Pergamon

Tetrahedron Letters 39 (1998) 9219–9222

TETRAHEDRON LETTERS

# Asymmetric Synthesis of β-Hydroxy Esters Having Three Consecutive Chiral Centers with a Reductase from Bakers' Yeast

Yasushi Kawai,* Kouichi Hida, Duc Hai Dao,† and Atsuyoshi Ohno

*Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan*
†*Course of Fundamental Studies and Energy Technology, Graduate School of Science and Technology, Niigata University, Ikarashi, Niigata 950-2181, Japan*

Received 7 September 1998; revised 1 October 1998; accepted 2 October 1998

**Abstract**

Asymmetric synthesis of methyl 2-alkyl-3-hydroxybutanoate having three consecutive chiral centers with a reductase from bakers' yeast has been investigated. When the enzyme is employed in the reduction of a β-keto ester substituted by a secondary alkyl group at α-position, the reaction affords the corresponding β-hydroxy ester in excellent stereoselectivity. 

*Keywords*: Enzymes and enzyme reactions; Asymmetric synthesis; Hydroxy acids and derivatives

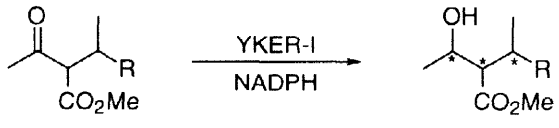
Optically pure α-alkyl-β-hydroxy esters are useful as chiral building blocks in the syntheses of various natural products, because they have two stereocenters and two functional groups that are readily convertible to other functions [1-6]. Although a number of asymmetric syntheses of α-alkyl-β-hydroxy esters by chemical [7-9] and biochemical [10-12] methods have been reported, there have been few reports on asymmetric syntheses of β-hydroxy esters having three consecutive stereocenters. They are important precursors of trisubstituted biologically active lactones [13, 14]. We have explored a novel synthetic route to such β-hydroxy esters by tandem introduction of three consecutive chiral centers. Microbes, *e. g.* bakers' yeast, are powerful tools for asymmetric syntheses. However stereoselectivity exerted by a microbe is not always satisfactory for artificial substrates. Poor stereoselectivity is often the result of simultaneous action of several enzymes that exert opposite stereochemistry toward the substrate [15-20]. In such a case, the use of an isolated enzyme is recommended for obtaining a pure enantiomer.

Recently, we reported the isolation of four β-keto ester reductases from bakers' yeast [16]

0040-4039/98/$ - see front matter 
*PII:* S0040-4039(98)02102-9

and the improvement of the stereoselectivity by employing an isolated enzyme in the reduction [17, 19]. One of them, Yeast Keto Ester Reductase-I (YKER-I), affords (*S*)-β-hydroxy esters in excellent enantioselectivities from various β-keto esters. Particularly, in the reduction of alkyl 2-alkyl-3-oxobutanoate, this enzyme can recognize the stereochemistry not only at the reaction center but also at the adjacent carbon atom to give the corresponding (2*R*,3*S*)-hydroxy esters in excellent enantio- as well as diastereoselectivities [17, 21-23]. In this paper, we would like to report the synthesis of optically pure β-hydroxy esters having three consecutive chiral centers by means of YKER-I (Scheme 1).

Scheme 1

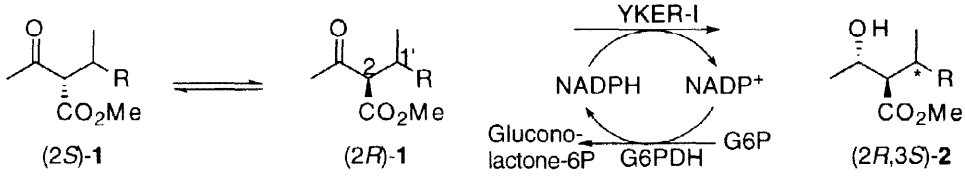



**1** **2**

Methyl 2-*sec*-alkyl-3-oxobutanoates **1** were prepared by acylation of the corresponding methyl esters with acetic anhydride in the presence of lithium diisopropylamide in THF.

