Asymmetric Synthesis of Fluorophenylalanines and (Trifluoromethyl)phenylalanines; the Use of Chiral Pyridoxamine-Like Pyridinophane-Zinc Complex as an Enzyme Mimic

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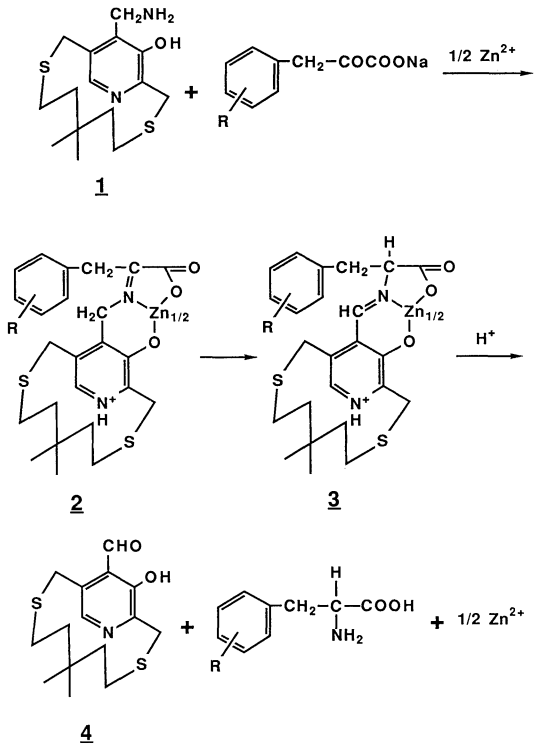
Transamination reactions in the presence of zinc(II) ion between (*R*)- or (*S*)-15-aminomethyl-14-hydroxy-5,5-dimethyl-2,8-dithia[9](2,5)pyridinophane, pyridoxamine analog with planar chirality, and *o*-, *m*-, and *p*-fluorophenylpyruvic acids or *o*-, *m*-, and *p*-(trifluoromethyl)phenylpyruvic acids gave the corresponding fluorophenylalanines or (trifluoromethyl)phenylalanines with 33—66% enantiomeric excess in moderate yields. The observed rate constants of these transaminations, k_{obsd}, were determined with a concomitant observation of of a linear relationship between log k_{obsd} and Hammett's constant, σ^0.

Fluorine-containing amino acids[1] have attracted considerable attention regarding their potential utility as medical and agricultural drugs, although none of these amino acids have been found in nature. Actually, an artificial synthesis of (*S*)-β-fluoroalanine disclosed its antimicrobial activity.[2] It recently was reported that (*S*)-enantiomers of *o*-, *m*-, and *p*-fluorophenylalanines and *p*-(trifluoromethyl)phenylalanine were successfully prepared from the corresponding 2-oxo acids through the transfer of an amino group from L-aspartic acid catalyzed by specific transaminase of microbial origin.[3] In order to move the equilibrium towards the fluorine-containing phenylalanines and oxaloacetic acid, that system was coupled with oxaloacetic acid decarboxylase for the removal of oxaloacetic acid derived from L-aspartic acid as the amino group donor. Consequently, an enzymatic production of those fluorine-containing chiral amino acids was accompanied by a loss of L-aspartic acid through its conversion into pyruvic acid and carbon dioxide. In this paper we present an application of our transaminase model system to asymmetric syntheses of *o*-, *m*-, and *p*-fluorophenylalanines and *o*-, *m*-, and *p*-(trifluoromethyl)phenylalanines. In our system there is no consumption of the amino group donor, since it can be recycled after a brief treatment. Some observations on the kinetics of the transamination reactions are also described.

In previous papers we have reported the syntheses of (*R*)- and (*S*)-15-aminomethyl-14-hydroxy-5,5-dimethyl-2,8-dithia[9](2,5)pyridinophanes (**1**)[4] and their homologs[5,6] as pyridoxamine analogs with planar chirality and their successful use in the presence of zinc ion for asymmetric syntheses of natural amino acids by a trasnamination reaction which mimicks vitamin B_6 enzymes.[6,7] The molecular ratio of zinc ion to chiral **1** and the solvent to be employed were crucial factors in increasing the enantiomeric excess values of the products. Following the obtained information, chiral **1**, its one half molecular zinc(II) ion, and an excess of sodium salt of *o*-, *m*-, or *p*-fluorophenylpyruvic acid or *o*-, *m*-, or *p*-(trifluoromethyl)phenylpyruvic acid were treated in methanol. The reaction temperature was precisely kept at 25.0 °C, although the ambient temperature had been employed for previous syntheses of the natural amino acids. Preparaton of phenylalanine from sodium phenylpyruvate in this manner was also re-conducted for a comparison. Prior to such transaminations, *o*-, *m*-, and *p*-(trifluoromethyl)phenylpyruvic acids were prepared by a similar method for the preparation of phenylpyruvic acid,[8] while sodium



