the increase in the selectivity was not remarkable. It should be noted that the reduction of **2** is catalyzed by a primary alcohol dehydrogenase(s), but the secondary alcohol dehydrogenase(s) must be involved for the reduction of **3**. The same phenomenon was also observed in the BY reduction of 5-methoxy-3-oxopentanoate where the e.e. of the product increases from the ethyl (60%) to the butyl (82%) ester, then decreases in the hexyl ester (71%).[14] The substrate with a long alkyl chain in the ester part did not give the highest value. These results suggests that the structural similarity in substrates does not necessarily result in similar results in multi-enzyme systems. It is unfortunate that complex structure of microbe does not allow to predict a simple relationship between the structure of a substrate and the stereoselectivity of the reduction.

Since an attempt to control the stereochemistry in yeast reduction by changing the length of alkyl chain in the ester group appeared unsatisfactory, we intended to look for another method which affected stereochemical course of the reduction. In order to change the electronic property in the alcohol moiety, an oxygen function was introduced and the substrate was subjected to the BY reduction. It was expected that the difference in affinities to enzyme(s) along with changing a hydrocarbon unit to an oxygen might affect the stereochemistry of the reduction. However, this modification was found to shift the specificity toward the *S*-configuration, if any. Thus the e.e. of products from the 2-ethoxyethyl derivative was 29% and that of the 2-methoxyethyl derivative was 47%. Both of these substrates are not suitable in order to produce the (2*R*)-hydroxy esters in high enantioselectivities.

In order to achieve the third way of our challenge to increase the e.e. of yeast reduction, we focused attention on the bulkiness of the alcohol moiety of the substrate. In contrast to the length, the bulkiness of the alcohol moiety was found to play an important factor to change the e.e. of the products. That is, the substitution of a methyl group at the 3-position of the butyl ester resulted in the hydroxy ester in 81% e.e. whereas the e.e. of the corresponding butyl ester was 53%. Encouraged by these observations, we prepared several substrates from bulky alcohols. Neither 3,3-dimethylbutyl ester (81% e.e.) nor 2-(trimethylsilyl)ethylester (79% e.e.) improved the stereoselectivity. In other words, bulkiness of isopropyl group is enough as a single substituent at the 2-position of the ester to shift

the e.e. to an asymptotic value of about 80%. However, on the other hand, double substitution at the 2-position increases the e.e. value further; the reduction of the 2,2-dimethylpropyl ester proceeded with 90% e.e.

The reason why the bulkiness at the 2-position of the ester shifts the stereospecificity of the reaction toward the *R*-configuration is not clear at present. One plausible mechanism is that more than two dehydrogenases are involved in the reduction and each of them reduces a substrate in high e.e. but in opposite configuration. When both (*R*)- and (*S*)-hydroxy ester-producing enzymes contribute to the reduction in comparable extents, the e.e. of the product becomes low. On the contrary, if a bulky ester is not reduced by the (*S*)-hydroxy ester-producing enzyme, only the (*R*)-hydroxy ester-producing enzyme opperates and consequently the (*R*)-hydroxy ester will be produced exclusively. Although several dehydrogenases contribute to the reduction in the BY reduction of ethyl 4-chloro-3-oxobutanoate, it was pointed out that the octyl ester is reduced only by the (*R*)-hydroxy ester-producing enzyme and thus produced (*R*)-hydroxy ester has high e.e. We believe that the same recognition is operating here in the reduction of 2,2-dimethylpropyl 2-methyl-3-oxopropionate as that seen in the reduction of octyl 4-chloro-3-oxobutanoate. To confirm our suggestion, further investigation is necessary.

Experimental

^{1}H NMR spectra were recorded on a JEOL FX-100 (100 MHz), a Varian VXR-200 (200 MHz), and a JEOL GX-400 (400 MHz) spectrometer in $CDCl_3$ with Me_4Si as an internal reference. Gas chromatography was recorded on a Yanaco G-2800 gas chromatograph. HPLC was performed with a Hitachi 655 Liquid Chromatography (pump) and IRICA 852-III spectrophotometer, using a Cosmosil 5SL (4.6×250) column. IR spectra were recorded on a Hitachi EPI-S2 infrared spectrometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter.