In the enzymatic reduction of **1**, the chiral carbon at 2-position remains racemic throughout the reaction due to enolization, and only (2*R*)-**1** is reduced with YKER-I to the corresponding (2*R*)-**2** almost in 100% chemical and enantiomeric yields [21]. On the other hand, the chiral center at 1'-position does not racemize throughout the reaction. Therefore, if YKER-I discriminates the chiral center at 1'-position, the reaction must cease after 50% conversion of the substrate. The selectivity, however, is not so strict as to cease the reaction at 50% conversion. In order to regulate the conversion of the substrate, a coupling system with glucose-6-phosphate (G6P) – glucose-6-phosphate dehydrogenase (G6PDH) was employed to regenerate the coenzyme of appropriate amount (Scheme 2). The esters **1** were reduced with YKER-I and NADPH in potassium phosphate buffer (KPB) at pH 7.0. The results are listed in Table 1.

Scheme 2




Moderate chemical yields of the products indicate that the coupling system employed for the coenzyme regenerating is effective to regulate the conversion of the substrate. The reduction of **1** with YKER-I affords only two stereoisomers out of eight possible stereoisomers of the hydroxy esters **2** in excellent enantioselectivities (>99% e.e.). The

Table 1
Asymmetric reduction of β-keto esters **1** with YKER-I[a]

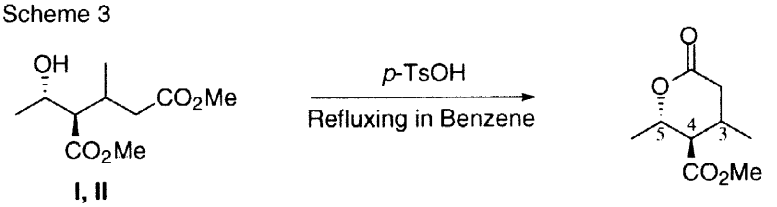
| Compd. | R | Conv. (%) | Ratio of Products (e.e.%) major : minor |
|---|---|---|---|
| **a** | Et[b] | 36 | 71 (>99) : 29 (>99) |
| **b** | Ph[b] | 46 | 55 (>99) : 45 (>99) |
| **c** | 4-MePh | 28 | 51 (>99) : 49 (>99) |
| **d** | 4-BrPh | 22 | 70 (>99) : 30 (>99) |
| **e** | $CH_2CO_2Me$[b] | 30 | 99 (>99) : 1 (–)[c] |

[a] Conditions: **1**: 50 μmol, NADPH: 1.2 μmol, YKER-I: 0.5 units, G6PDH: 5units, G6P: 25 μmol, KPB (pH 7.0, 10 mM): 2 ml. [b] Trace amount of another stereoisomer (≤1%) was detected.
[c] E.e. was not determined.

stereoisomers except for those of **2a** are readily separatable each other by column chromatography on silica gel, and we can obtain enantiomerically pure isomers of **2** without difficulty. The diastereoselectivity of the reaction depends highly on the variation of the substituent R: when R is an alkyl or aryl group, diastereoselectivity of the reaction is unsatisfactory (**1a-1d**). However, in the reduction of **1e**, the diastereoselectivity is much improved and practically only one stereoisomer of **2e** was obtained in more than 98% purity out of possible eight stereoisomers.

In order to determine the absolute configuration of **2e-I** (major) and **II** (minor), a mixture was prepared by bakers' yeast reduction of **1e** and subjected to column chromatography on silica gel with an eluent of hexane/ethyl acetate (3/1) to isolate pure **I** and **II**. Each diastereomer was converted into the corresponding δ-lactone in refluxing benzene in the presence of *p*-toluenesulfonic acid (Scheme 3).

Scheme 3




**I, II**

Configurations of **I** and **II** were determined by the coupling constants between H3 and H4, and H4 and H5. NOE was observed in the spectrum of the lactone prepared from **I** and the results fully consisted with this configuration (Fig. 1). As aforementioned, (*S*)-hydroxy esters are always produced by the reduction with YKER-I. If the conservation of stereochemistry of the hydroxy group of **2e** on lactonization is accepted, which is a quite reasonable assumption, the absolute configurations of lactones from **I** and **II** are (3*R*, 4*R*, 5*S*)

and (3*S*, 4*R*, 5*S*), respectively. *R* configuration at 4-position of these lactones also agree with the fact that the configuration at the vicinal position of the reaction center is always *R* in the reduction of α-alkyl-β-keto esters with this enzyme.

