Kynureninase in Organic Synthesis : Preparation of γ-Hydroxy-α-L-Amino Acids

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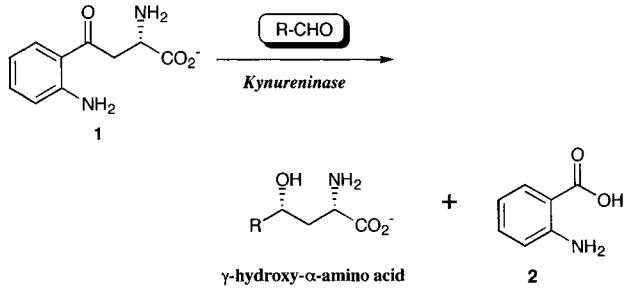
Abstract: Kynureninase, which is known to catalyze the transaldol reaction between benzaldehyde and kynurenine, accepted many kinds of other aromatic aldehydes and propargyl aldehydes as the substrates to afford novel γ-hydroxy-α-L-amino acids. The L-configuration of the α-carbons was confirmed by an enzymatic method using both D- and L-amino acid oxidases. The stereochemistry of the newly formed chiral center (γ-position) in major isomers was determined to be *R*-configuration by the observed NOEs in the NMR spectroscopy of lactones derived from the γ-hydroxy-α-L-amino acids.

Recently, we reported a facile method for the preparation of β-hydroxy-α-L-amino acids by using L-threonine aldolase (LTA) catalyzed reaction,[1] and succeeded in short step synthesis of some bioactive compounds by using this enzymatic reaction as a key step.[2] Enzymatic aldol reaction[3] including LTA catalyzed reaction is a practical method for achieving asymmetric carbon-carbon bond formation on a large scale, since these reactions require neither absolute non-aqueous atmosphere nor extremely low temperature.

γ-Hydroxy-α-L-amino acids as well as β-hydroxy-α-amino acids are useful building blocks for the synthesis of many kinds of bioactive compounds. The anion which possesses a negative charge on the β-carbon of L-alanine is an essential species for the aldol reaction to prepare the γ-hydroxy-α-L-amino acids. Fortunately, kynureninase (L-kynurenine hydrolase, E.C. 3.7.1.3) was shown to generate the anion in its natural reaction from kynurenine (**1**) to anthranilic acid (**2**) and alanine. The reaction mixture of benzaldehyde and kynurenine (**1**) in the presence of kynureninase afforded γ-phenyl-γ-hydroxy-α-L-amino-butyric acid with anthranilic acid.[4] It seems that the β-anion of alanine generated during the enzymatic bond cleavage between β- and γ-carbons of kynurenine (**1**) attacked the carbonyl group of benzaldehyde before being trapped with a proton from the aqueous medium. Kynureninase is one of the best-researched enzymes in terms of biochemical study, however, the substrate specificity of the kynureninase catalyzed carbon-carbon bond formation has not been studied at all. In this article, we intend to report the substrate specificity of this aldol reaction (precisely transaldol reaction) catalyzed by kynureninase from *Pseudomonas fluorescens* (ATCC 11250). Moreover, the stereochemistry of the products is discussed in order to utilize this reaction widely and practically in organic synthesis.



Scheme1. Kynureninase-catalyzed Carbon-carbon Bond Formation

Kynureninase (*ca.* 7 units) prepared according to a method in the literature[4] was added into a mixture of an aldehyde (2.0 mmol) and kynurenine (**1**, 0.25 mmol) in Tris. buffer(pH 8, 65 mM, 40 mL) containing pyridoxal-5'-phosphate (65 mmol), and this reaction mixture was gently shaken at 30 °C for 8 hours. Dimethylsulfoxide (DMSO) can be used as a cosolvent up to 10% of volumetric concentration unless aldehydes dissolve well in the buffer solution at the reaction temperature (30 °C). Phenylacetaldehyde, phenylpropylaldehyde, *N*-phthalimido-acetaldehyde, benzyloxyacetaldehyde, 1,1-dimethylethyl-2,2-dimethyl-4(*R*)-formyloxazolidine-3-carboxylate (Garner's aldehyde)[5] were not consumed in the reaction and more than 90 % of these aldehydes were recovered from the reaction medium. The neighboring sp^3-carbons to the aldehyde group seemed to interrupt the enzyme-substrate (ES) complex formation due to the van der Waals repulsion which can not be relieved by rotation even in the case of methylene carbon. Neither oxygen nor nitrogen functional groups in the aldehydes affected the progress of the reaction.[2b] Therefore, several aldehydes bearing sp^2-carbon at the α-position were next tested as the substrates. Many aromatic aldehydes were converted to the corresponding γ-hydroxy-α-amino acids in good yields (Table 1) although the appropriate products were not obtained from *p*-dimethylaminobenzaldehyde, 1-methylindole-3-carboxaldehyde, and cinnamic aldehyde. Furthermore, phenylpropargylaldehyde (**3a**) which carrys *sp*-carbon at the α-position was smoothly transformed into γ-phenylpropargyl-γ-hydroxy-α-amino acid (**3b**) in spite of the low chemical yield (20%). The substrate specificity of kynureninase implies that the catalytic site of kynureninase should be narrow and sterically hindered since only the flat and/or straight molecules can be incorporated as the substrates.

