# Stereochemical Control on Yeast Reduction of α-Keto Esters. Reduction by Immobilized Bakers' Yeast in Hexane[1]

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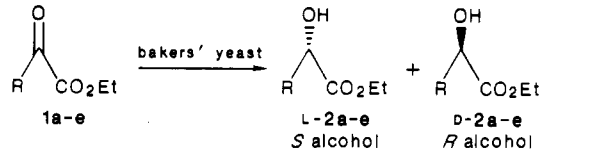
Ethyl 2-oxoheptanoate has been reduced by three methods: free bakers' yeast (FBY) in water, immobilized bakers' yeast (IMBY) in water, and IMBY in hexane. It has been found that the stereochemistry of reduction of α-keto esters by bakers' yeast is controlled by appropriate choice of reaction conditions.

Chiral α-hydroxy esters have been used widely as chiral building blocks because of their ease of transformation into other functional groups. For example, chiral α-hydroxy esters have been converted into chiral halo esters,[2] chiral glycols,[3] chiral epoxides,[4] and chiral amino acids.[5] In this paper, we report a new method for preparing chiral α-hydroxy esters by bakers' yeast. Bakers' yeast is a potential candidate in preparing chiral alcohols[6,7] because it is a cheap and easily obtainable *reagent*, and the reduction by bakers's yeast is very efficient in many cases. For synthetic purposes, chiral alcohols of desired configurations with high enantiomer excesses (ee) are required. However, while some ketones on reduction with bakers' yeast give alcohols of high optical purity, there are many others that afford alcohols with low ee. So, a development of methodology for stereochemical control in the yeast reduction is required. The stereochemical control has been achieved by several methods. The first is the modification of substituent(s) in ketones.[8-10] Change in the size of ester group in a β-keto ester is also effective in enhancing optical purity of the reduction products.[11-13] The second category of stereochemical control is to subject the yeast to stress by changing the reaction conditions. The change in the conditions will affect the metabolic pathway and will result in the change in ee of the alcohol. Two reports from our laboratory have contributed to this category.[14,15] If the low ee stems from the existence of plural dehydrogenases in the yeast that participate in the reduction and each dehydrogenase gives a chiral alcohol of a specific configuration in a high ee, then the inhibition of a particular enzyme(s) might be effective to the stereochemical control. We found that allyl alcohol has an effect of this kind in the reduction of β-keto esters.[14] Recently, we reported that immobilization of yeast can cause a change in stereochemical consequence of the reduction of β-keto esters by bakers's yeast and favor the formation of alcohols of the D configuration.[15,16] We believe that the stereochemical control by immobilization is due to the effect of change in the reaction conditions at the surface of the yeast or a change in the concentrations of substrates. If the expectation is correct, a drastic change of the reaction conditions may have an appreciable effect on the stereochemistry of products. Based on this idea, we investigated the reduction of α-keto esters in an organic solvent.

Since yeast is a living material in an aqueous solution, every enzyme and coenzyme is recycled by complex sequences of metabolizing reaction pathways. Therefore, the reduction of a substrate with yeast in an aqueous medium can proceed appreciably with a limited (though not "catalytic") amount of yeast. On the other hand, yeast cannot survive in an organic solvent, and its metabolic pathways no longer operate in an organic solvent. Consequently, NADPH, which is the necessary coenzyme for the reduction of α-keto esters, is not regenerated automatically in yeast in an organic solvent and the amount of a substrate that can be reduced is quite limited to the

**Table I. Reduction of α-Keto Esters by Bakers' Yeast**




1a-e L-2a-e S alcohol D-2a-e R alcohol

R = $CH_3(CH_2)_n$: a, n = 0; b, n = 1; c, n = 2; d, n = 3; e, n = 4

| substr | ee, %[a]/chemical yield, %[b]/confign[c] FBY in water[d] | IMBY in water[d] | IMBY in hexane[d] |
|---|---|---|---|
| 1a | 91/47/S | 87/43/S | 94/33/S |
| 1b | 75/42/S | 66/42/S | 36/28/S |
| 1c | 31/36/S | 39/36/S | 32/27/S |
| 1d | 50/29/S | 78/20/S | 47/41/R |
| 1e | 30/23/S | 63/31/S | 54/36/R |

[a] Enantiomeric ratios were determined by GLC analyses (OV-1701, 25 m, 180–205 °C) of their (+)-α-methoxy-α-(trifluoromethyl)phenylacetates (MTPA ester). [b] Isolated yield. Satisfactory analytical data (±0.4% for C, H, N) were obtained for all products listed in the table. [c] The absolute configuration was determined as described in the Experimental Section. [d] Reaction conditions are described in the Experimental Section.

