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Enantioselective Formation of Amino Acids by Isomerization of Mixed Ligand Copper(II) *Schiff*-Base Complexes [1]

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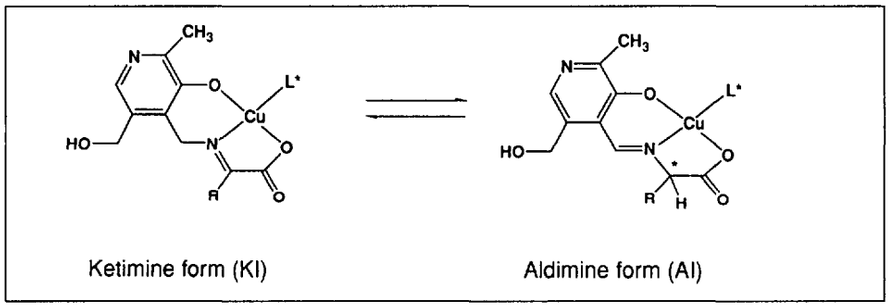
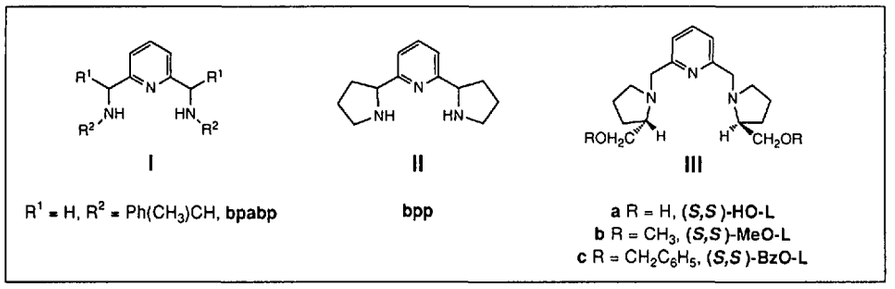
Abstract. The formation of optically active phenylalanine from phenylpyruvic acid and pyridoxamine in the presence of various chiral Cu^{II} complexes was investigated as a function of the reaction conditions. The enantioselectivity of the reaction, as well as the pH- and ligand-dependant racemization of the product, is discussed in terms of possible reaction pathways and the most likely structure of intermediate [Cu(L)aldimine] complexes.

Scheme 1





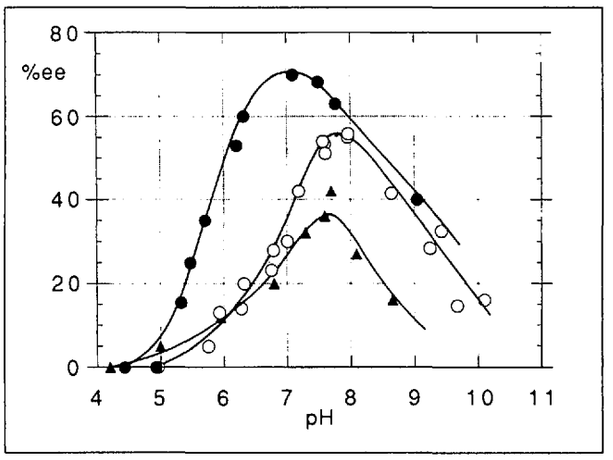


Fig. 1. *Measured % ee of (R)-phenylalanine as a function of pH, formed between pyridoxamine and phenylpyruvate in the presence of [Cu((S,S)-HO-L)]* (▲; reaction time 6 h), *[Cu((S,S)-MeO-L)]* (○), *and [Cu((S,S)-BzO-L)]* (●; reaction time 15 min). Conditions as given in the text and in the *Exper. Part.*

Introduction

Isomerization of Cu^{II} *Schiff*-base complexes formed by pyridoxamine and α-keto acids, yields pyridoxal and the corresponding α-amino acids. Chiral amino acids can be obtained under diastereoface-differentiating conditions which can be achieved either by implanting a chiral center in the *Schiff* base [3–9], or by employing a substrate-independent chiral ligand attached to Cu^{II}, see *Scheme 1* [10][11].