Organic reagents were purchased from Nakarai Chemical Co., Tokyo Kasei Co., and Aldrich Chemical Co. unless otherwise indicated. Solvents and commercially available starting materials were generally used without additional purification unless otherwise indicated. Pyridine and benzene were refluxed on calcium hydride for 1 day and distilled before the use. Satisfactory results were obtained from elemental analyses of all of the products.

Preparation of Esters of 2-Methyl-3-oxopropionate. The corresponding ethyl ester was prepared according to the reference.[6] The other esters were prepared by transesterification with the ethyl ester. Two methods were used.

Method A. Ethyl 2-methyl-3-oxopropionate (10 mmol) and alcohol (50 mmol) were heated for an appropriate time and distilled under reduced pressure.

Method B. Ethyl 2-methyl-3-oxopropionate (10 mmol) was added to 15 mmol of alcohol in 20 ml of benzene and the solution was refluxed for an appropriate time, then distilled under reduced pressure, affording the desired ester.

Ethyl 2-Methyl-3-oxopropionate (2a): bp 60 °C/20 mmHg (1 mmHg=133.322 Pa); ^{1}H NMR ($CDCl_3$-TMS) δ=1.29 (aldehyde form, a), 1.32 (enol form, e) (3H, t, J=3.4 Hz (a), 3.4 Hz (e)), 1.34 (a), 1.66 (e) (3H, d, J=7.2Hz (a), 1.6Hz (e)), 3.37 (a), 6.98 (e) (1H, qd, J=7.2 Hz, 1.5Hz (a), dq, J=12.5Hz, 1.5Hz (e)), 4.23 (2H, q, J=7.3Hz (a,e)), 9.77 (a), 11.31 (e) (1H, d, J=1.5Hz (a), 12.5Hz (e)); IR (neat), 1727, 1673 cm^{-1}.

Butyl 2-Methyl-3-oxopropionate (2b): Method A; bp 85 °C/26 mmHg; ^{1}H NMR ($CDCl_3$-TMS) δ=0.93 (a), 0.94 (e) (3H, t, J=7.3 Hz (a), 6.9 Hz (e)) 1.30—1.77 (4H, m), 1.34 (a), 1.66 (e) (3H, d, J=7.2 Hz (a), 1.2 Hz (e)), 3.38 (a), 6.98 (e) (1H, qd, J=7.2 Hz, 1.4 Hz (a), dq, J=12.4 Hz, 1.2 Hz (e)), 4.17 (2H, t, J=6.5 Hz (a,e)), 9.77 (a), 11.31 (e) (1H, d, J=1.4 Hz (a), 12.4 Hz (e)); IR (neat), 1730, 1673 cm^{-1}.

Octyl 2-Methyl-3-oxopropionate (2c): Method A; bp 135—140 °C/20 mmHg; ^{1}H NMR ($CDCl_3$-TMS) δ=0.86 (3H, b (a,e)), 1.12—1.40 (12H, m (a,e)), 1.34 (a), 1.66 (e) (3H, d, J=7.3 Hz (a), 1.2 Hz (e)), 3.38 (a), 6.98 (e) (1H, qd, J=7.3 Hz, 1.5 Hz (a), dq, J=12.5 Hz, 1.2 Hz (e)), 4.15 (2H, t, J=6.6 Hz (a,e)), 9.77 (a), 11.31 (e) (1H, d, J=1.5 Hz (a), 12.5 Hz (e)); IR (near), 1735, 1674 cm^{-1}.