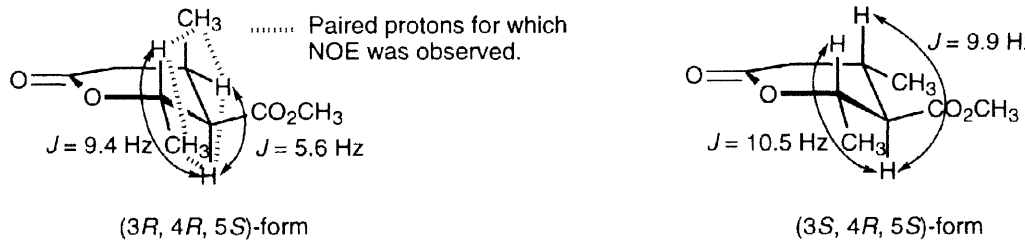



Fig. 1. Determination of configurations of Lactones from **I** and **II**.

Now, we have succeeded in developing a method of tandem introduction of three consecutive chiral centers in a reaction. Further application of the present system will be reported elsewhere.

**Acknowledgement**. The present work was partially supported by a Grant-in-Aid for Scientific Research No. 10740337 from the Ministry of Education, Science, Sports and Culture, Japan.

**References**.

[1] Uematsu T, Umemura T, Mori K. Agric. Biol. Chem. 1983; 47: 597-601.
[2] Brooks DW, Kellogg RP. Tetrahedron Lett. 1982; 23: 4991-4994.
[3] Chiba T, Nagatsuma M, Nakai T. Chem. Lett. 1985; 1343-1346.
[4] Katsuki T, Yamaguchi M. Tetrahedron Lett. 1987; 28: 651-654.
[5] Meyers AI, Amos RA. J. Am. Chem. Soc. 1980; 102: 870.
[6] Mori K. Tetrahedron 1989; 45: 3233-3298.
[7] Fráter G, Müller U, Günter W. Tetrahedron 1984; 40: 1269-1277.
[8] Noyori R, Ikeda T, Ohkuma T, Widhalm M, Kitamura M, Takaya H, Akutagawa S, Sayo N, Saito T, Taketomi T, Kumobayashi H. J. Am. Chem. Soc. 1989; 111: 9134-9135.
[9] Morrison JD. Asymmetric Synthesis. In: Ed. Vol. 3, New York: Academic Press, 1985.
[10] Santaniello E, Ferraboschi P, Grisenti P, Manzocchi A. Chem. Rev. 1992; 92: 1071-1140.
[11] Kawai Y, Kondo S, Tsujimoto M, Nakamura K, Ohno A. Bull. Chem. Soc. Jpn. 1994; 67: 2244-2247.
[12] Kawai Y, Takanobe K, Ohno A. Bull. Chem. Soc. Jpn. 1995; 68: 285-288.
[13] Sibi MP, Ji J. Angew. Chem. Int. Ed. Engl. 1997; 36: 274-276.
[14] Pilli RA, Murta MM. J. Org. Chem. 1993; 58: 338-342.
[15] Shieh W, Gopalan AS, Sih CJ. J. Am. Chem. Soc. 1985; 107: 2993-2994.
[16] Nakamura K, Kawai Y, Nakajima N, Ohno A. J. Org. Chem. 1991; 56: 4778-4783.
[17] Nakamura K, Kawai Y, Miyai T, Honda S, Nakajima N, Ohno A. Bull. Chem. Soc. Jpn. 1991; 64: 1467-1470.
[18] Nakamura K, Kondo S, Kawai Y, Nakajima N, Ohno A. Biosci. Biotech. Biochem. 1994; 58: 2236-2240.
[19] Nakamura K, Kondo S, Nakajima N, Ohno A. Tetrahedron 1995; 51: 687-694.
[20] Kawai Y, Takanobe K, Ohno A. Bull. Chem. Soc. Jpn. 1997; 70: 1683-1686.
[21] Kawai Y, Tsujimoto M, Kondo S, Takanobe K, Nakamura K, Ohno A. Bull. Chem. Soc. Jpn. 1994; 67: 524-528.
[22] Kawai Y, Hida K, Nakamura K, Ohno A. Tetrahedron Lett. 1995; 36: 591-592.
[23] Nakamura K, Miyai T, Kawai Y, Nakajima N, Ohno A. Tetrahedron Lett. 1990; 31: 1159-1160.