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level of NADPH which exists in the yeast subjected to the reduction in an organic solvent. The use of organic solvent may also cause a serious damage of the cell membrane, resulting in a scattering of the contents of the cell toward the outside. However, immobilization of a biocatalyst has been known to enhance the stability of the catalyst against denaturation by organic solvents.[17] In order to test the effect of immobilization, we investigated the reduction of α-keto esters by bakers' yeast in aqueous and organic solvents.

## Results

Five α-keto esters (**1a–e**) were reduced by the following three methods: that is, free bakers' yeast (FBY) in water, immobilized bakers' yeast (IMBY) in water, and IMBY in hexane. Results are summarized in Table I.

**Immobilization of Bakers' Yeast.** IMBY which was used in the present reduction was made from polyurethane prepolymer (PU). Gels of polysaccharides such as calcium alginate[18] or carrageenan[19] have been used usually to immobilize microbes by entrapment. However, these polysaccharides were not suitable for the reaction in hexane, because a certain amount of water was oozed from gels during the reduction. Therefore, we chose PU as an immobilizing material. Bakers' yeast was immobilized by mixing it with PU, and the resultant polymer was cut into small pieces and rinsed with water to give IMBY, which was wiped with a dry cloth to reduce the volume of water in IMBY.

**Determination of the Absolute Configuration of the Product.** The absolute configuration of the product was determined by comparing the sign of optical rotation of the product with that of the authentic compound or by comparing the retention time on gas chromatography of the MTPA derivative[20] with that of the authentic compound. Detailed descriptions can be seen in the Experimental Section.

**Reduction of Ethyl 2-Oxoalkanoate by FBY–Water.** Five ethyl 2-oxo-*n*-alkanoates ($RCOCO_2Et$) were reduced by FBY in water. One millimole of a substrate was reduced with 2 g of bakers' yeast and 2.5 g of glucose in 75 mL of water. With this method, *S* alcohols were obtained in all cases. The enantiomer excess (ee) of the product alcohol decreased with the increase in the length of alkyl group in the acid part of the substrate. Ethyl pyruvate (**1a**) was reduced to ethyl lactate (**2a**) in 91% ee, whereas ethyl 2-oxoheptanoate (**1e**) gave the corresponding alcohol in only 30% ee. Since bakers' yeast has some saccharides in its cell, it can produce NADPH along the pentose phosphate pathway and has reducing power without added glucose. Since it is expected that the activity of the pentose phosphate pathway or the lebel of NADPH affects the stereochemistry of reduction, the effect of glucose concentration was also investigated. The results will be discussed later in connection with the mechanism.

**Reduction of Ethyl 2-Oxoalkanoate by IMBY–Water.** One millimole of a substrate was reduced in water with IMBY containing 5 g of bakers' yeast. Glucose (2.5 g) was added and the substrate concentration was kept the same as that described for FBY. Although the *S* alcohol was formed in all the reductions, a trend in ee changed here from that in FBY–water system. That is, the difference in ee among the substrates studied leveled off and the ee for the substrates with a short alkyl chain decreased while those for the substrates with a long alkyl chain increased.