1-Methylethyl 2-Methyl-3-oxopropionate (2d): Prepared from 1-methylethyl propionate and 1-methylethyl formate according to the reference:[6] bp 60 °C/20 mmHg; ^{1}H NMR ($CDCl_3$-TMS) δ=1.27 (a), 1.28 (e) (6H, dd, J=1.1 Hz, 6.2 Hz (a), d, J=6.4 Hz (e)), 1.33 (a), 1.65 (e) (3H, d, J=7.2 Hz (a), 1.2 Hz (e)), 3.34 (a), 6.97 (e) (1H, qd, J=7.2 Hz, 1.6 Hz (a), dq, J=12.4 Hz, 1.2 Hz (e)), 5.02—5.18 (1H, m (a,e)), 9.77 (a), 11.38 (e) (1H, d, J=1.6 Hz (a), 12.4 Hz (e)); IR (neat), 1735, 1675 cm^{-1}.

2-Methylpropyl 2-Methyl-3-oxopropionate (2e): Method A; bp 75 °C/27 mmHg; ^{1}H NMR ($CDCl_3$-TMS) δ=0.93 (a), 0.95 (e) (6H, d, J=6.7 Hz (a), 6.7 Hz (e)), 1.36 (a), 1.68 (e) (3H, d, J=7.2 Hz (a), 1.2Hz (e)), 1.80—2.00 (1H, m (a,e)), 3.39 (a), 6.98 (e) (1H, qd, J=7.2 Hz, 1.5 Hz (a), dq, J=12.5 Hz, 1.2 Hz (e)), 3.95 (2H, d, J=6.6 Hz (a,e)), 9.78 (a), 11.30 (e) (1H, d, J=1.5 Hz (a), 12.5 Hz (e)); IR (neat), 1732, 1677 cm^{-1}.

3-Methylbutyl 2-Methyl-3-oxopropionate (2f): Method A; bp 97—99 °C/20 mmHg; ^{1}H NMR ($CDCl_3$-TMS) δ=0.91 (a), 0.92 (e) (6H, d, J= 4.0 Hz (a), 4.9 Hz (e)), 1.12—1.78 (3H, m (a,e)), 1.36 (a), 1.68 (e) (3H, d, J=7.5 Hz (a), 1.0Hz (e)), 3.37 (a), 6.98 (e) (1H, qd, J=7.2 Hz, 1.5 Hz (a), dq, J=12.5 Hz, 1.0 Hz (e)), 4.05—4.33 (2H, m (a,e)), 9.78 (a), 11.31 (e) (1H, d, J=1.5 Hz (a), 12.5 Hz (e)); IR (neat), 1733, 1677 cm^{-1}.

4-Methylpentyl 2-Methyl-3-oxopropionate (2g): Method A; bp 119 °C/20 mmHg: ^{1}H NMR ($CDCl_3$-TMS) δ=0.88 (a), 0.89 (e) (6H, d, J=6.6 Hz (a), 6.6 Hz (e)), 1.15—1.77 (5H, m (a,e)), 1.34 (a), 1.67 (e) (3H, d, J=7.3 Hz (a), 1.1 Hz (e)), 3.38 (a), 6.98 (e) (1H, qd, J=7.3 Hz, 1.5 Hz (a), dq, J=12.5 Hz, 1.1 Hz (e)), 4.05—4.26 (2H, m (a,e)), 9.78 (a), 11.31 (e) (1H, d, J=1.5 Hz (a), 12.5 Hz (e)); IR (neat), 1728, 1672 cm^{-1}.

2-Ethylbutyl 2-Methyl-3-oxopropionate (2h): Method A; bp 115.5 °C/20 mmHg; ^{1}H NMR ($CDCl_3$-TMS) δ=0.85—0.94 (6H, m (a,e)), 1.12—1.58 (4H, m (a,e)) 1.36 (a), 1.67 (e) (3H, d, J=7.3 Hz (a), 1.3 Hz (e)) 3.37 (a), 6.98 (e) (1H, qd, J=7.2 Hz, 1.5 Hz (a), dq, J=12.4 Hz, 1.3 Hz (e)), 3.98—4.18 (1H, m (a,e)), 4.10 (2H, q, J=5.6 Hz (a,e)), 9.77 (a), 11.31(e) (1H, d, J=1.5 Hz (a), 12.4 Hz (e)); IR (neat), 1735, 1677 cm^{-1}.

