Asymmetric Synthesis of α-Amino-β-hydroxy Acids Using a Chiral Pyridoxal-Like Pyridinophane-Zinc Complex as an Enzyme Mimic; Scope and Limitation

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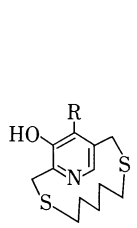
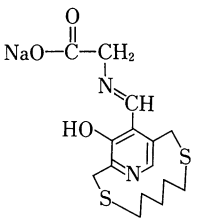
A chelate complex (**4**) with high homogeneity was precipitated upon stirring a mixture of zinc(II) ion and a Schiff base produced from glycine and (*R*)- or (*S*)-15-formyl-14-hydroxy-2,8-dithia[9](2,5)pyridinophane, chiral pyridoxal-like pyridinophane. A four-coordinated zinc chelate complex was newly proposed as the structure of **4**. Aldol condensations between **4** and several aldehydes were attempted at pH 10.0. Only small linear chain aldehydes, such as acetaldehyde and propionaldehyde, could react with **4** under these conditions to give the corresponding α-amino-β-hydroxy acid in the range of 27—77% enantiomeric excess.

Since the pioneering studies of Snell[1] and Martell,[2] many studies have been carried out that mimic various vitamin B_6-dependent enzymes.[3] We have succeeded in constructing a transaminase model which uses zinc(II) and 15-aminomethyl-14-hydroxy-2,8-dithia[9](2,5)pyridinophane (**1**)[4] or derivatives of **1**[5] as pyridoxamine-like pyridinophane derivatives with planar chirality. The application of this type of chiral system was further extended to the asymmetic syntheses of allothreonine and threonine by simulating the reaction of vitamin B_6-dependent aldolase, such as serine hydroxymethyltransferase.[6] A zinc(II) chelate complex of the Schiff base derivative of glycine and the (*R*)- or (*S*)-enatiomer of 15-formyl-14-hydroxy-2,8-dithia[9](2,5)pyridinophane (**2**) was a key substrate for the aldolase model reaction, and showed some characteristic features. This paper deals with a further examination of such an aldolase model reaction in order to confirm its scope and limitations.



1 R = CH_2NH_2

2 R = CHO



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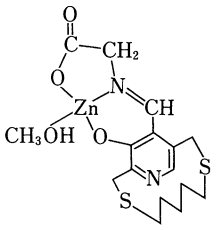

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As described in a previous paper,[6] the treatment of a Schiff base derivative with zinc acetate (0.5 mole equivalents) resulted in two chelate complexes showing absorption maxima at 308 and 397 nm, respectively, of which the latter product could undergo a subsequent aldol condensation. In this study the zinc complex was isolated in a different way. After glycine had been coupled with (*R*)- or (*S*)-**2** in the presence of potassium methoxide in benzene–methanol or sodium methoxide in methanol, a mixture containing the resulting Schiff base (**3**) was stirred overnight with 0.6 mole equivalents of zinc acetate. Almost a pure zinc chelate complex, showing absorption maximum at 397 nm, was precipitated as an amorphous powder. In contrast to the previous study, results of elemental analyses strongly suggested that this complex had a 1:1 composition of the Schiff base and a zinc ion, like **4**. Since the use of 0.75 or 1 mole equivalent of zinc acetate to the Schiff base only resulted in the formation of impurities without any increase in the yield of the desired product, the 0.6:1 ratio of zinc to the Schiff base seemed to be nearly optimum for the preparation of the chelate complex. An excess of chiral **2** could be recovered in high yield after acidic hydrolysis of the filtrate. In order to obtain further evidence for the composition of **4**, a continuous variation method[7] was employed; i.e., the absorbances at 397 nm were plotted versus the concentration of the resulting **3**, while the

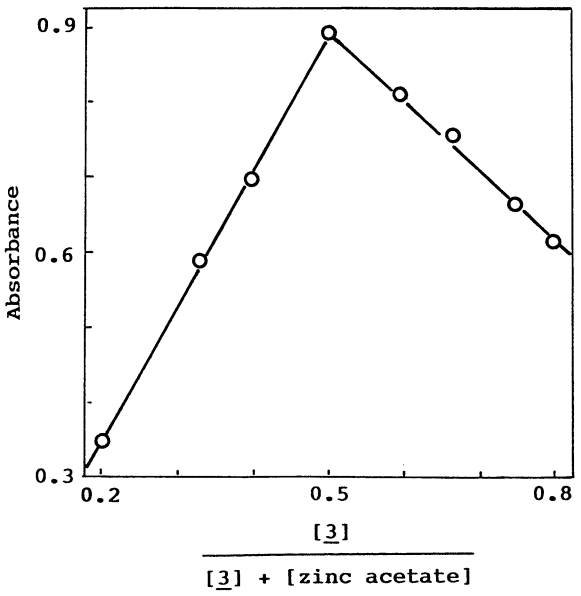
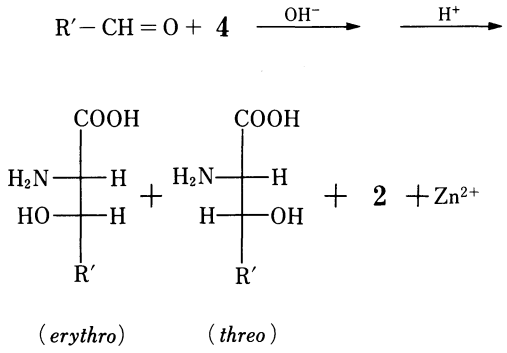