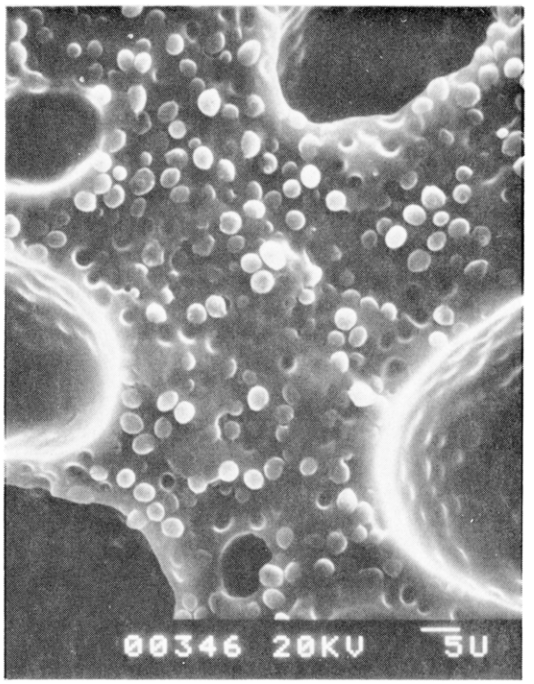


**Figure 1.** Scanning electron micrograph of immobilized bakers' yeast by polyurethane (×1000).

**Reduction of Ethyl 2-Oxoalkanoate by IMBY–Hexane.** The reaction conditions employed for this system were the same as described for the IMBY–water system except for the fact that the solvent was changed from water to hexane. The reaction required a longer period (24–72 h) than the other methods (5 h for FBY–water and 6 h for IMBY–water). In the case of the reduction of **1a**, the ee of the alcohol (94%; $S/R$ = 32/1) increased slightly from that from the FBY–water (91%; $S/R$ = 21/1) and the IMBY–water (87%; $S/R$ = 14/1). In the reduction of **1b**, although the *S* alcohol was obtained, the ee decreased to 36%. The reduction of **1c** showed no difference in ee from those from the FBY–water and the IMBY–water. On the contrary, the *R* alcohols were obtained from the reductions of **1d** and **1e**. Thus, the use of hexane as solvent exerted a large effect in shifting the configuration of the product toward *R*.

## Discussion

Several factors can be proposed as candidates to explain the shift of configuration depending on the reaction conditions. First of all is the effect of immobilization of yeast. Since yeast cells are surrounded tightly by polymers on immobilization as evidenced by scanning electron microsope photography (Figure 1), it is reasonable to expect that the cell membrane of the yeast is chemically influenced by the immobilizing polymer which is in contact with the cell within the resolution of the electron microscope. As a consequence, the concentration and/or the rate of uptake of substrate though the cell will differ from those of the FBY. In addition, if the dehydrogenase involved in the reaction is one of the membrane enzymes, the activity would be affected by immobilization. In fact, it has been reported that immobilization of yeast by PU changes the stereochemical consequence in the reduction of β-keto esters.[15] For example, ethyl 4-chloro-3-oxobutanoate was reduced to the corresponding L-hydroxy ester by FBY[16] while the D enantiomer was obtained by IMBY. Since the

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**Table II. Effect of Glucose Concentration on the ee of Product of the Reduction of 1d with FBY in Water[a]**

| glucose, g/L | chemical yield, % | ee, % |
|---|---|---|
| 0 | 25 | >97 |
| 33 | 29 | 51 |
| 67 | 18 | 37 |
| 133 | 24 | 20 |
| 200 | 28 | 15 |

[a] Reaction conditions: [1d] = 13 mM, [BY] = 26 g/L, reaction time = 6 h. *S* alcohol was obtained in every case.

configuration of the D hydroxy ester corresponds to the *R* configuration in α-hydroxy esters, immobilization will shift the ee of the reduction product toward *R* if the same effect is to be expected in the present reduction. Indeed, this was the case for **1a** and **1b** where the IMBY–water system gave more *R* hydroxy esters relative to the FBY system (vide supra). That is, the ee of the *S* hydroxy ester decreased. However, this phenomenon is not seen in the reduction of **1d** and **1e**, where the ee of the *S* hydroxy ester increases when the FBY system is substituted by the IMBY–water system. This has been elucidated to stem from enantioselective decompositions of the product, which will be described in a later section.

The second factor is the effect of glucose concentration on the reduction. Although the actual reducing agent in the present system is NADPH, the amount of NADPH in the yeast cell is limited to a quite low level. In order to undergo the reduction continuously, therefore, it is necessary to activate another biological pathway to reduce $NADP^+$ into NADPH. Yeast has some saccharides in the cell and $NADP^+$ is reduced to NADPH by the pentose phosphate pathway which originates from glucose-6-phosphate. Therefore, yeast has a certain reducing power in its cell. The addition of glucose to the reaction mixture will activate the pentose phosphate pathway and, as a consequence, the concentration of NADPH will increase and then will change the ee of the product hydroxy ester because it is expected that *R* hydroxy ester producing enzymes and their *S* counterparts differ in Michaelis constant and $V_{max}$ with respect to NADPH. In fact, it has been found that the glucose concentration exerts a large effect on ee. Table II shows the effect of glucose concentration on the reduction of **1e**. The reduction without glucose gave the *S* hydroxy ester with a high ee, whereas the ee decreased to 20% (*S*) with 200 g/L of glucose. Since glucose is insoluble to hexane in the IMBY–hexane system, the glucose might exist in the immobilizing layer. A simple calculation with the amount of glucose added and the volume of water in the immobilizing layer elucidates the glucose concentration in the IMBY–hexane system to be 167 g/L. This concentration is the condition which affords the *S* hydroxy ester with 20% ee based on the results listed in Table II. The value is nearly identical with the observed one.