Allyl 2-Methyl-3-oxopropionate (2i): Method A; bp 70 °C/15 mmHg; ^{1}H NMR ($CDCl_3$-TMS) δ=1.36 (a), 1.69 (e) (3H, d, J=7.2 Hz (a), 1.2 Hz (e)), 3.42 (a), 7.00 (e) (1H, qd, J=7.2 Hz, 1.4 Hz (a), dq, J=11.4 Hz, 1.2 Hz (e)), 4.56—4.70 (2H, m (a,e)), 5.26—5.39 (2H, m (a,e)), 5.83—6.05 (1H, m (a,e)), 9.78 (a), 11.22 (e) (1H, d, J=1.4 Hz (a), 11.4 Hz (e)); IR

(neat), 1727, 1674 cm^{-1}.

2-Ethoxyethyl 2-Methyl-3-oxopropionate (2j): Method A; bp 107—109 °C/15 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=1.15—1.24 (3H, m (a,e)), 1.35 (a), 1.69 (e) (3H, d, J=7.3 Hz (a), 1.0 Hz (e)), 3.43—3.73 (m, (a,e)), 6.99 (dq, J=12.6, 1.0 (e)) (5H), 4.24—4.41 (2H, m (a,e)), 9.79 (a), 11.18 (e) (1H, d, J=1.4 Hz (a), 12.6 Hz (e)); IR (neat), 1743, 1675 cm^{-1}.

2-Methoxyethyl 2-Methyl-3-oxopropionate (2k): Method A; bp 134 °C/20 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=1.34 (a), 1.68 (e) (3H, d, J=7.3 Hz (a), 1.0 Hz (e)), 3.36 (3H, d, J=1.5 Hz (a,e)), 3.52—3.76 (m (a,e)), 6.92 (dq, J=12.5, 1.0 (e)) (3H), 4.27—4.36 (2H, m (a,e)), 9.74 (a), 11.18 (e) (1H, d, J=1.5 Hz (a), 12.5 Hz (e)); IR (neat), 1729, 1673 cm^{-1}.

2,2-Dimethylpropyl 2-Methyl-3-oxopropionate (2l): Method A; bp 90 °C/20 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.95 (9H, d, J=4.1 Hz (a,e)), 1.36 (a), 1.70 (e) (3H, d, J=7.2 Hz (a), 1.2 Hz (e)), 3.42 (a), 7.05 (e) (1H, qd, J=7.2 Hz, 1.5 Hz (a), dq, J=12.6 Hz, 1.2 Hz (e)), 3.77—3.96 (2H, m (a,e)), 9.81 (a), 11.29 (e) (1H, d, J=1.5 Hz (a), 12.6 Hz (e)); IR (neat), 1732, 1678 cm^{-1}.

3,3-Dimethylbutyl 2-Methyl-3-oxopropionate (2m): Method B; Kugelrohr distillation; bp 75 °C/20 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.97 (9H, s (a,e)), 1.14—1.18 (2H, m (a,e)) 1.35 (a), 1.67 (e) (3H, d, J=7.3 Hz (a), 1.5 Hz (e)), 3.38 (a), 6.99 (e) (1H, qd, J=7.3 Hz, 1.5 Hz (a), dq, J=12.2 Hz, 1.5 Hz (e)), 4.25 (2H, t, J=7.3 Hz (a,e)), 9.78 (a), 11.33 (e) (1H, d, J=1.5 Hz (a), 12.2 Hz (e)); IR (neat), 1745, 1677 cm^{-1}.

2-(Trimethylsilyl)ethyl 2-Methyl-3-oxopropionate (2n): Method A; bp 113.5—114 °C/20 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.06 (9H, d, J=1.0 Hz (a,e)), 0.95—1.14 (2H, m (a,e)), 1.35 (a), 1.67 (e) (3H, d, J=7.3 Hz (a), 1.5 Hz (e)), 3.37 (a), 6.99 (e) (1H, qd, J=7.3 Hz, 1.5 Hz (a), dq, J=12.2 Hz, 1.5 Hz (e)), 4.29 (2H, m (a,e)), 9.78 (a), 11.37 (e) (1H, d, J=1.5 Hz (a), 12.2 Hz (e)); IR (neat) 1728, 1673 cm^{-1}.