Scheme 1.

salts of *o*-, *m*-, and *p*-fluorophenylpyruvic acids were supplied by a company. The Schiff base isomerization from initially formed ketimine (**2**) to aldimine (**3**) within zinc chelate complex is well-known to constitute an essential part of these transaminase model reactions. These isomerizations towards the production of fluorine-containing amino acids were monitored by the change in the electronic absorption spectrum. In contrast to the cases of the usual amino acid formations so far studied,[9] there were several absorption maxima in the region 269—309 nm ascribable to **2** and the phenylpyruvate used when the fluorine-containing phenylpyruvates were employed as the amino group acceptors. Although the changing pattern of these absorptions through the reaction was diverse, the absorptions of **3** appearing at 398—401 nm as a single peak were regularly enhanced with time for all fluorine-containig substrates. On the basis of the enhancements of these absorbance, the observed rate constants of the isomerization reaction, k_{obsd}, were determined[9,10] (Table 1). In the case of enzymatic transaminations, [3] there was a large difference in the reaction rates between *p*-fluorophenylpyruvic acid (or phenylpyruvic acid) and *p*-(trifluoromethyl)phenylpyruvic acid as substrates. In contrast, this model transamination system essentially lacked such a difference. On the other hand, k_{obsd} of the *o*-isomers were considerably smaller than those of the corresponding *m*- and *p*-isomers, owing to a steric hindrance of the substituent group. A linear relationship was expected between log k_{obsd} and one of the Hammett's constant, σ^0,[11] regarding substituent groups on the benzene ring of phenylpyruvic acids, since those benzene rings and the C=N double bond were separated by a methylene group within zinc chelate complexes of Schiff bases, such as **2** and **3**. This was actually the case; i.e., the reaction constant, ρ, was 0.50 and the correlation coefficient, r, was 0.96 in a roughly linear relationship between log k_{obsd} and σ^0 (Fig. 1).

Table 1. Observed Rate Constant, k_{obsd}, and Hammett's Constant, σ^0

R	$k_{obsd}/10^{-5}$ s^{-1}	σ^0
H	6.0	0
o-F	6.9	
m-F	8.2	0.35
p-F	7.6	0.21
o-CF_3	6.7	
m-CF_3	9.5	0.47
p-CF_3	12.0	0.53

The transamination reactions between (*R*)- or (*S*)-**1** and *o*-, *m*-, and *p*-fluorophenylpyruvic acids and *o*-, *m*-, and *p*-(trifluoromethyl)phenylpyruvic acids were conducted in a similar way to that of the previous studies.[6,7] Thus, a solution of sodium salt of fluorine-containing phenylpyruvic acid (or a mixture of equimolecular amounts of the phenylpyruvic acid and sodium methoxide), (*R*)- or (*S*)-**1**, and zinc acetate (4:2:1 in molecular ratio) in methanol was stirred at 25.0 °C. For a comparison to the previous studies ,[6,7] 24 h was chosen as the reaction time, although monitoring the reaction through the electronic absorption change showed that a shorter period had been

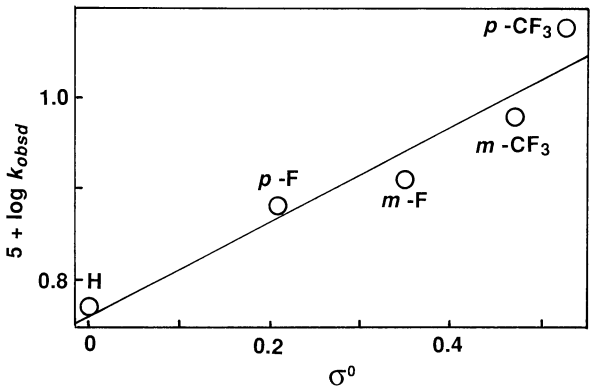


Fig. 1. Relation between the rate constant of the reaction, k_{obsd}, and Hammett's constant, σ^0.

