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UNIVERSITE DE NEUCHATEL
FACULTE DES SCIENCES

SYNTHESE ENANTIOSELECTIVE D'ACIDES AMINES CATALYSEE PAR DES COMPLEXES DU CUIVRE(II) DANS LA REACTION DE TRANSAMINATION

THESE PRESENTEE A LA FACULTE DES SCIENCES PAR

ROBERT DESCHENAUX

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DE NEUCHATEL

POUR L'OBTENTION DU GRADE DE
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IMPRIMATUR POUR LA THÈSE

Synthèse énantiosélective d'acides aminés catalysée par des complexes du cuivre(II) dans la réaction de transamination

de Monsieur *Robert Deschenaux*

UNIVERSITÉ DE NEUCHATEL

FACULTÉ DES SCIENCES

La Faculté des sciences de l'Université de Neuchâtel, sur le rapport des membres du jury,

MM. les professeurs K. Bernauer, E. Stutz et H. Kagan (Orsay)

autorise l'impression de la présente thèse.

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by **Klaus Bernauer***, **Robert Deschenaux**[2]) and **Toshiaki Taura**[3])

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Summary

Enantioselective synthesis of phenylalanine was performed by reacting phenylpyruvic acid with pyridoxamine followed by ketimine-aldimine isomerization of the *Schiff* base formed catalyzed by an optically active copper(II)-complex. By UV and CD measurements it was shown that the enantiomeric excess strongly depends on the reaction conditions and on the reaction time. In favorable cases it reached values up to 80%. The selectivity of the reaction is discussed on the basis of possible structures of the intermediate mixed ligand complex.

Introduction. – Stereoselective interactions in metal complexes may be used *a*) for selective binding of one antipode to achieve separation of a racemic mixture, and *b*) to perform an asymmetric synthesis by means of a stereospecific reaction taking place in the coordination sphere of a mixed ligand complex. The crucial step in both types of reaction is the selective formation of a reactive diastereoisomeric complex obtained by combining a chiral or prochiral substrate (S) and a chiral and nonracemic auxiliary ligand (L*) with a metal ion, the substrate then being transformed into the chiral product (P*) *(Eqn. 1)*:

$$M + L^* \rightleftharpoons ML^* + S \rightleftharpoons ML^*S \rightarrow ML^* + P^* \qquad (1)$$

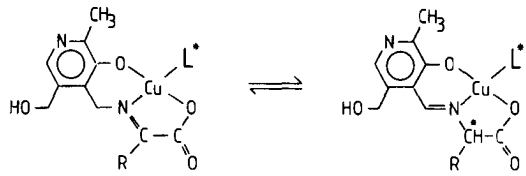
The optical yield of the reaction (% ee) depends on the way in which the stereochemical information is conducted through the reaction chain from the first inducing center, located in the auxiliary ligand, up to the final chiral center in the product P*. Several steps may be involved in such an information transfer: *i.* induction from the backside ligand center, which is usually an asymmetric C-atom, through asymmetric coordination atoms to result in a definite absolute configuration of the ML*-complex; *ii.* orientated and non-statistical binding of the substrate

[1]) Part VI, see [1].
[2]) Part of PhD thesis of *R. D.*, Université de Neuchâtel.
[3]) Present address: Aichi Prefectural University, Nagoya, 467 Japan.

ligand forming the ML*S-complex; *iii.* evolution of the ML*S-complex towards the product P*, either by an intramolecular or by an intermolecular reaction. In the latter case the entering reactant may also need to be orientated in a specific way at the moment it reacts with the substrate ligand. As in each of these steps some factors favoring statistical arrangement may interfere, the structural information can be lost in different ways, and this loss could be important if several effects accumulate.

One of the most important conditions for successful planning of an asymmetric synthesis is therefore total elimination of such factors at any of the reaction steps where they may appear.

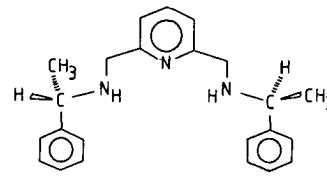
The problem of selective formation of labile ML*-type complexes was reviewed recently [2]. In the following study some results are presented on the asymmetric formation of amino acids by ketimine-aldimine isomerization in mixed ligand Cu(II)-*Schiff* base complexes formed from keto acids *(Eqn. 2)*. It is well-known that such isomerization reactions occur readily in weakly acidic solutions when pyridoxamine or analogous compounds are used in the *Schiff*-base formation [3], and indeed the reaction has also been used to obtain optically-active amino acids [4].