In previous communications, we have reported [10][11] that the reaction using **I** as an auxiliary ligand shows quite high stereoselectivity, but the amount of the isolated amino acids was much smaller due to subsequent racemization of the isomerization product. We now present some results on the stereoselectivity of the isomerization of the Cu^{II} *Schiff*-base complex of pyridoxamine and phenylpyruvic acid in the presence of **II**, and **IIIa–c** as auxiliary ligands. The synthesis of the ligands, their Cu^{II} complex formation equilibria in solution, as well as the X-ray crystal structures of the Cu^{II} complexes with **II** and **IIIa–c** are discussed in previous [12][13] and forthcoming [14] publications.

Results

Fig. 1 shows the % ee of the isolated amino acid for the systems with **IIIa–c** as a function of pH. The stereoselectivity of the reaction generally increases from pH ≤ 4 and reaches a maximum at pH ≈ 7.5 before decreasing again.

Although the behavior of the three ligands seems very similar, an important difference exists. Whereas (*R*)-phenylalanine is configurationally stable in the presence of the ligand **IIIa**, complete racemization of the initially optically active aminoacid is observed with **IIIb** and **IIIc**. The points reported in *Fig. 1* for these two ligands are % ee values measured after 15 min reaction time at 25°, corresponding to an isomerization of 12 and 30%, respectively, which is sufficiently high to determine the enantiomeric ratio precisely.

The different behavior of **IIIa** and **IIIb** is shown in *Fig. 2*. Typical results of all the five ligands studied so far are given in the *Table*, together with some thermodynamic data of the corresponding Cu^{II} complexes.

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Discussion

A quantitative analysis of the system is difficult, because only few of the numerous equilibrium and rate constants which control the systems are known. On the other hand, the different behavior can be rationalized in a qualitative way by the four different pathways *A–D* (*Scheme 2*):

Pathway *A*: **1** → **2** (**9**) → **3** (**10**) → **11**
Pathway *B*: **1** → **4** → **5** → **11**
Pathway *C*: **1** → **6** → **7** → **5** → **11**
Pathway *D*: **1** → **6** → **7** → **8** → **11**

Pathways *A* and *B* lead to racemic products, whereas pathway *C* gives an optically active product, followed by racemization. A stable optically active product is obtained by pathway *D* (grey background in *Scheme 2*).

In basic solution pathway *A* is favored by both $[CuLOH]^+$ formation and stabilization of the free *Schiff* base by deprotonation of pyridoxamine. Pathway *B*, on the other hand, becomes relatively more important in acidic solution due to the different relative influence of protonation on the formation of the different Cu^{II} complexes involved. The factors which control the relative importance of pathway *C* and *D* can be discussed on the basis of the data given in the *Table*. Pathway *D* seems clearly preferred for ligands showing high complex-formation constants. On the other hand, the stereoselectivity of the reaction seems to decrease as the stability of the complex with the optically active ligand increases. This could be due to a lower amount to the ketimine mixed ligand complex, the key intermediate for the formation of an optically active product, which, together with a slower reaction rate would then allow a relatively higher contribution of pathway *A*.

Finally, the question arises, whether the preferential formation of one enantiomer of phenylalanine can be understood on the basis of structural models of the reacting mixed ligand Cu^{II} complexes. The situation is schematically represented in *Fig. 3* for the optically active triamines **II** and **III**.

The C_2 symmetry of these ligands allows only one geometrical arrangement of the tridentate *Schiff* base, which must adopt a meridional coordination mode. In the ketimine form, the α-C-atom of the keto acid moiety has sp^2 geometry and the substituent carried by this C-atom lies in the coordination plane of the *Schiff* base ligand, whereas the six-membered ring is puckered, locating the pyridine moiety of the *Schiff* base outside of this plane. On isomerization, the substituent moves out of this plane, whereas the six-membered ring becomes almost coplanar.