Table 2. Transamination Reaction

R	Enantiomer of **3**	Time h	C.y.[a] %	E.e.[b] %	Major enantiomer
H	*R*	24	76	61	*S*
o-F	*R*	24	92	65	*S*
o-F	*S*	8	90	63	*R*
m-F	*R*	24	85	58	*S*
p-F	*R*	24	82	66	*S*
o-CF_3	*S*	24	86	33	*R*
o-CF_3	*S*	8	80	41	*R*
m-CF_3	*R*	24	65	46	*S*
p-CF_3	*R*	24	84	33	*S*

a) C.y.: chemical yield. b) E.e.: enantiomeric excess.

sufficient for its completion. In order to isolate and characterize the isomerization products, the zinc chelate complex in the reaction mixture was decomposed by the addition of acid and the resulting (*R*)- or (*S*)-15-formyl-14-hydroxy-5,5-dimethyl-2,8-dithia[9](2,5)pyridinophane (**4**) with original chirality was recovered by extraction with an organic solvent. The aqueous solution underwent ion exchange chromatographies for further separation, giving the fluorine-containing phenylalanine. Table 2 shows the chemical yields, kind of major enantiomer, and the enantiomeric excesses of these fluorine-containing amino acids obtained and those of phenylalanine as a reference. The chemical yields of most of the fluorine-containing phenylalanines were higher than that of phenylalanine. Employment of (*R*)-**1** gave (*S*)-amino acids in excess, and vice versa, suggesting that preparations of fluorine-containing amino acids also obey the general rule reported previously.[7] All fluorophenylalanines were obtained in the range 58—66% of enantiomeric excesses, which was essentially equivalent to the enantiomeric excess of phenylalanine (61%). On the other hand, the enantiomeric excesses of (trifluoromethyl)phenylalanines (33—46%) were much less than that of phenylalanine. In both series, *o*- and *p*-substituted phenylalanines were obtained in almost the same enantiomeric excess after the reaction had been conducted for 24 h. A comparison of *m*-isomer to *o*- and *p*-isomers showed that the enantiomeric excess value of *m*-fluorophenylalanine was smaller than those of the corresponding *o*- and *p*-isomers, whereas this was not the case in the (trifluoromethyl)phenylalanine series (*m*- > *o*- and *p*-). With an exception concerning *o*-(trifluoromethyl)-phenylalanine, the slower reaction (the smaller k_{obsd}) resulted in a larger enantiomeric excess value of the product. In order to check the corelation between the enantiomeric excess values and the reaction times, preparations of *o*-fluorophenylalanine and *o*-(trifluoromethyl)phenylalanine were again attempted using a reaction time of 8 h, 1/3 as long as the previous one employed. The enantiomeric excess of *o*-(trifluoromethyl)phenylalanine increased from 33% to 41% by shortening the reaction time, whereas that of *o*-fluorophenylalanine kept almost the same value. This difference might be rationalized by considering the degree of racemization, to which the amino acid residues formed within the zinc chelate complex will be apt to undergo. A fluorine atom strongly withdraws an electron since it has the greatest electronnegativity among the elements. However, when it is located directly on an aromatic ring, it can also donate an electron by mesomeric effect. On the contrary the trifluoromethyl group just serves as a powerful electron-withdrawing group. Thus, racemization would be affected in different degrees by fluoro and trifluoromethyl groups.

Since chiral 15-formyl-14-hydroxy-2,8-dithia[9](2,5)-pyridiophane (**4**) recovered after the isomerization reaction is readily converted into the original pyridoxamine analog (**1**) keeping the full chirality,[4] it can be reused for another run. The optimum reaction conditions for the maximum enantiomeric excess of the amino acids susceptible to racemization might be attained by taking the rates of both transamination and racemization into consideration.

Experimental

General. Melting points were determined in a capillary tube using a Ishii melting-point apparatus and are uncorrected. IR and UV-VIS absorption spectra were recorded on a Shimadzu IR 27G and a Varian-Cary 2290 spectometers, respectively. ^{1}H NMR spectra were obtained with a JOEL JNM GSX 500S (500 MHz) using TMS as the internal reference. Optical rotations were measured with a Perkin-Elmer Model 241MC polarimeter.

Materials. Sodium salts of *o*-, *m*-, and *p*-fluorophenylpyruvic acids were donated by Asahi Glass Co., Ltd.

Prepatations of *o*-, *m*-, and *p*-(Trifluoromethyl)phenylpyruvic Acids. A suspension of α-acetylamino derivative of *o*-, *m*-, or *p*-(trifluoromethyl)cinnamic acid[12] in 1 mol dm^{-3} hydrochloric acid (1:20 wt/v) was refluxed for 3 h while being stirred. After standing overnight in an ice box, the resulting precipitates of the corresponding pyruvic acid were recrystallized from water.

o-Isomer: Yield 93%; mp 142—141 °C; UV (MeOH) 288 nm (ε 1.7×10^4); IR (KBr) 3450 (OH) and 1690 cm^{-1} (broad, C=O); ^{1}H NMR (DMSO-d_6) δ=6.62 (1.18H, d, *J*=1.8 Hz, Ph-CH$_2$), 7.41 (1H, t, *J*=7.7 Hz, Ph-H), 7.65 (1H, t, *J*=7.7 Hz, Ph-H), 7.71 (1H, d, *J*=8.1 Hz, Ph-H), 8.37 (1H, d, *J*=8.1 Hz, Ph-H), 9.7 (broad, 1H, s, COOH), and 13.5 (broad, 0.82H, s, C=C-OH).