The third factor which affects the stereochemistry of the reduction is enantioselective decomposition of the produced 2-hydroxy esters catalyzed by bakers' yeast. In the reduction of **1d** and **1e** by FBY, the prolonged reaction time gave the *S* hydroxy ester in higher ee although the chemical yield was reduced. Apparently, an enantioselective decomposition (probably by hydrolysis) of the *R* hydroxy ester occurred, leaving the *S* hydroxy ester unhydrolyzed. The hydrolysis also proceeded in the IMBY–water system, which was not the case in the IMBY–hexane system because the hydrolysis was inhibited in the nonaqueous solvent. Since the IMBY contains considerable amounts of water, the protection from the hydrolysis in hexane as a bulk solvent seems to suggest that a particular

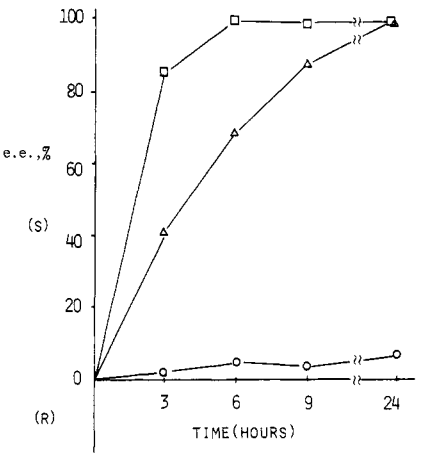


**Figure 2.** Enantiomeric excess (ee) of recovered α-hydroxy esters from the reaction of racemic α-hydroxy esters with bakers' yeast: (○) **2a**; (△) **2c**; (□) **2e**.

(hydrolytic) enzyme (or enzymes) is inhibited in this system.

One may expect, based on the above discussion, that the chemical yield of the product from the IMBY–hexane system should be much higher than those from the other systems because the product is not decomposed under the reaction conditions. However, the yield from the IMBY–hexane system is not higher than those from the other methods. This is due to the unstability of the starting material. α-Keto esters are not so stable under the reduction conditions and the reaction with the IMBY–hexane system requires longer time so that the starting material decomposes competitively during the reaction and the yield of α-hydroxy esters does not increase.

The present discussion inevitably predicts that the *R* hydroxy ester is the preferred product over its *S* enantiomer in the reduction of **1d** and **1e** with the IMBY–hexane system. The value of ee itself, however, may be shifted to some extent from that of the FBY system due to the immobilization effect. Although the *true* ee of the reduction process in the FBY system without enantiomeric decomposition of the product is not clear, it is evident that the reduction itself gives more *R* hydroxy ester than the amount indicated in Table I.

In fact, the hydrolysis of racemic **2e** with the FBY system gave the *S* hydroxy ester in a high ee with 40% chemical yield, whereas the same reaction with the IMBY–hexane system did not give the *S* hydroxy ester in a reasonable ee; almost racemic hydroxy ester was recovered in a good yield. Apparently, the enantioselective decomposition was inhibited in the latter case. Figure 2 shows the dependence of ee on the reaction period for the hydrolyses of racemic **2a**, **2c**, and **2e** with the FBY. The hydrolysis of racemic α-hydroxy esters has been studied in detail and it has been found that the reaction is composed of more complicated processes than a simple hydrolysis. The details will be reported elsewhere. On the contrary, the reduction of **1a** in the IMBY–hexane system did not result in an increase in the production of the *R* hydroxy ester. The difference in the result between **1a** and **1e** is explainable with the idea of lack of enzymes that decompose **2a** enantiospecifically. That is, in contrast to other hydroxy esters, the hydrolysis of racemic **2a** with the FBY system did not give the chiral hydroxy ester. The decrease in the ee from the IMBY–water system (87%; $S/R = 14/1$) compared to that of the FBY system (91%; $S/R = 21/1$) is due to the effect of immobilization. The reduction shifts the specificity to give more *R* hydroxy ester than the FBY system does as expected from the results

with β-keto esters (vide infra).[15] It is interesting to note that an organic solvent, hexane, shifts the configuration back to the *S* direction (94% ee; $S/R = 32/1$).[21] It is expected that the substrate concentration around the bakers' yeast in the IMBY–hexane system is lower than that in the IMBY–water system because of the difficulty of permeation of substrate into the aqueous immobilizing layer from the hexane solution.

