Stereochemical Control in Microbial Reduction. XV. Preparation of (2*R*,3*S*)-2-Allyl-3-hydroxybutanoate

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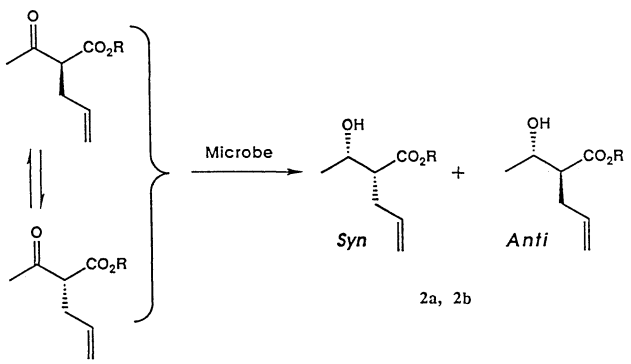
The reduction of ethyl 2-allyl-3-oxobutanoate mediated by *Mucor javanicus* affords the corresponding (2*R*,3*S*)-*syn*-hydroxy ester. The stereoselectivity is excellent and complementary to the reduction mediated by bakers' yeast where the (2*S*,3*S*)-*anti*-hydroxy ester is obtained.

It was reported in a previous paper from our laboratory that the reduction of alkyl 2-alkyl-3-oxobutanates with bakers' yeast (*Saccaromyces cerevisiae*) yields the corresponding (3*S*)-2-alkyl-3-hydroxy esters with various *syn*/*anti*[1] ratios depending on the structure of the alkoxyl group in the β-keto ester.[2] Namely, the reduction of alkyl 2-allyl-3-oxobutanoate affords the (2*S*,3*S*)-*anti*-hydroxy ester in an excellent diastereomeric excess when the alkoxyl group is sufficiently bulky such as those in *t*-butoxy or 1,1-dimethylpropoxy esters. Since the β-keto ester of this type can keep the configuration at its 2(or α)-position to be racemic throughout the reduction by fast enolization under the reduction conditions, all molecules of the substrate subjected to the reduction can be converted into one product out of the four possible diastereomeric isomers, which means that the stereoselectivity of the reduction can be kept constant up to 100% completion of the reduction. This is a notable advantage of the present reduction over the others popularly employed for reduction, oxidation, or hydrolysis of diastereomeric esters mediated by a microbe or an isolated enzyme, where the reaction has to be quenched before 50%, or practically before 30%, completion in order to obtain a good stereoselectivity.[3–6]

It was also reported that when the reduction prefers to afford the *syn*-product such as the reduction of 2-methyl-3-oxobutanoate, the alkoxyl group in the substrate should have a methylene moiety next to the alkoxyl-oxygen, such as in 2,2-dimethylpropoxyl group, in order to yield one product in an excellent diastereomeric excess.[2] Based on the evidence mentioned above, we proposed "the principle of the smallest volume" for the stereochemistry of the reduction with bakers' yeast.[2]



Scheme 1.

Since 2-allyl-3-hydroxybutanoate is a useful chiral building block in organic synthesis because both the alkoxycarbonyl and allyl groups in this molecule can be converted into a lot of valuable functional groups by simple transformations, an attempt to prepare other diastereoisomers than the (2*S*,3*S*)-*anti* isomer of this ester will be worthwhile. However, the *syn*/*anti* stereochemical preferency exerted by bakers' yeast seems to be an inherent characteristic of an ester; that is, although the ratio can be controlled quantitatively, it is difficult to obtain another diastereoisomer of the product by reversing the preferency in stereochemical relationship.

The preparation of (2*S*,3*S*)-*syn*-isomer of 2-allyl-3-hydroxybutanoate has been succeeded by the use of an enzyme system obtained from bakers' yeast.[7] Nevertheless, a living microbial system is much more desirable than an isolated enzyme system because the latter is more unstable than the former and requires the continuous supply of coenzyme, NAD(P)H.

We, therefore, subjected several microbes as alternative biocatalysts for the reduction of this ester, and found that *Mucor Javanicus* cultivated in a potassium-free medium afforded satisfactory *syn*-preferency. Several reports have indicated that stereochemical results from microbial transformations can be controlled by selecting a suitable reaction conditions such as those that change the reaction medium or those that change relative activity lebels of the working enzymes.[8–13] This report will describe a detail in the preparation of ethyl (2*R*,3*S*)-2-allyl-3-hydroxybutanoate in an excellent stereoselectivity.