(2)

The choice of this system was based on the fact that the reaction proceeds by intramolecular rearrangement and enantiomeric excess is therefore only determined by diastereoselective differentiation of the various possible transition states. A special problem arose from the fact that the tridentate *Schiff* base is unsymmetrical. Its structure is such that in any arrangement where the N-atom of the *Schiff* base, the metal ion and the auxiliary ligand do not exhibit a twofold rotational axis, a mixture of *cis*- and *trans*-isomers may be formed in the reacting complex. Such geometrical isomerism is indeed one of the possible factors favoring random selection of the configuration of the amino-acid unit in the transition state. For this reason the optically-acive ligand L* must show C_2-symmetry and as a first example the tridentate compound 2,6-bis[(3*S*)-3-phenyl-2-azabutyl]pyridine (**1**) was chosen which has two identical molecular halves and which coordinates exclusively in a peripheric manner.

Results. – When an α-keto acid (KA) reacts with pyridoxamine (PM) in the presence of Cu^{2+}-ions and in a weakly acidic solution, two consecutive reactions



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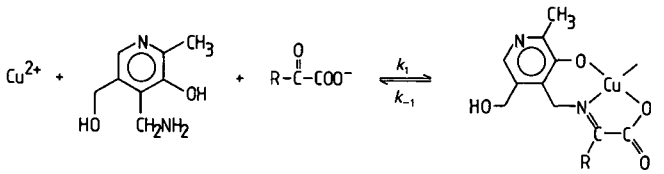
are observed: the formation of a Cu-ketimine complex *(Eqn. 3)* followed by the isomerization of the Cu-ketimine- into the corresponding Cu-aldimine complex *(Eqn. 4)*.



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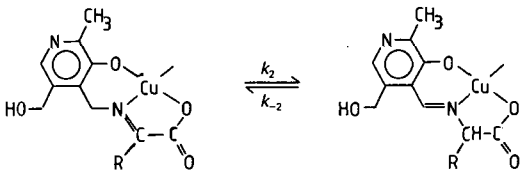


(4)

Both reactions can be followed by measurements of absorption spectra. Whereas the visible spectra of both the ketimine- and aldimine complexes are very similar, the aldimine complex shows a strong absorption band in the UV region with a maximum absorption around 390 nm [5].

The reaction rate of the Cu^{2+}-ketimine formation, measured at the wavelength corresponding to the isosbestic point ($\lambda = 692$ nm) of the spectra of Cu^{2+}-ketimine and Cu^{2+}-aldimine, follows first-order kinetics with respect to keto acid concentration. Ketimine-aldimine isomerization, on the other hand, depends only on the concentration of the ketimine complex. It therefore shows an increase in reaction rate with increasing keto-acid concentration when the latter is small, reaching, in general, a limiting rate at high keto acid concentrations. This limiting rate corresponds to the real rate of the isomerization. This behavior is shown in *Fig. 1* using pyruvic acid as an example

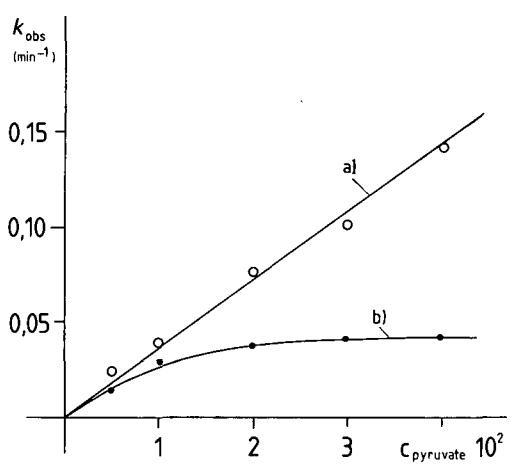


Fig. 1. *Observed rate for the reaction between Cu^{2+}, pyridoxamine and pyruvic acid: a) Cu^{2+}-ketimine formation* ($\lambda = 692$ nm); *b) Cu^{2+}-ketimine – Cu^{2+}-aldimine isomerization* ($\lambda = 395$ nm) ($C_{Cu^{2+}} = C_{pyridoxamine} = 2 \cdot 10^{-3}$; pH = 5.0; acetate buffer; $\mu = 0.1$; t = 25°)

In *Table 1*, the observed rate constants for several keto acids are given. From these values - which must be considered as approximate - it is seen that 2-oxo-3-methylbutyric acid and phenylpyruvic acid show a particular behavior in that their rates increase proportionally with increasing keto-acid concentration for both *Schiff*-base formation and isomerization, even at the highest keto-acid concentration. In these two cases, the *Schiff*-base formation is therefore the rate limiting step of the overall transamination reaction.