The reduction of **1c** in the IMBY–hexane system gives the *S* hydroxy ester with an ee which is not so much different from those from the FBY (31% ee; $S/R = 1.9/1$) and the IMBY–water (39% ee; $S/R = 2.3/1$) system. The hydrolysis of a racemic mixture of **2c** with the FBY system revealed that **2c** is decomposed enantiospecifically but the rate of decomposition is much slower than that with **2e**. This fact means that the reduction of **1c** with bakers' yeast affords the *S* hydroxy ester in about 30% ee and then the hydroxy ester produced by the reduction is decomposed enantiospecifically. However, the hydrolysis proceeds so slowly that it does not affect the stereochemical consequence significantly.

**Conclusion.** The IMBY–hexane system seems to be a useful method for a stereochemical control in yeast reduction. The method is effective for the protection of the product from the hydrolytic decomposition and for the synthesis of the *R* enantiomer of long-chain α-hydroxy esters. Although the ee from the IMBY–hexane system is not enough for synthetic purposes at present, it has been elucidated that the yeast reduction is surely controlled to give the *R* enantiomer of the α-hydroxy ester.

## Experimental Section

$^1$H NMR spectra were recorded on a JEOL FX-100 (100 MHz) spectrometer in $CDCl_3$ with $Me_4Si$ as internal reference. For analytical purposes, a Yanaco G-2800 (capillary column) or a G-1800 gas chromatograph was used, whereas for preparative separation, a Varian Aerograph Model 920 gas chromatograph was employed. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Electron microscopic observation was done by a Hitachi S-450 scanning electron microscope.

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 elemental analyses of all the products were obtained.

**Preparation of Ethyl 2-Oxoheptanoate (1e).** A modification of Kagan's method[22] was adopted for the synthesis. To 95 mL of absolute ethanol in a 500-mL round-bottomed flask fitted with a magnetic stirrer was added thinly sliced sodium metal (6.35 g, 0.28 mol) at 0 °C piece by piece, and the resulting mixture was stirred until the sodium was dissolved at room temperature. To the alcoholic solution was added a mixture of diethyl oxalate (165 g, 1.1 mol) and ethyl hexanoate (39.75 g, 0.25 mol) quickly. Then the ethanol ($bp_{100}$ 60 °C) and diethyl oxalate were removed under reduced pressure ($bp_{0.4}$ 58–60 °C). Acetic acid (16.5 mL) and water (20 mL) were added to the solid residue. The solution was extracted with ether (2 × 150 mL) and the organic layer was washed with water (100 mL), saturated aqueous sodium hydrogen carbonate (50 mL), and water (2 × 50 mL), successively. The solvent was evaporated under reduced pressure. In a 300-mL round-bottomed flask equipped with a magnetic stirrer and a reflux condenser were placed this residual oil, water (150 mL), and concentrated hydrochloric acid (75 mL). The solution was refluxed for 6 h. The mixture was cooled to room temperature and was separated to organic and the aqueous layers. The aqueous layer was extracted with ether (2 × 150 mL) and the solvent was removed under reduced pressure. The combined organic portion was distilled, giving 2-oxoheptanoic acid (17.17 g, 48%); $bp_{0.4}$ 65–67 °C (lit.[22] $bp_{18}$ 111–113 °C).

In a 30-mL round-bottomed flask equipped with a magnetic stirrer and a reflux condenser were added 2-oxoheptanoic acid (17.17 g, 0.12 mol), *p*-toluenesulfonic acid (0.07 g, cat.), absolute ethanol (9 mL), and toluene (2.4 mL). Then the mixture was refluxed for 6 h. After the addition of triethanolamine (0.06 ml), excess ether and toluene were removed under reduced pressure. Then the residual oil was distilled, giving ethyl 2-oxoheptanoate (16.07 g, 78%): $bp_{0.4}$ 58 °C (lit.[22] $bp_{11}$ 97–102 °C); $^1$H NMR ($CDCl_3$–TMS) δ 0.71–1.83 (m, 12 H, $C_4H_9$, $CH_3$), 2.64–3.05 (m, 2 H, $CH_2CO$), and 4.31 (q, $J$ = 7.3 Hz, 2 H, $OCH_2$); IR (neat) 1745 (s, C=O) and 1760 (s, C=O) cm$^{-1}$.