Finally, the stereochemistry of α- and γ-carbons and the ratios of the isomeric products were determined. All of the products were obtained as diastereomixtures; however, the absolute configuration at the α-positions was confirmed to retain L-configuration by means of experiments in which prepared γ-hydroxy-α-amino acids were subjected to reactions with D- and L-amino acid oxidase.[6] These compounds were substrates for L-amino acid oxidase but monitoring with TLC showed them to be inactive toward D-amino acid oxidase. The ratios of the diastereomers were evaluated by deriving lactones from the γ-hydroxy-α-amino acids. For instance, the amino group of γ-furyl-γ-hydroxy-α-amino acid (**4**) was protected with *tert*-butyloxycarbonyl group to afford *N*-Boc amino acid (**5**), from which cesium salt was prepared by adding Cs_2CO_3 in aqueous methanol. Addition of methyl iodide into the DMF solution of **5** afforded γ-lactones (**6a**,[7] **6b**[8]), which can be separated by

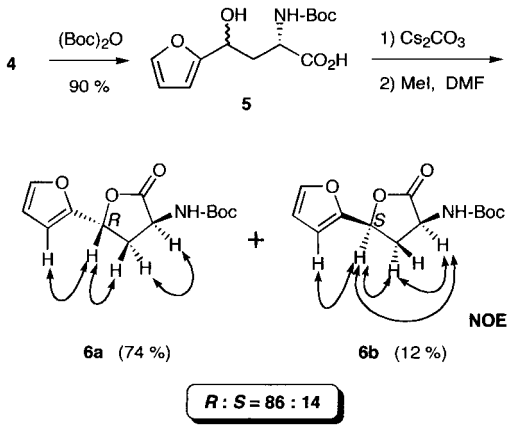
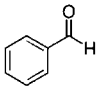
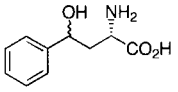
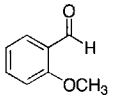
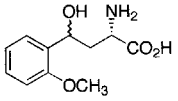
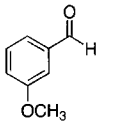
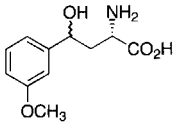
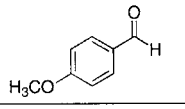
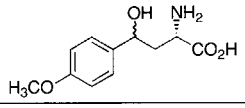
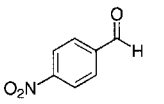
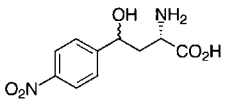
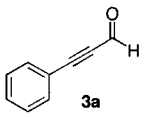
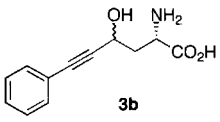
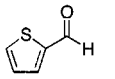
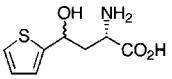
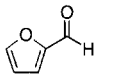
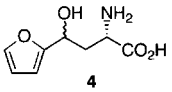


Fig. 1. Determination of Relative Stereochemistry of Compound 4

Table 1. Substrate-Specificity of Aldol Reaction Catalyzed by Kynureninase

Entry	Substrate	Product	Diastereo-ratio *R* : *S*	Chemical Yield
a			91 : 9	50 %
b			96 : 4	46 %
c			96 : 4	69 %
d			96 : 4	50 %
e			88 : 12	52 %
f	 **3a**	 **3b**	63 : 37	20 %
g			95 : 5	46 %
h		 **4**	96 : 4	55 %

silica gel chromatography.[9] The result of NOE experiments showed that the furyl and the *Boc* groups orientate *trans* in the major lactone (**6a**) while showing *cis* -orientation in minor lactone (**6b**). As a result, the *R/S* ratio of γ-carbon of L-amino acid (**4**) was determined to be 86 : 14 (Fig. 1). One isomer of methyl esters was also obtained as a product of lactonization in some cases (Table 1, entry *e*, *g*, *f*). The ester was estimated to have the same configuration as a minor lactone because the *cis*-substituents in the minor lactone can interrupt the lactonization and compete with methylation of the carboxylate.