As is easily recognized from *Fig. 3*, the formation of phenylalanine with (*R*)-configuration is favored when ligand **II** shows the (+)-(*R,R*)- and the ligands **IIIa–c** the (*S,S*)-configuration. One may argue that the ligands of type **III** can yield three different diastereoisomeric complexes according to the absolute configuration (*R*) or (*S*) of the coordinated N-atoms of the pyrrolidine rings. However, it can be seen from model considerations that the coordination of the tridentate *Schiff* base can only occur on the diastereoisomer represented in *Fig. 3*, in which the coordinated asymmetric N-atom shows (*S*)-configuration for the case of (*S,S*)-configuration of the ligands. In the other two diaster-

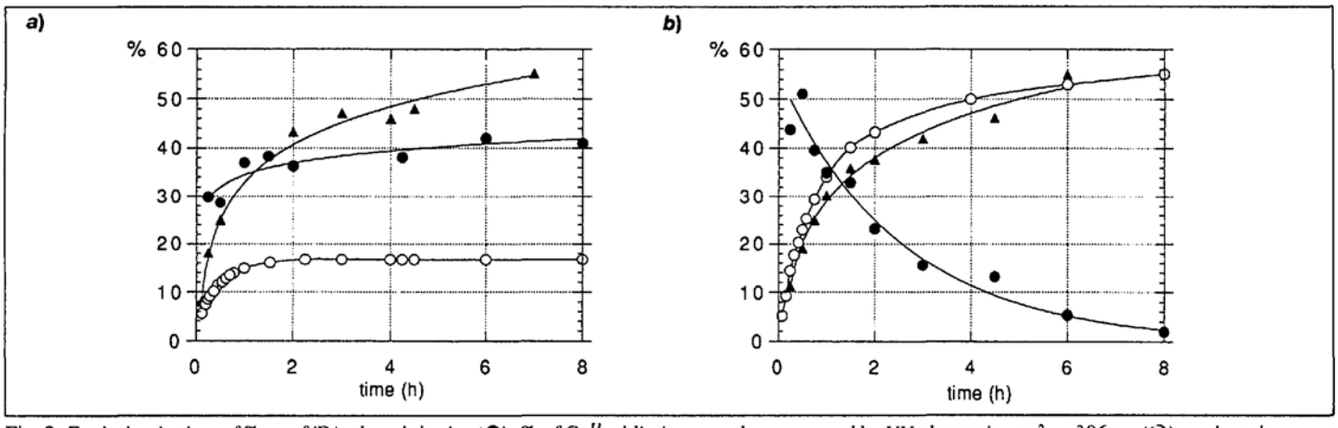


Fig. 2. *Evolution in time of % ee of* (R)*-phenylalanine* (●), *% of* Cu^{II}*-aldimine complex measured by UV absorption at* λ = *386 nm* (○), *and total amount of phenylalanine formed* (▲): a) *[Cu((S,S)-HO-L)]*. b) *[Cu((S,S)-MeO-L)]*. Conditions as given in the *Exper. Part*.

Table. *Enantioselective Formation of* (R)*-Phenylalanine by the Isomerization of* Cu^{II}*-Pyridoxylimino-Phenylpyruvate in the Presence of Various Tridentate Optically Active Ligands*

Ligand	pH	Max. Abs. λ = 386 nm	Selectivity % ee measured	extrapolated[a])	Racemization	Main pathway	log K[b])	log kOH[c])	Ref.
(*R,R*)-**I**	5	weak	45 (*R*)[d])	= 80 (*R*)	fast	*C*	10.5	7.7	[10]
(*R,R*)-**II**	5	Medium	0	–	–	*B*	16.8	8.5	[12][13]
	8	weak	24 (*R*)[e])	–	–	*D*			
	12	weak	0	–	–	*A*			
(*S,S*)-**IIIa**	7.7	15%[h])	42 (*R*)[f])	–	–	*D*	13.1	8.0	this work
(*S,S*)-**IIIb**	7.9	≈ 55%[h])	59 (*R*)[g])	≈ 70 (*R*)	fast	*C*	12.2	8.6	this work
(*S,S*)-**IIIc**	7.1	≈ 100%[h])	70 (*R*)[h])	≈ 90 (*R*)	fast	*C*	11.7	8.7	this work

[a]) Extrapolated to $t = 0$ for reactions followed by racemization. [b]) For the equilibrium CuL/Cu·L. [c]) For the equilibrium $CuL(H_2O)/CuL(OH)\cdot H$. [d]) Measured after a reaction time to attain maximum CD intensity at λ = 386 nm. [e]) Observed after 90% conversion. [f]) Observed after 60% conversion. [g]) Observed after 15 min of reaction. [h]) Compared to the reaction without ligand.

eoisomers, one or both of the CH_2OR substituents are placed in a position, giving strong steric interactions with any coordinated group situated in the apical positions of the coordination sphere of the mixed ligand Cu^{II} complex.