**Ethyl 2-oxohexanoate (1d)** was prepared from ethyl valerate and diethyl oxalate: yield 25%; $bp_{30}$ 96 °C; $^1$H NMR ($CDCl_3$)–TMS) δ 0.92 (t, $J$ = 6.9 Hz, 3 H, $CH_3$), 1.10–1.84 (m, 7 H, $C_3H_7$), 2.87 (t, $J$ = 7.7 Hz, 2 H, $CH_2CO$), and 4.30 (q, $J$ = 7.7 Hz, 2 H, $OCH_2$); IR (neat) 1740 (s, C=O) and 1760 (s, C=O) cm$^{-1}$.

**Ethyl 2-oxopentanoate (1c)** was prepared from ethyl butanoate and diethyl oxalate: yield 53%; $bp_{15}$ 74–74.5 °C; $^1$H NMR ($CDCl_3$–TMS) δ 0.70–1.88 (m, 8 H, $C_2H_5$, $CH_3$), 2.81 (t, $J$ = 7.1 Hz, 2 H, $CH_2CO$), and 4.31 (q, $J$ = 6.8 Hz, 2 H, $OCH_2$); IR (neat) 1745 (s, C=O) and 1760 (s, C=O) cm$^{-1}$.

**Ethyl 2-oxobutanoate (1b)** was prepared from 2-oxobutanoic acid (3 g, 29 mmol), ethanol (25 ml), benzene (12 mL), and *p*-toluenesulfonic acid (0.06 g): yield 82%; $bp_{18}$ 65 °C; $^1$H NMR ($CDCl_3$–TMS) δ 0.92–1.52 (m, 6 H, $CH_3$, $CH_3$), 2.86 (q, $J$ = 6.3 Hz, 2 H, $CH_2CO$), and 4.32 (q, $J$ = 6.3 Hz, 2 H, $OCH_2$); IR (neat) 1735 (s, C=O) and 1750 (s, C=O) cm$^{-1}$.

**Immobilization of Bakers' Yeast by Polyurethane Prepolymer.** A triol type prepolymer (MW = 3000: the content of ethylene glycol was 70%) was used as a polyurethane prepolymer (PU). This PU was provided by Dai-ichi Kogyo Seiyaku Co. Ltd.

A suspension of bakers' yeast (5 g) in water (5 mL) was agitated well with a PU (5 g) by a spatula at room temperature until it became impossible to be agitated. The mixture was kept at 4 °C for about 30 min. The polymer was cut into small cubes (about 5 mm edge) with scissors and rinsed with water for about 30 min. Then the excess water was wiped away by a dry cloth. As a result, about 25 g of immobilized bakers' yeast (IMBY) was obtained. In comparison with the total weight of starting materials (15 g), the resulting IMBY gained 10 g in weight. This happened because of the incorporation of water during the rinse of IMBY. So, 25 g of IMBY consists of 5 g of bakers' yeast, 5 g of PU, and 15 g of water.

**Reduction of Ethyl 2-Oxopropionate (1b). With FBY in Water.** In a 100-mL round-bottomed flask equipped with a magnetic stirrer and a porous silicon stopper were placed **1b** (130 mg, 1 mmol), FBY (2 g), glucose (2.5 g), and water (73 mL). The mixture was stirred at 30 °C for 4 h. After filtration over Hyflo Super-Cell, the solution was extracted with ether (3 × 50 mL). The combined organic layer was washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The residue was subjected to preparative GLC (PEG, 1.5 m, 120 °C), giving **2b** (55.2 mg, 42%), $[\alpha]^{24}_D$ −6.25° (*c* 1.04, EtOH).

The alcohol was converted to the corresponding (*R*)-MTPA ester by Mosher's method.[20] Capillary GLC of the (*R*)-MTPA ester (OV 1701, 25 m, 185 °C) revealed that the ee of the hydroxy ester was 75% and the absolute configuration was *S*.

**With IMBY in Water.** In a 100-mL conical flask with a porous silicon stopper were placed ethyl 2-oxobutanoate (130 mg, 1 mmol), IMBY (25 g), glucose (2.5 g), and water (50 mL). The whole system was shaken at 30 °C for 3 h. Then the mixture was separated by filtration and the IMBY was washed with ether. The solution was extracted with ether (3 × 50 mL). The combined organic layer was worked up in the same manner as described above to give ethyl 2-hydroxybutanoate (55.3 mg, 42%). The ee of the hydroxyl ester was 66%, and the absolute configuration was *S*.

