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ENANTIOSELECTIVE REDUCTION OF ETHYL 3-METHYL-2-OXOBUTANOATE BY AN ENZYMATIC SYSTEM FROM CALLUS OF *CATHARANTHUS ROSEUS*

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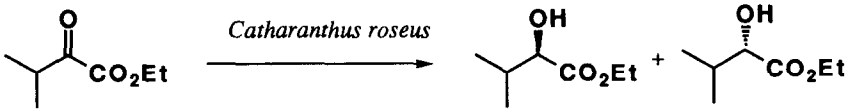
Abstract: Ethyl 3-methyl-2-oxobutanoate was reduced enantioselectively to the corresponding (*R*)-hydroxy-ester by an enzyme obtained from callus of *Catharanthus roseus*.

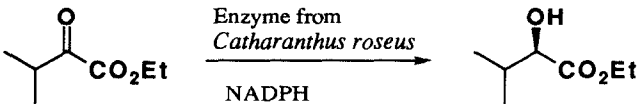
Application of biological systems to asymmetric synthesis has been developed widely in the last decade.[1] However, the progress of biotransformations have been limited to the use of isolated hydrolytic enzymes such as proteases and lipases[2] as well as microbial oxidations and reductions.[3] On the contrary, the use of plant cell cultures are relatively rare although plants have been expected to possess enzymes which show different properties from microbes. Until now, only a little information has been known about isolated enzymes from plant cell cultures for the use to organic synthesis.[4] In this paper, we would like to report that partially purified enzymatic fraction from the membrane portion of *Catharanthus roseus* reduced ethyl 3-methyl-2-oxobutanoate(**1**) enantioselectively to the corresponding (*R*)-hydroxy ester ((***R***)-**2**) while original callus showed low enantioselectivity. The product, optically active 3-methyl-2-hydroxybutanoate, is a versatile and important candidate as a chiral building block to synthesize natural products and other biologically active compounds.



1 ***R*-2** ***S*-2**

The reduction of **1** with the cell suspension of the callus from *C. roseus* proceeded smoothly. However, unfortunately, the cell gave (***R***)-**2** in only 16 % ee,[5] this value is too low for practical use. To increase the selectivity and to study the enzymatic system of the plant, we intend to separate the enzymes from *C. roseus*. Cells of callus from *C. roseus* (1.5 kg) were destructed by liquid nitrogen and enzymes were extracted with

500 ml of 10 mM potassium phosphate buffer (pH 7.2) containing 0.02 % 2-mercaptoethanol (base buffer solution). Centrifugation of the enzymatic solution gave supernatant fraction (SF) and membrane fraction (MF). MF was washed with 500 ml of the buffer solution and centrifuged. The supernatant was collected and mixed with the 1st SF. Both of these two fractions (MF and SF) could oxidize NADPH in the presence of **1**, but NADH was not oxidized. This fact indicates that the enzymes that participate in the reduction of **1** in the plant cell of *C. roseus* use only NADPH, not NADH. To determine the enantioselectivity of these fractions, each fraction was reacted with a mixture of **1** and NADPH in 100 mM of potassium phosphate buffer (pH 5.0) at 30 oC for 3 hrs. As the result, both of the fractions exhibited (*R*)-preference in the reduction, and the selectivity of MF is better (53.5 % ee) than SF (18.5 % ee). SF affords similar enantioselectivity with plant cell. This fact indicates that the enzyme(s) which affords (*S*)-**2** on reduction locates mainly in SF.



1 ***R*-2**

Table 1. Enantioselectivity of each enzyme reduction system.

Reduction system	e.e. (config.)
Callus	16.0 % (R)
Membrane fraction (MF)	53.5 % (R)
Soluble fraction (SF)	18.5 % (R)
Solubilized membrane fraction (MF-2)	100 % (R)

Table 2 shows the substrate specificities of both fractions. Both of the enzymes could reduce α-keto esters but they could not reduce the corresponding acids. Among the substrates tested for enzyme activity, **1** showed the highest activity in both of SF and MF. Fig. 1 shows the pH dependency of the enzyme activity. Both of these enzymes shows maximum activity at acidic solution. MF exhibits maximum activity at pH 4.0 while SF showed the highest activity at pH 5.0.

It was found that the dehydrogenase(s) of SF is not stable and the enzyme activity was diminished during further purification. The further purification of MF was done by solubilizing the enzyme fraction with the buffer containing 1 % Triton X-100 and separated with DEAE-Toyopearl anion exchange chromatography gradient elution with the buffer containing 0-0.5 M potassium chloride. The enzyme fraction (MF-2) was gathered and enantioselectivity of the enzyme was examined. To our astonishment, this partially purified enzyme gave only (**R**)-**2** enantioselectively on reduction of **1**. A simple separation of the crude enzyme fraction make it possible to reduce an α-keto ester enantioselectively. Although the dehydrogenase which gave (***R***)-**2** selectively is not purified at the present time, the partially purified enzyme could be used for synthetic purposes.

Fig. 1. Effect of pH on enzyme activity

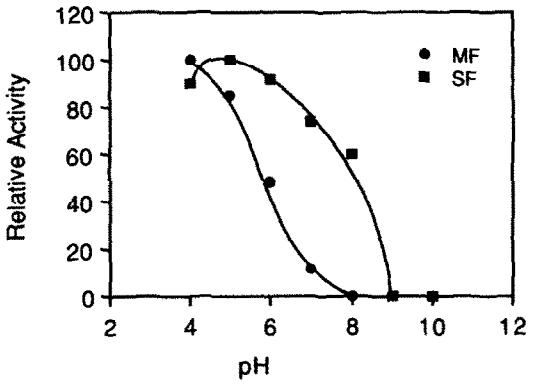
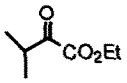
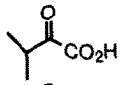
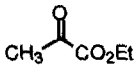
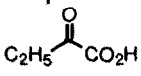
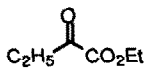
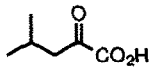
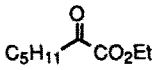
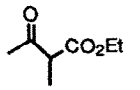
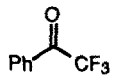
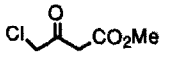
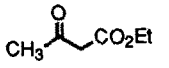
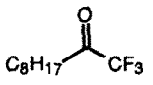


Table 2. Substrate specificity of the enzymes (relative activities)

substrate	Activity SF	Activity MF	substrate	Activity SF	Activity MF
α-keto esters			**α-keto acids**		
	100	100		0	0
	70	59		0	0
	68	65		0	0
			others		
	50	50	CH_3CHO	0	0
β-keto esters			Glyceraldeahyde	0	0
	29	0		65	6
	12	0			
	0	0		0	0

Thus we have found that the NADPH-dependent dehydrogenase of the membrane fraction reduce **1** to the corresponding *R*-hydroxy ester stereoselectively. The further purification of the enzyme and application to the synthesis of natural products are under investigation.

References

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4) Hirata, T.; Tang,Y.; Okano, K.; Suga, T. *Phytochem.*, **1989**, *28*, 3331.
5) The ee was determined by a chiral HPLC (column : Sumichiral OA 2500 I, solvent : Hexane/isopropanol = 200/1, detection : 210 nm, flow rate: 0.8 ml/min, retention time: *R* =13.7 min, *S* = 14.4 min).

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