In addition, the prepared amino acids are useful starting materials in the preparation of not only novel peptides but also many types of bioactive compounds. γ-Furyl-γ-hydroxy-α-amino acid (**4**), for example, was converted to a synthetic precursor of 3-deoxy-D-*manno*-2-octusolonic acid (KDO)[10], which are often present at a non-reducing terminal of endotoxic oligosaccharide. The aminocarbonylation of **4** with L-amino acid oxidase provided an α-keto acid which bears γ-hydroxyl group with *R*-configuration and is composed of eight carbons. This keto acid bears all of the structural features of KDO.

Further study and application are now in progress.

Acknowledgements

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References

1. Vassilev, V. P.; Uchiyama, T.; Kajimoto, T.; Wong, C.-H. *Tetrahedron Lett.* **1995**, *36*, 4081.
2. a) Uchiyama, T.; Vassilev, V. P.; Kajimoto, T.; Wong, W.; Lin, C.-C.; Huang, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 5395.
   b) Vassilev, V. P.; Uchiyama, T.; Kajimoto, T.; Wong, C.-H. *Tetrahedron Lett.* **1995**, *36*, 5063.
   c) Shibata, K.; Shingu, K.; Vassilev, V. P.; Nishide, K.; Fujita, T.; Node, M.; Kajimoto, T.; Wong, C.-H. *Tetrahedron Lett.* **1995**, *37*, 2791.
   d) Miura, T.; Fujii, M.; Shingu, K.; Koshimizu, I.; Naganoma, J.; Kajimoto, T.; Ida, Y. *Synlett*, *submitted*.
3. For example, Tetrahedron Organic Chemistry Series Vol. 12 "*Enzymes in Synthetic Organic Chemistry*" C.-H. Wong & G. M. Whitesides, Pergamon.
4. Phillips, R. S.; Dua, R. K. *J. Am. Chem. Soc.* **1991**, *113*, 7385.
5. Garner, P.; Park, J. M. *J. Org. Chem.* **1987**, *52*, 2361.
6. 10 mM substrate in 1 mL of Tris. buffer (10 mM, pH 8.5), 5 units of amino acid oxidase, 30 °C, 60 hours.
7. Compound (**6a**): ^{1}H-NMR ($CDCl_3$) δ 1.47 (9H, s, *t*-Bu), 2.56 (1H, m, CH*H*), 2.95 (1H, m, C*H*H), 4.72 (1H, m, HN-C*H*), 5.10 (1H, brs, N*H*), 5.60 (1H, d, *J* = 8.9 Hz, O-C*H*), 6.36 (1H, dd, *J*= 3.4, 1.8

Hz, O-CH=C*H*), 6.42 (1H, d, *J*= 3.4 Hz, O-C=C*H*), 7.42 (1H, dd, *J*= 1.8, 0.9 Hz, O-C*H*=). ^{13}C-NMR ($CDCl_3$) δ 28.3, 34.1, 49.8, 71.9, 80.8, 109.5, 110.6, 143.6, 150.8, 155.4, 174.9.

8. Compound (**6b**): ^{1}H-NMR ($CDCl_3$) δ 1.47 (9H, s, *t*-Bu), 2.48 (1H, m, CH*H*), 3.02 (1H, m, C*H*H), 4.57 (1H, m, HN-C*H*), 5.13 (1H, brs, N*H*), 5.40 (1H, dd, *J* = 11.3, 5.5 Hz, O-C*H*), 6.40 (1H, dd, *J*= 3.6, 1.8 Hz, O-CH=C*H*), 6.51 (1H, d, *J*= 3.6 Hz, O-C=C*H*), 7.47 (1H, dd, *J*= 1.8, 0.9 Hz, O-C*H*=).
9. Wang, S. S.; Gisin, B. F.; Winter, D. P.; Makofske, R.; Kuresha, D.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.*, **1977**, *42*, 1286.
10. Ungar, F. M. *Adv. Carbohydr. Chem. Biochem.*, **1981**, *38*, 323.