**With IMBY in Hexane.** The reaction was run under the same conditions as described for the reaction in the IMBY–water system, except for the use of hexane in place of water, and the reaction time was 1 day. Ethyl 2-hydroxybutanoate (36.3 mg) was obtained in 28% chemical yield. The ee of the hydroxy ester was 40%, and the absolute configuration was *S*.

(21) The difference in $S/R$ ratio between these three systems is experimentally significant.

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**Determination of Absolute Configuration. Ethyl 2-Hydroxyheptanoate (2e).** Ethyl 2-hydroxyheptanoate (*S*) obtained from the reduction of **1e** with the FBY system was converted into 1,2-dihydroxyheptane to compare the sign of rotation with that reported in the reference.[22] In a round-bottomed flask equipped with a magnetic stirrer were placed 0.39 g (2.2 mmol) of ethyl 2-hydroxyheptanoate, whose ee was already determined to be larger than 97%, and 40 mL of absolute ethanol. A solution of sodium borohydride (0.08 g, 2.2 mmol) in ethanol (5 mL) was added to the cooled solution in an ice bath. The solution was refluxed for 6 h. After being cooled to room temperature, the solution was acidified with 2 M hydrochloric acid and the solvent was removed under reduced pressure. The residual oil was extracted with ether (3 × 15 mL). The organic layer was washed with saturated aqueous sodium hydrogen carbonate and brine, successively, and dried over sodium sulfate. After removal of the ether, the residual oil was purified by a preparative GLC (PEG, 1.5 m, 170 °C) to give 1,2-dihydroxyheptane (78.4 mg, 27%): $[\alpha]^{24}_D$ −17.24° (0.98, EtOH); $^1$H NMR ($CDCl_3$–TMS) δ 0.80–1.06 (m, 3 H, $CH_3$), 1.10–1.69 (m, 8 H, $C_4H_8$), 1.82–2.23 (m, 2 H, OH), and 3.30–3.96 (m, 3 H, CH, $CH_2$). Since the optical rotation of (*R*)-1,2-dihydroxyheptane was reported to be +16.8° (EtOH),[22] the absolute configuration of **2e** obtained by the reduction of **1e** with the FBY system was established as *S*.

**Ethyl 2-Hydroxyhexanoate (2d).** The same procedure as described above gave 38.3 mg (11%) of 1,2-dihydroxyhexane: $[\alpha]^{24}_D$ −9.70° (*c* 1.00, EtOH); $^1$H NMR ($CDCl_3$–TMS) δ 0.79–1.11 (m, 3 H, $CH_3$), 1.15–1.73 (m, 6 H, $C_3H_6$), 1.80–1.43 (m, 2 H, OH), and 3.31–3.87 (m, 3 H, CH, $CH_2$).

Since the optical rotation of (*R*)-1,2-dihydroxyhexane was reported to be +15.2° (EtOH),[23] the absolute configuration of ethyl 2-hydroxyhexanoate obtained by reduction of **1e** with FBY in water was established as *S*. The relatively smaller rotation value of (*S*)-1,2-dihydroxyhexane derived from **2d** is due to a partial racemization of **2d** during the reaction of **2d** with $NaBH_4$.

**Ethyl 2-Hydroxypentanoate (2c).** In a 200-mL round-bottomed flask equipped with a magnetic stirrer and a dropping funnel were placed (*S*)-2-aminopentanoic acid (1 g, 8.5 mmol), 1 M hydrochloric acid (9.5 mL), acetic acid (19 mL), and water (38 mL). A solution of sodium nitrite (6.65 g, 85 mmol) in 12 mL of water was added dropwise to the solution through a dropping funnel at 0 °C. The solution was stirred for an hour at 0 °C and then kept overnight with stirring at room temperature. Ninhydrin reaction of the solution appeared negative. To the solution was added concentrated hydrochloric acid (10 mL), and the evolution of nitrogen dioxide gas was recognized. The solution was concentrated under reduced pressure to give a yellow solid, which was extracted with hot acetone. Removal of the solvent under reduced pressure gave a crude product, which was subjected to the following reaction without further purification.