Experimental

1. *Apparatus.* Optical rotations: *Perkin-Elmer 241* polarimeter. UV/VIS Spectra: *Uvicon 820* spectrophotometer. HPLC Analyses: pump *Perkin-Elmer* series *10*, detector *Perkin-Elmer Tridet*, integrator *LCI-100*.

2. *Products.* All the commercially available products were of anal. grade. The optically active ligands and the corresponding Cu^{II} complexes used in this work have been obtained according to [12] for **II** and [14] for **IIIa–c**.

(+)-1-(9-Fluorenyl)ethyl chloroformate was obtained according to [15], except for the optical resolution of 1-(9-fluorenyl)ethanol which was achieved using (*S*)-camphanyl chloride in place of D-camphor-10-sulfonate. The crude ester (yield 84%) was recrystallized 8 times in EtOH giving 22% of pure (+)-1-(9-fluorenyl)ethyl (*S*)-camphanate ester. The optical purity was checked by HPLC on a chiral stationary *Pirkle*'s phase ((*R*)-dinitrobenzoylphenylglycine coupled to aminopropylsilica). M.p. 158°, $[\alpha]_{589} = +49$ ($c = 0.1$, EtOH).

1.2 g (30 mmol) of $LiAlH_4$ was added portionwise to a soln. of 1.0 g (2.56 mmol) of the (+)-1-(9-fluorenyl)ethyl (*S*)-camphanate ester in 50 ml of dried Et_2O and the suspension was stirred for 1 h at r.t. After addition of 2 ml of AcOEt and 2 ml of H_2O, the suspension was filtered and the solid well washed with CH_2Cl_2. The org. solns. were combined, dried ($MgSO_4$), concentrated, and purified by chromatography (Al_2O_3 *507C*, 50 x 2 cm, petroleum ether/CH_2Cl_2 40:60). The fractions containing (+)-1-(9-Fluorenyl)ethanol were combined and the solvent eliminated under vacuum. The crude product was recrystallized in ligroin giving 0.29 g (1.39 mmol; 54%), of fine white crystals. $[\alpha]_{589} = +30$ ($c = 0.1$, CH_2Cl_2).

3. *Reactions.* For reactions in the presence of ligand (*R,R*)-**I**, see [10].

3.1. *General.* All solns. were prepared in bidistilled H_2O except for reactions in the presence of ligand (*S,S*)-**IIIc**, where a 70:30 bidistilled H_2O/EtOH *p.a.* mixture was used, due to low solubility of this ligand and its complexes in water. Solns. were degassed, kept under N_2, and thermostated at 25° during the reaction. The ionic strength was fixed at 0.1 by the buffer solns. used in the different pH domains (acetate, citrate, borate, or phosphate).

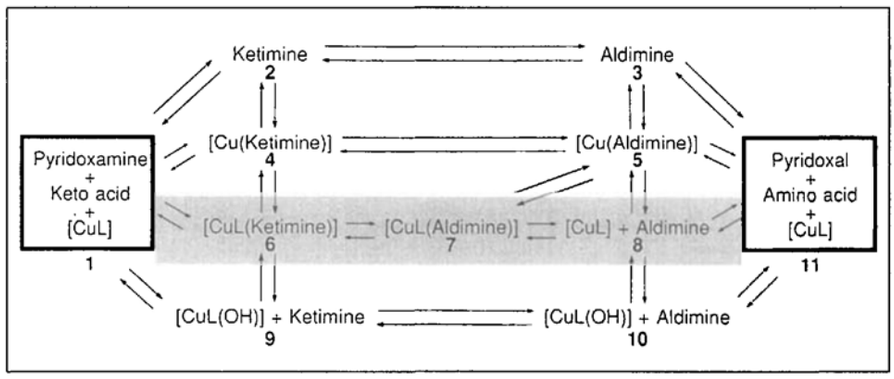
3.2. *Isomerization in the Presence of* (R,R)-**II**. To 1 ml (0.02 mmol) of a 0.02M soln. of [Cu((*R,R*)-**II**)]$(ClO_4)_2$ [13] was added 1 ml (0.02 mmol) of a 0.02M soln. of pyridoxamine HCl (*Merck p.a.*), 3 ml (0.3 mmol) of a 0.1M soln. of sodium phenylpyruvate (*Fluka p.a.*) and 2 ml of a 0.5M buffer soln. The mixture was diluted to 10 ml.