Results and Discussion

Ethyl and 2,2-dimethylpropyl (neopentyl) esters of

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Table 1. Reduction of Ethyl and 2,2-Dimethylpropyl 2-Allyl-3-oxobutanoate with Various Microbes

Microbe	C-Source	N-Source	*Syn/Anti*		Yields/%	
			2a	**2b**	**2a**	**2b**
C. tropicalis	Glucose	Corn steep liquor	29/71	20/80	66	46
	Alkane[a)]	Aorn steep liquor	8/92	10/90	86	64
A. oryzae	Glucose	Yeast extract	21/79	35/65	46	19
	Glucose	Malt extract	30/70	51/49	—	19
M. javanicus	Glucose	KNO_3	49/51	53/47	77	46

a) A mixture of C_{10}—C_{13} alkanes.

2-allyl-3-oxobutanoic acid (**1a** and **1b**) were employed as the substrates. The 2,2-dimethylpropyl ester was chosen as a substrate because this alkoxyl group generally resulted in satisfactory *syn*-preferency in the reduction with bakers' yeast. Three microbes, *Candida tropicalis* 6052, *Aspergillus oryzae* M61, and *Mucor javanicus* IAM 6101 cultivated under different conditions were tested as the biocatalysts.

The first attempt was to investigate the effect of nutrition on the distortion of enzyme-levels in a microbe. When the reaction is catalyzed by a multi-enzyme system, the distortion of enzyme-levels will result in the change in stereochemical consequence.[9)] Thus, the carbon- and nitrogen-sources were changed on cultivation. The results are summarized in Table 1.

It is interesting to note that the stereoselectivity of the reduction is affected by the change in nutrition in significant amount; namely, the result from the reduction with *C. tropicalis*, a peterotroph, is satisfactory to afford the *anti*-isomer. Similar trend has been observed in the reduction of β-keto esters with methylotrophic microbes.[12)] Since we are interestsed in synthesizing the *syn*-isomer in the present research and *C. tropicalis* has a tendency to afford the *anti*-isomer preferentially, this microbe was discarded in further studies, and the effort to produce a desirable biocatalyst was further continued using *A. orizae* and *M. javanicus*.

It is known that the sort of metal ion present in the reaction solution is a candidate to improve the stereoselectivity.[14)] Therefore, a part of potassium ion present in the cultivating solution was substituted by sodium ion, and it was found that the preferency in the *syn*-product becomes more prominent in a solution with higher proportion of sodium ion as shown in Table 2. Since chemical yield of the product is another important factor to be taken into account in organic synthesis, *M. javanicus* seems more preferable than *A. orizae* for the present purpose. Therefore, finally, *M. javanicus* was cultivated in a 100% sodium ion medium and the concentration of sodium ion in the cultivating solution was increased to yield (2*R*,3*S*)-2-allyl-3-hydroxybutanoate in satisfactory stereoselectivity (80% d.e.; *syn/anti*=90/10) and chemical yield

Table 2. Effect of Alkali Metal Ion in the Cultivating Medium of a Microbe on the Stereoselectivity of the Reduction

Microbe	$[Na^+]/[K^+]$	*Syn/Anti*		Yield/%	
	M/M[a)]	**2a**	**2b**	**2a**	**2b**
A. oryzae[b)]	100/ 0	62/38	69/31	53	15
	90/ 10	51/49	69/31	62	17
	70/ 30	60/40	68/32	71	17
	50/ 40	44/56	59/41	52	12
	30/ 70	45/55	63/37	68	16
	10/ 90	45/55	61/39	52	14
	0/100	30/70	51/49	—	19
M. javanicus[c)]	100/ 0	71/29	70/30	83	72
	91/ 9	67/33	—	70	—
	73/ 27	61/39	71/29	85	62
	54/ 46	56/44	62/38	92	63
	33/ 67	55/45	59/41	68	65
	11/ 89	54/46	62/38	82	68
	0/100	49/51	53/47	77	46

a) The ratio of total concentrations of ions in the cultivating media. b) For cultivation, a solution which contains 8.2 g dm^{-3} (0.061 M) of KH_2PO_4 and 2.3 g dm^{-3} (0.013 M) of K_2HPO_4 and the other solution which contains 1.9 g dm^{-3} (0.016 M) of NaH_2PO_4 and 9.4 g dm^{-3} (0.067 M) of Na_2HPO_4 were mixed each other to obtain a 200 ml aqueous solution. See Experimental for other nutritions. c) For cultivation, a solution which contains 5.0 g dm^{-3} (0.099 M) of KNO_3 and 10.0 g dm^{-3} (0.037 M) of KH_2PO_4 and the other solution which contains 5.0 g dm^{-3} (0.118 M) of $NaNO_3$ and 10.0 g dm^{-3} (0.042 M) of NaH_2PO_4 were mixed each other to obtain a 200 ml aqueous solution. See Experimental for other nutritions.