Cyclohexylmethyl 2-Methyl-3-oxopropionate (2o): Method B; bp 139 °C/20 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.90—1.78 (11H, m (a,e)), 1.36 (a), 1.69 (e) (3H, d, J=7.3 Hz (a), 1.0 Hz (e)), 3.40 (a), 6.99 (e) (1H, qd, J=7.3 Hz, 1.5 Hz (a), dq, J=12.7 Hz, 1.0 Hz (e)), 3.99 (2H, d, J=6.3 Hz (a,e)), 9.80 (a), 11.31 (e) (1H, d, J=1.5 Hz (a), 12.7 Hz (e)); IR (neat), 1727, 1675 cm^{-1}.

2-Cyclohexylethyl 2-Methyl-3-oxopropionate (2p): Method B; bp 150 °C/20 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.97—1.77 (13H, m (a,e)), 1.36 (a), 1.68 (e) (3H, d, J=7.3 Hz (a), 1.0 Hz (e)), 3.39 (a), 6.99 (e) (1H, qd, J=7.2 Hz, 1.5Hz (a), dq, J=12.7 Hz, 1.0 Hz (e)), 4.22 (2H, t, J=6.6 Hz (a,e)), 9.79 (a), 11.33 (e) (1H, d, J=1.5 Hz (a), 12.7 Hz (e)); IR (neat) 1730, 1675 cm^{-1}.

Preparation of Racemic 3-Hydroxy-2-methylpropionate. Sodium borohydride (9.5 mg, 0.25 mmol) was added to a stirred and cooled solution of one mmol of 2-methyl-3-oxopropionate and 10 ml of ethanol in an ice bath. The resulted solution was stirred at room temperature for 1 h and the ethanol was removed under reduced pressure, then the residual solution was acidified with dilute hydrochloric acid. The organic portion was extracted with ether (30 ml), and the ether layer was washed with water, aqueous sodium hydrogencarbonate, and water (10 ml each) respectively. After dried over anhydrous sodium sulfate, the solvent was evaporated from the ether solution. Esters of 3-hydroxy-2-methylpropionate were obtained by Kugelrohr distillation.

Ethyl 2-Methyl-3-hydroxypropionate (1a): Kugelrohr distillation; bp 90 °C/21 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=1.26 (3H, t, J=7.1 Hz), 1.17 (3H, d, J=7.4 Hz), 2.41 (1H, m), 2.64 (1H, m), 4.15 (2H, q, J=7.1 Hz); IR (neat), 1739, 3470 cm^{-1}.

Butyl 2-Methyl-3-hydroxypropionate (1b): Kugelrohr distillation; bp 120 °C/21 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.93 (3H, t, J=7.2 Hz), 1.17 (3H, d, J=7.3 Hz), 1.29—1.70 (4H, m), 2.28—2.34 (1H, m), 2.57—2.74 (1H, m), 3.67—3.74 (2H, m), 4.11 (2H, t, J=6.6 Hz); IR (neat), 1737, 3480 cm^{-1}.

Octyl 2-Methyl-3-hydroxypropionate (1c): Kugelrohr distillation; bp 150 °C/21 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.88 (3H, t, J=5.9 Hz), 1.18 (3H, d, J=6.8 Hz), 1.28 (12H, m), 2.34 (1H, m), 2.70 (1H, m), 3.71 (2H, d, J=6.1 Hz), 4.11 (2H, t, J=6.8 Hz); IR (neat), 1738, 3460 cm^{-1}.

1-Methylethyl 2-Methyl-3-hydroxypropionate (1d): Kugelrohr distillation: bp 95 °C/24 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=1.24 (6H, m), 1.15 (3H, d, J=7.3 Hz), 2.37 (1H, m), 2.52—2.68 (1H, m), 3.64—3.72 (2H, m), 4.94—5.12 (1H, m); IR (neat), 1733, 3490 cm^{-1}.