Fig. 1. Application of a continuous variation method for determining the composition of the zinc complex of **3** at 397 nm. The total concentration of **3** and zinc acetate was 0.25 mM.

total concentration of **3** and zinc acetate was maintained at 0.25 mM (1 M=1 mol dm^{-3}). As Fig. 1 shows, such an examination also revealed that a complex having a 1:1 molecular ratio of zinc to the Schiff base was formed in a methanolic solution, suggesting that a four-coordinated zinc chelate has the structure of **4**.

Aldol condensations between **4** and various aldehydes were tested at 10.0 and at 27.0 °C. The basicity of the reaction medium was fixed at pH 10.0, since a higher basicity had been shown to decrease the enantiomeric excesses of amino acids produced. At first, the applicability of various aldehydes to the aldol condensation with **4** was examined at 27.0 °C by using TLC. Propionaldehyde reacted as smoothly as acetaldehyde did, but the reactivity seemed to somewhat decrease in the case of butyraldehyde. Reactions of octanal and formaldehyde with **4** showed only a faint ninhydrin-positive spot other than glycine. An examination of



the various aldehydes disclosed that this reaction was applicable only to simple linear chain aldehydes. Next, the reactions of acetaldehyde and propionaldehyde with **4** were examined in detail. Complex **4** and a large excess amount of aldehyde was stirred in a mixture of aqueous buffer solution (sodium hydrogencarbonate–sodium hydroxide, pH 10.0) and methanol at 27.0 or 10.0 °C. After stirring for 12 or 24 h, the reaction was ceased by the addition of diluted hydrochloric acid. Compound **2** was liberated while maintaining full chirality, and was recovered almost quantiatively by extraction with an organic solvent. The remaining aqueous solution underwent successive ion exchange chromatography on a strongly acidic resin: first in hydrogen form (eluted with aqueous ammonia for isolation of amino acids) and then in pyridinium form (eluted with pyridine–formic acid buffer solution at pH 3.1 for removal of glycine). The ratio of erythro to the threo isomer of the isolated α-amino-β-hydroxy acid mixture was determined by measuring the ratio of the signals due to the α-protons in their ^1H NMR spectra. Enantiomeric excesses of the acids were determined by means of HPLC on a chiral stationary phase after esterification and *N*-3,5-dinitrobenzoylation in the same way as described in a previous paper.[6] The retention times measured for each amino acid are shown in Table 1.

The results of aldol reactions of **4** with acetaldehyde and propionaldehyde are summarized in Table 2, which shows the following characteristics: (i) Erythro isomers are dominant over threo ones regarding both chemical yields and enantiomeric excesses. (ii) The reaction temperature influences both the chemical yields and enantiomeric excesses. Namely, a lower temperature increases enantiomeric excesses, whereas it decreases chemical yields. (iii) There is no remarkable difference between acetaldehyde and propionaldehyde in the reaction products with **4**. Table 2 also

Table 1. Retention Time

Amino acid	Retention time/min
(*R*)-Threonine	13.9
(*R*)-Allothreonine	16.3
(*S*)-Threonine	18.5
(*S*)-Allothreonine	22.8
(*R*)-*threo*-β-Hydroxynorvaline	17.3
(*R*)-*erythro*-β-Hydroxynorvaline	20.3
(*S*)-*threo*-β-Hydroxynorvaline	22.0
(*S*)-*erythro*-β-Hydroxynorvaline	26.8

Conditions:
Stationary phase: Sumipax OA-1000
Mobile phase: Hexane–1,2-dichloroethane–ethanol; 10:4:1 for threonine derivatives; 12:4:1 for norvaline derivatives
Flow rate: 1 ml min^{-1}
Detector: UV 254 nm/0.04 AVFS
Temperature: Room temperature

Table 2. Aldol Condensation between **4** and Aldehydes

Reaction conditions					Products (amino acids)			
Config of **2**	Aldehyde	Temp °C	pH	Time h	Total yield %	E[a] T	e.e.[b] %	Abs config
S	EtCHO	27.0	10.0	24	85	1.2	E 50 T 27	*S*
S	EtCHO	27.0	10.0	12	75	1.4	E 63 T 49	*S*
S	EtCHO	10.0	10.0	24	54	1.4	E 75 T 54	*S*
S	MeCHO	27.0	10.0	12	83	1.6	E 73 T 50	*S*
S	MeCHO	10.0	10.0	24	25	1.8	E 77 T 50	*S*
R	MeCHO	10.0	10.0	24	24	1.8	E 69 T 45	*R*

a) E: Erythro isomer; T: Threo isomer. b) Enantiomeric excess.

illustrates that the use of (*S*)-**2** in the aldol condensation produces (*S*)-amino acid in excess and vice versa, and a longer reaction time decreases the enantiomeric excesses. Although there is no rationalization yet for these experimental results, it is noteworthy that these types of aldol condensation reactions could proceed using a zinc chelate complex, though neither iron(III) nor copper(II) chelate complexes are effective.