3.3. *Isomerization in the Presence of* (S,S)-**IIIa**, (S,S)-**IIIb**, *and* (S,S)-**IIIc**. Crystallized [Cu(L)Cl]ClO_4 obtained as described in [14] (0.1 mmol for L = (*S,S*)-**IIIa** and (*S,S*)-**IIIb**, 0.022 mmol for (*S,S*)-**IIIc**) was dissolved in 30 ml of the corresponding solvent (see *3.1*). 5 ml of the 1M buffer soln. and 5 ml of a soln. containing 1 equiv. of pyridoxamine HCl were added. If necessary, the pH was adjusted to the desired value with dil. HCl or NaOH. The reaction was started by addition of 5 ml of a soln. containing 7.5 equiv. of sodium phenylpyruvate and adjusting the total volume to 50 ml.

4. *Analyses.* 4.1. *Isolation and Derivatization of Amino Acids.* At various reaction times aliquots containing ~ 0.005 mmol of the amino acids were withdrawn from the soln. The pH was fixed at 2 with dil. HCl and 0.003 mmol of (*R*)-alanine was added as an internal standard. The soln. was introduced onto a small chelating ion-exchange column (*Acryl-IDA*, Na^+, 4 x 1.5 cm) and eluted with H_2O The [CuL] complex is retained on the column while the amino acids are eluted. The eluent was neutralized, concentrated to ~10 ml, and the amino acids derivatized with (+)-1-(9-fluorenyl)ethyl chloroformate, as described in [15].

4.2. *HPLC Measurements.* A 5-µl sample of the soln. of derivatized amino acids (see *4.1*) was injected onto a column (*Nucleosil 100-5C*$_8$ reversed phase, 250 x 4 mm), equipped with a precolumn (*Nucleosil 120-5C18* reversed phase, 30 x 4 mm). The mobile phase consisted of THF (55%) and 0.1M AcOH buffer, pH 4.75 (45%); flow rate was 0.6 ml/min. Typical retention times were 6.1 min for the (*R*)-alanine derivative, 8.3 min for the (*R*)-phenylalanine derivative, 9.5 min for the (*S*)-phenylalanine derivative, and 11.5 min for the hydrolyzed (+)-1-(9-fluorenyl)ethyl chloroformate.

Scheme 2



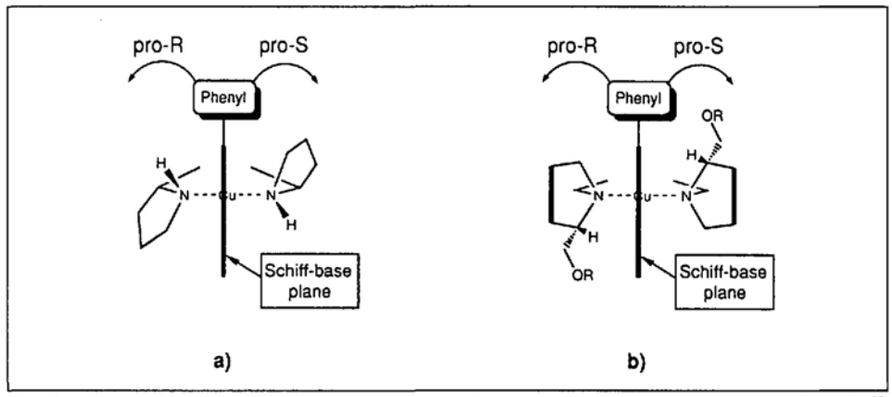


Fig. *3. Schematic representation of possible stereochemical pathways in the evolution of the* Cu^{II}/*ligand/ketimine intermediate complexes:* a) *ligand* = (*R,R*)-**II**, b) *ligands* = (*S,S*)-**IIIa–c**.

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