2-Methylpropyl 2-Methyl-3-hydroxypropionate (1e): Kugelrohr distillation; bp 120 °C/25 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.93 (6H, d, J=6.7 Hz), 1.185 (3H, d, J=7.3 Hz), 1.65—2.05 (1H, m), 2.34 (1H, t, J=5.7 Hz), 2.58—2.75 (1H, m), 3.70 (2H, t, J=5.1 Hz), 3.89 (2H, m); IR (neat), 1737, 3480 cm^{-1}.

3-Methylbutyl 2-Methyl-3-hydroxypropionate (1f): Kugelrohr distillation; bp 130 °C/27 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.92 (6H, d, J=6.4 Hz), 1.17 (3H, d, J=7.3 Hz), 1.48—1.75 (3H, m), 2.28 (1H, m), 2.58—2.74 (1H, m), 3.65—3.77 (2H, m), 4.14 (2H, t, J=6.8 Hz); IR (neat), 1738, 3480 cm^{-1}.

4-Methylpentyl 2-Methyl-3-hydroxypropionate (1g): Kugelrohr distillation; bp 140 °C/27 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.88 (6H, d, J=6.6 Hz), 1.17 (3H, d, J=7.3 Hz), 1.22—1.28 (1H, m), 1.46—1.71 (4H, m), 2.31 (1H, m), 2.57—2.74 (1H, m), 4.09 (2H, t, J=6.8 Hz); IR (neat), 1738, 3490 cm^{-1}.

2-Ethylbutyl 2-Methyl-3-hydroxypropionate (1h): Kugelrohr distillation; bp 125 °C/22 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.89 (6H, t, J=7.6 Hz), 1.18 (3H, d, J=7.3 Hz), 1.28—1.43 (4H, m), 1.47—1.59 (1H, m), 2.32 (1H, m), 2.58—3.76 (1H, m), 3.68 (2H, m), 4.02—4.06 (2H, m); IR (neat), 1739, 3490 cm^{-1}.

Allyl 2-Methyl-3-hydroxypropionate (1i): Kugelrohr distillation; bp 105 °C/23 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=1.19 (3H, d, J=7.2 Hz), 2.27 (1H, m), 2.60—2.80 (1H, m), 3.70 (2H, m), 4.60 (2H, m), 5.20—5.40 (2H, m), 5.80—6.00 (1H, m); IR (neat), 1738, 3470 cm^{-1}.

2-Ethoxyethyl 2-Methyl-3-hydroxypropionate (1j): Kugelrohr distillation; bp 140 °C/23 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=1.18 (3H, d, J=7.3 Hz), 1.20 (3H, d, J=7.0 Hz), 2.35—2.55 (1H, m), 2.55—2.88 (1H, m), 3.53 (2H, q, J=7.0 Hz), 3.61—3.74 (4H, m), 4.25—4.33 (2H, m); IR (neat), 1738, 3480 cm^{-1}.

2-Methoxyethyl 2-Methyl-3-hydroxypropionate (1k): Kugelrohr distillation; bp 130 °C/26 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=1.58 (3H, d, J=7.2 Hz), 2.44 (1H, m), 3.38 (3H, s), 3.60 (2H, t, J=4.7 Hz), 3.71 (2H, m), 4.24—4.35 (2H, m); IR (neat), 1740, 3480 cm^{-1}.

2,2-Dimethylpropyl 2-Methyl-3-hydroxypropionate (1l): Kugelrohr distillation; bp 120 °C/25 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.95 (9H, d, J=4.1 Hz), 1.21 (3H, d, J=7.3 Hz), 2.36 (1H, m), 2.60—2.80 (1H, m), 3.73 (2H, m), 3.82 (2H,

d, J=1.5 Hz); IR (neat), 1737, 3490 cm^{-1}.

3,3-Dimethylbutyl 2-Methyl-3-hydroxypropionate (1m): Kugelrohr distillation; bp 130 °C/23 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.95 (9H, s), 1.17 (3H, d, J=7.3 Hz), 1.57 (2H, t, J=7.3 Hz), 2.33 (1H, m), 2.55—2.75 (1H, m), 3.71 (2H, m), 4.18 (2H, t, J=7.3 Hz); IR (neat) 1740, 3490 cm^{-1}.