Experimental

General. The melting point was determined in a capillary tube using a Büchi melting-point apparatus and is uncorrected. IR and UV-VIS absorption spectra were obtained with Shimadzu IR 27G and Varian-Cary 2290 spectrometers, respectively. ^{1}H NMR spectra were taken on a JEOL JNM GSX 500S (500 MHz) in a D_2O solution. TLC was performed by precoated aluminium sheets from E. Merck (silica gel 60 F_{254} 0.2 mm-thick for *N*-acylamino acid methyl esters, and cellulose F_{254} 0.1 mm-thick for amino acids). The solvent systems were as follows: chloroform–methanol (50:1 v/v) for silica gel, and ethanol–water–concentrated aqueous ammonia (18:1:1 v/v) for cellulose (developed repeatedly for 2—3 times). Silica-gel column chromatography was performed using silica gel 60 (Merck, No. 7734, 0.063—0.200 mm in particle size). HPLC was carried out with a Hitachi 635A Liquid Chromatograph on a chiral Sumipax OA-1000 column (5 μm in particle size, and 4 mm ID×250 mm in column size).

Preparation of Metal Complex. A typical procedure is described. Solid (*S*)-**2** (567 mg, 2 mmol) was added in one portion to a solution of glycine (150 mg, 2 mmol) and sodium methoxide (130 mg, 2.4 mmol) in methanol (48 ml). After 1.5 h, solid zinc acetate dihydrate (263 mg, 1.2 mmol) was added to the resulting solution of **3** and stirred overnight. Meanwhile, precipitates of **4** appeared, which were separated by filtration (403 mg, 77% based on zinc acetate): MP>280 °C; UV-VIS (MeOH) 397 nm (ε 7.3×10^3); IR (KBr disk) 1615 cm^{-1} (C=O). Found: C, 43.79; H, 4.93; N, 6.63; S, 14.78; Zn, 15.30%. Calcd for $C_{15}H_{18}N_2O_3S_2ZnCH_3OH$: C, 44.09; H, 5.09; N, 6.43; S, 14.71; Zn, 15.00%.

The filtrate was acidified to pH 1—2 by adding diluted hydrochloric acid and extracted with ethyl acetate. From the organic extract (*S*)-**2** was recovered (0.28 g).

Aldol Condensation. A typical procedure is described. A solution of **4** (131 mg, 0.3 mmol) in 0.05 M sodium hydrogencarbonate–sodium hydroxide buffer solution (adjusted to pH 10.0, 75 ml) and methanol (25 ml) was treated with 20% solution of propionaldehyde in methanol (10 ml, 34.4 mmol) for 24 h at 27.0 °C. Then, dilute hydrochloric acid was added to the reaction mixture and evaporated. The residue was extracted with ethyl acetate–water at pH 1—2, and the aqueous extract was concentrated to ca. 10 ml and placed on an Amberlite CG 120 column (H^+ form, 100—200 mesh, 50 ml). The column was eluted with water (1 l) and then with 0.1 M aqueous ammonia; the latter eluate was concentrated. At this stage, the reactivity of the aldehyde was qualitatively determined by a comparison of ninhydrin-positive spots of amino acids on cellulose TLC plate using a 0.1-fold scale of the starting materials. The residue was dissolved in a 0.1 M pyridine–formic acid buffer solution (pH 3.1, 2 ml) and placed on an Amberlite CG 120 column (pyridinium form, 100—200 mesh, 50 ml). The column was chromatographed with 0.1 M pyridine–formic acid (pH 3.1) to give a mixture of *erythro*- and *threo*-β-hydroxynorvalines[8] (33.8 mg, 85%) and then glycine unreacted (1.3 mg, 6%).

Determination of the Enantiomeric Excess. A solution of a mixture of *erythro*- and *threo*-β-hydroxynorvalines (10 mg) in 10% hydrogen chloride–methanol (5 ml) was stirred at 50 °C for 2 h and the solvent was evaporated. The residue was dissolved as much as possible in THF (5 ml), and the mixture was treated with triethylamine (0.05 ml) and solid 3,5-dinitrobenzoyl chloride (30 mg) for 1 h at room temperature. After evaporation of the solvent, the residue was dissolved in chloroform, washed successively with 1 M hydrochloric acid, 5% sodium hydrogencarbonate, and water, and dried over anhydrous magnesium sulfate. After concentration of the solvent, the residue was placed on a silica-gel column and eluted with chloroform–methanol (50:1 v/v) as the eluent. The mixture of methyl esters of *N*-3,5-dinitrobenzoyl amino acids produced was dissolved in chloroform (5 ml) and injected into HPLC (2 μl). The conditions of HPLC and the retention times were described in Table 1.

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