Table 3. Effect of the Concentration of Sodium Ion in the Cultivating Medium of *M. javanicus* on the Stereoselectivity of the Reduction

$[Na^+]$	*Syn* (2*R*,3*S*)/*Anti* (2*S*,3*S*)		Chemical yield/%	
M[a)]	**2a**	**2b**	**2a**	**2b**
0.08	74/26	—	84	—
0.16[b)]	71/19	74/26	83	72
0.32	84/16	63/37	81	52
0.48	84/16	61/39	98	46
0.64	90/10	78/22	82	49

a) As NaH_2PO_4. b) Standard concentration for cultivation.

(82%). Although the value of 80% d.e. is not yet asymptotic, the microbe could not survive any more at higher Na^+-concentrations than 0.64 M, and this is the maximum value to be achieved at present. It is probable to have a chance to improve the value in future by investigating better conditions for cultivation. The results are summarized in Table 3. The stereoselectivity of the reduction (3*S*-selectivity) was found to be more than 99.6%, or practically quantitative.

Experimental

Instruments. ^1H NMR spectra were recorded on a Varian VXR-200 spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. Gas chromatography was recorded on a Yanaco G-1800 and a G-2800 gas chromatographs (PEG 20M, 1.5 m; 120 °C). Optical rotations were measured with a Perkin-Elmer 241 polarimeter.

Materials. Commercially available reagents were purchased from Nacalai Tesque Co., Tokyo Kasei Co., and Aldrich Chemical Co. unless otherwise indicated. Solvents and purchased reagents were generally used without additional purification unless otherwise indicated. Preparation of ethyl and 2,2-dimethylpropyl 2-allyl-3-oxobutanoates was described in a previous paper.[2]

General Procedure for Cultivation of Microbes. *Candida tropicalis.* In a 1 l of deionized and distilled water, 5.0 g of $NH_4H_2PO_4$, 2.5 g of KH_2PO_4, 1.0 g of $MgSO_4 \cdot 7H_2O$, 20 mg of $FeCl_3$ and 1.0 ml of corn steep liquor were dissolved. To this aqueous solution, either 16.0 g of glucose or a mixture of each 2.5 ml of decane, undecane, and dodecane and 0.5 ml of Tween 80 were added as the carbon source. The pH of the solution was kept at 6.2. The solution was sterilized for 20 min at 120 °C in an autoclave, then the microbe was cultivated. Celite (10 g) was added to the mixture. Filtration of the mixture gave 17 g (wet weight) of the Celite-supported microbe, which was subjected to the reduction.

A. orizae. In a 1 l of deionized and distilled water, 9.0 g of KH_2PO_4, 1.0 g of $MgSO_4 \cdot 7H_2O$, 25.0 g of glucose, 20.0 g of either yeast extract or malt extract, and 3 g of polypeptone were dissolved. The pH of the solution was kept at 6.2. The solution was sterilized and the Celite-supported microbe was obtained as described above. A part of potassium ion was substituted by sodium ion for modified cultivation.

M. javanicus. In a deionized and distilled water, 5.0 g of KH_2PO_4 10.0 g of KNO_3, 2.5 g of $MgSO_4 \cdot 7H_2O$, 0.25 mg of $FeSO_4 \cdot 7H_2O$, 2.5 mg of $ZnSO_4 \cdot 7H_2O$, 33.4 g of glucose, and 0.113 ml of thiamine solution (40 ml/100 ml ethanol) were dissolved to keep the pH of the solution at 4.5. The solution was sterilized and the Celite-supported microbe was obtained as described above. A part of potassium ion was substituted by sodium ion for modified cultivation.

Reduction of Ethyl or 2,2-Dimethylpropyl 2-Allyl-3-oxobutanoate (1a or 1b) by the Aid of a Microbe. In general, in a 100 ml flask were placed 25 ml of deionized and distilled water, 2 g of glucose, 17 g of the Celite-supported microbe, and 1 mmol of a substrate, and the whole mixture was kept at 30 °C in an incubator for 2 days. The organic portion was extracted with ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was diluted to 25 ml by acetone and an appropriate amount of hydroquinone dipropyl ether (an internal standard for GLC) was added to 1 ml of an aliquot of the acetone solution. The aliquot was subjected to GLC analysis to measure chemical yield of the product (**2a** or **2b**) and the *syn/anti* isomer ratio in the product.

Determination of Diastereomeric Composition. The reduced product from the 2,2-dimethylpropyl ester was converted into the corresponding ethyl ester. The hydroxyl group in thus obtained hydroxy ester was acylated by MTPA chloride,[15] and the vapor-phase chromatogram of the MTPA ester was compared with those of the authentic samples. The authentic samples of racemic and (3*S*)-2-allyl-3-hydroxybutanoates were prepared by allylation of ethyl esters of racemic and (*S*)-3-hydroxybutanoate, respectively.[2,16]

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1) For simplicity of the expression, we use the terms *syn*- and *anti*-isomers for the *erythro*- and *threo*-isomers, respectively.

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