2-(Trimethylsilyl)ethyl 2-Methyl-3-hydroxypropionate (1n): Kugelrohr distillation; bp 135 °C/24 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.05 (9H, s), 1.01 (2H, m), 1.17 (3H, d, J=7.3 Hz), 2.35 (1H, m), 2.54—2.74 (1H, m), 3.71 (2H, d, J=5.9Hz), 4.21 (2H, m); IR (neat), 1738, 3500 cm^{-1}.

Cyclohexylmethyl 2-Methyl-3-hydroxypropionate (1o): Kugelrohr distillation; bp 145 °C/22 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.70—1.90 (11H, m), 1.18 (3H, d, J=7.3 Hz), 2.40 (1H, t, J=6.1Hz), 2.58—2.77 (1H, m), 3.71 (2H, dd, J=5.4Hz, 6.4Hz), 3.93 (2H, d, J=6.4 Hz); IR (neat), 1739, 3480 cm^{-1}.

2-Cyclohexylethyl 2-Methyl-3-hydroxypropionate (1p): Kugelrohr distillation; bp 160 °C/23 mmHg; ^1H NMR ($CDCl_3$-TMS) δ=0.62—1.82 (13H, m), 1.18 (3H, d, J=7.3 Hz), 2.38 (1H, t, J=5.9 Hz), 2.56—2.76 (1H, m), 3.71 (2H, t, J=5.9 Hz), 4.15 (2H, t, J=6.8 Hz); IR (neat), 1736, 3480 cm^{-1}.

Reduction of 2 with Bakers' Yeast. One millimole of **2** was added to a suspension of 5 g of BY in 15 ml of water and the mixture was stirred at room temperature until no starting material was detected on gas chromatography (PEG, 1 m, 80 °C). Then Hiflo-Super-Cell (10 g) and ethyl acetate (30 ml) was added to the mixture and the resulted suspension was filtered. The organic portion was concentrated under reduced pressure and the residue was subjected to column chromatography (silica gel, eluent; hexane-ethyl acetate=9 : 1) to give the corresponding alcohol. The NMR and IR spectra were the same with the corresponding hydroxy ester obtained by sodium borohydride reduction. The optical rotational values, e.e.s, and chemical yields of each hydroxy ester are listed in the Table.

Reduction of 2a with Immobilized Bakers' Yeast. One millimole of **2a** in 50 ml of water was reacted for two days with immobilized BY prepared from 10 g of polyurethane prepolymer, 10 g of BY, and 10 g of water.[8] After filtration and extraction from the aqueous phase with ethyl acetate, **1a** was obtained in 45% chemical yield with 55% e.e. The e.e. was slightly improved from the reduction of **2a** by free BY (45% e.e.).

Determination of e.e. of the Reduced Product. The reduced product (**1a—p**) was converted into the corresponding (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) ester by the reaction of **1** and MTPA-chloride in the presence of pyridine in benzene. The e.e. was determined by HPLC analysis of the MTPA ester.

Determination of Absolute Configuration. The absolute configuration of the reduction product was determined by converting **1** into the corresponding ethyl ester and comparing its rotational value with that reported.

A mixture of 1 mmol of **11**, ethanol (10 ml) and catalytic amount of *p*-toluenesulfonic acid was heated at reflux temperature for 4 days. Ethanol was distilled off under reduced pressure and the residue was subjected to column chromatography (silica gel, eluent; hexane-ethyl acetate=9 : 1), giving ethyl 3-hydroxy-2-methylpropionate. The hydroxy ester obtained from the yeast reduction of 2,2-dimethylpropyl 2-methyl-3-oxopropionate (**11**) afforded the corresponding ethyl ester in 56% yield; $[\alpha]_D^{20}$ −20.26 (*c* 3.41, MeOH). Since the optical rotational value of (*R*)-ethyl 3-hydroxy-2-methylpropionate is −21.6, the absolute configuration of **11** was determined to be *R*.

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