Found: C, 51.62; H, 2.99; F, 24.32%. Calcd for $C_{10}H_7F_3O_3$: C, 51.74; H, 3.04; F, 24.55%.

m-Isomer: Yield 73%; mp 132—134 °C; UV (MeOH) 286 nm (ε 1.9×10^4); IR (KBr) 3550 (OH), 1700, and 1670 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6) δ=6.52 (1.22H, s, Ph-CH$_2$), 7.59 (1H, d, *J*=3.6 Hz, Ph-H), 7.60 (1H, d, *J*=3.2 Hz, Ph-H), 7.98 (1H, t, *J*=3.6 Hz, Ph-H), 8.22 (1H, s, Ph-H), 9.7 (broad, 1H, s, COOH), and 13.4 (broad, 0.78H, s, C=C-OH).

Found: C, 51.30; H, 3.04; F, 24.28%. Calcd for $C_{10}H_7F_3O_3$: C, 51.74; H, 3.04; F, 24.55%.

p-Isomer[13]: Yield 92%; mp 179—181 °C; UV (MeOH) 291 nm (ε 2.1×10^4); IR (KBr) 3500 (OH) and 1700 cm^{-1} (broad, C=O); ^{1}H NMR (DMSO-d_6) δ=6.48 (1.18H, s, Ph-CH$_2$), 7.71 (2H, d, *J*=8.3 Hz, Ph-H), 7.97 (2H, d, *J*=8.3 Hz, Ph-H), 9.8 (broad, 1H, s, COOH), and 13.4 (broad, 0.82H, s, C=C-OH).

Found: C, 51.73; H, 3.03; F, 24.07%. Calcd for $C_{10}H_7F_3O_3$: C, 51.74; H, 3.04; F, 24.55%.

The Determination of the Observed Rate Constant of the Reaction, k_{obsd}. Stocked solutions of (*RS*)-**1** (1 mmol dm^{-3} in methanol, 5 cm^3) and zinc acetate dihydrate (1 mmol dm^{-3} in methanol, 2.5 cm^3) were immediately added to a solution of sodium salt of fluorine-containing phenylpyruvic acid (or equimolecular amounts of sodium methoxide and the acid) (0.25 mmol dm^{-3}, 40 cm^3) kept at 25.0 °C and filled up to 50 cm^3 with methanol in a volumetric flask. The reaction

mixture was kept at 25.0 °C in a stoppered quartz cell and the absorption spectra were recorded at regular time intervals. The observed rate constant of the reaction, k_{obsd}, was determined from the slope of the straight line in the plots of $\ln A - \ln (A - A_t)$ against the time, t, where A and A_t indicate the absorbance at the absorption maxima around 400 nm at the completion of the reaction (72 h for convenience) and at the time, t, respectively.[9,10]

Transamination. A suspension of (*R*)- or (*S*)-**1** (93.7 mg, 0.3 mmol), sodium salt of fluorine-containing phenylpyruvic acid (0.6 mmol) (or the acid (0.6 mmol) and sodium methoxide (0.6 mmol)), and zinc acetate dihydrate (32.9 mg, 0.15 mmol) in methanol (150 cm^3) was stirred at 25.0 °C for 8 h or 24 h. After addition of 1 mol dm^{-3} hydrochloric acid (6 cm^3), the resulting mixture was concentrated to dryness under reduced pressure and extracted with water–ethyl acetate. The organic extract was washed with water, dried over anhydrous magnesium sulfate, and concentrated. The residue was placed on a silica-gel column (Merck, No. 7734, 0.063–0.200 mm in particle size) and eluted with chloroform, giving chiral **4** (for example, preparation of phenylalanine: 76.1 mg (81%), $[\alpha]_D^{23} = +421°$ (*c* 0.381, $CHCl_3$), ($[\alpha]_D^{24} = -422°$ for (*S*)-**4**[4])). The aqueous extract was concentrated to ca. 10 cm^3 and put on a column packed with Dowex 50W ×8 (100—200 mesh, H^+ form, 50 cm^3). The column was successively eluted with water (1 dm^3) and 0.1 mol dm^{-3} aqueous ammonia. The latter eluate was concentrated to give an amino acid-containing residue, which was put on a column packed with Amberlite CG 50 (100—200 mesh, H^+ form, 20 cm^3). The column was eluted with water to collect the amino acid-containing fractions, and evaporated. Enantiomeric excess of the product obtained was calculated on the basis of its optical rotation.

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