In a 100-mL round-bottomed flask equipped with a magnetic stirrer were placed the resulted oil, absolute ethanol (50 mL), and a catalytic amount of *p*-toluenesulfonic acid (0.15 g). The solution was stirred for 1 day at room temperature. After the addition of triethanolamine (0.15 mL), the solvent was removed under reduced pressure. Then the residual oil was distilled to give ethyl (*S*)-2-hydroxypentanoate (0.57 g, 44%): $bp_{22}$ 110 °C; $[\alpha]^{24}_D$ −5.95° (*c* 1.90, EtOH); $^1$H NMR ($CDCl_3$–TMS) δ 0.94 (t, *J* = 6.4 Hz, 3 H, $CH_3$), 1.28 (t, *J* = 6.9 Hz, 3 H, $CH_3$), 1.08–1.86 (m, 4 H, $C_2H_4$), 2.72 (d, *J* = 5.4 Hz, 1 H, OH), 4.00–4.38 (m, 1 H, CH), and 4.28 (q, *J* = 6.8 Hz, 2 H, $OCH_2$); IR (neat) 1735 (s, C=O) $cm^{-1}$.

From the sign of optical rotation, **2c** which was obtained by the reduction of **1c** with FBY was determined to be *S*.

**Ethyl 2-Hydroxybutanoate (2b).** The absolute configuration of the alcohol was determined by the same method as described for **2c**.

From (*S*)-2-aminobutanoic acid, ethyl (*S*)-2-hydroxybutanoate was obtained in 52% yield: $bp_{22}$ 100 °C; $[\alpha]^{24}_D$ −7.88° (*c* 1.46, EtOH); $^1$H NMR ($CDCl_3$–TMS) δ 0.94 (t, *J* = 7.3 Hz, 3 H, $CH_3$), 1.28 (t, *J* = 7.0 Hz, 3 H, $CH_3$), 1.42–2.01 (m, 2 H, $CH_2$), 2.77 (d, *J* = 5.29 Hz, 1 H, OH), 3.97–4.38 (m, 1 H, CH), and 4.23 (q, *J* = 7.15 Hz, 2 H, $OCH_2$); IR (neat) 1735 (s, C=O) $cm^{-1}$.

From the sign of optical rotation, **2b** obtained by the reduction of **1b** with the FBY system was determined to be *S*.

**Ethyl Lactate (2a).** The absolute configuration of **2a** obtained from the FBY system was determined by comparing its rotation value with that reported.[24]

The hydroxy ester obtained from the reduction of **1a** with FBY: $[\alpha]^{24}_D$ −8.51°; lit.[24] ethyl (*S*)-lactate: $[\alpha]^{24}_D$ −9.36°, EtOH.

From the sign of optical rotation, **2a** obtained by the reduction of **1a** with FBY was determined to be *S*.

**Electron Microscopic Observation.** The IMBY was fixed with 2% glutaraldehyde. The fixed IMBY was dried by using $CO_2$ critical point drying technique and coated with gold. Then the IMBY was observed in a scanning electron microscope operated at 20 kV.

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**Registry No.** **1a**, 617-35-6; **1b**, 15933-07-0; **1c**, 105-54-4; **1d**, 5753-96-8; **1e**, 123-66-0; (*S*)-**2a**, 687-47-8; (*S*)-**2b**, 88271-13-0; (*S*)-**2c**, 88945-70-4; (*S*)-**2d**, 93097-40-6; (*R*)-**2d**, 113747-69-6; (*S*)-**2e**, 93219-13-7; (*R*)-**2e**, 111137-20-3; glucose, 50-99-7; water, 7732-18-5; hexane, 110-54-3; diethyl oxalate, 95-92-1; ethyl hexanoate, 123-66-0; 2-oxoheptanoic acid, 13088-48-7; ethyl valerate, 539-82-2; ethyl butanoate, 105-54-4; 2-oxobutanoic acid, 600-18-0.

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# Regiospecific Quassinoidal A-Ring Synthesis via an Olefin Oxidation Strategy[1]

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A two-step method for oxidation of olefins to α-diketones is presented. Tricyclic olefin **12** was converted to three stereodefined 1,2-diols **14**, **15**, and **16**. Swern oxidation of each of these substrates gave the same enolized α-diketone **17**; base-catalyzed isomerization of this material quantitatively afforded an isomerized α-diketone **7B** bearing the substitution pattern found in the antileukemia agent bruceantin (**1A**). The four α-diketones prepared are reasonably cytotoxic against P388 mouse leukemia.

Bruceantin (**1A**) is a highly oxygenated triterpenoid whose topography, functionality, and potential pharmacological application as an antileukemia agent has spawned intense synthetic interest.[1,2] A group of related glycosides

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