Table 1. *Observed Pseudo-First-Order and First-Order Rate Constants for Cu^{2+}-Ketimine Formation* ($k_1 = k_{obs}$/[ketoacid]) *and Cu^{2+}-Ketimine – Cu^{2+}-Aldimine Isomerization* (k_2) ($C_{Cu^{2+}} = C_{pyridoxamine} = 2 \cdot 10^{-3}$ M; acetate buffer ($\mu = 0.1$); t = 25°)

Keto acid RCOCOOH	k_1 (sec^{-1})	k_2 (sec^{-1})	k_1 (sec^{-1})	k_2 (sec^{-1})
R–	pH = 4.4		pH = 5.0	
–H	> 1.2	$1.8 \cdot 10^{-3}$		
$-CH_3$	$9.9 \cdot 10^{-2}$	$8.2 \cdot 10^{-4}$	$6.6 \cdot 10^{-2}$	$7.3 \cdot 10^{-4}$
$-CH_2CH_3$	$8.7 \cdot 10^{-2}$	$\sim 6.3 \cdot 10^{-4}$		
$-CH(CH_3)_2$	$3.3 \cdot 10^{-3}$	$> 1.4 \cdot 10^{-4}$	$4.2 \cdot 10^{-3}$	$> 1.5 \cdot 10^{-4}$
$-CH_2CH(CH_3)_2$			$2.1 \cdot 10^{-2}$	$5 \cdot 10^{-4}$
$-CH_2C_6H_5$[a])		$> 1.6 \cdot 10^{-3}$	$4.2 \cdot 10^{-2}$	$> 1.8 \cdot 10^{-3}$

[a]) 30% EtOH.

With the exception of phenylpyruvic acid, where special effects seem to occur, it may be concluded that the isomerization reaction is only slightly affected by the presence and the nature of the substituent in the keto acid, whereas the formation rate of the Cu^{2+}-ketimine species varies by several orders of magnitude for the different keto acids. When the reaction mixture contains an optically active ligand, the UV-absorption band is only very slightly modified, but the CD spectrum shows a band near to 390 nm, the intensity of which depends strongly on the nature of the substituent R of the keto acid used. During the isomerization reaction, the intensity of this CD band reaches a maximum, then decreases and finally completely disappears. *Fig. 2* shows the variation in UV and CD intensity during the formation of phenylalanine as a product of the reaction between pyridoxamine and phenylpyruvic acid.

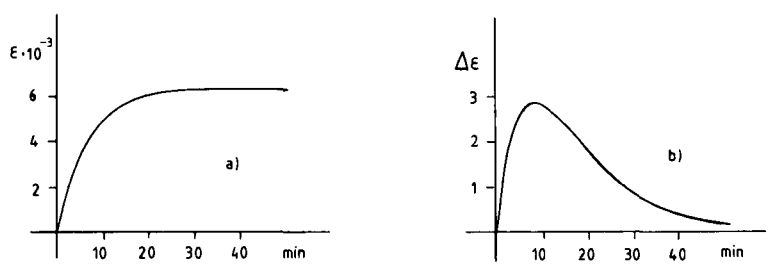


Fig. 2. *Variation at $\lambda = 395$ nm in UV-* (a) *and CD-absorption* (b) *during the reaction of the Cu^{2+}/pyridoxamine/phenylpyruvic acid/ligand system* ($C_{Cu^{2+}} = C_{pyridoxamine} = 2 \cdot 10^{-3}$; $C_{(S,S)^{-1}} = 5.5 \cdot 10^{-3}$; $C_{phenylpyruvic\ acid} = 3 \cdot 10^{-2}$; pH = 5.0; acetate buffer; $\mu = 0.1$; 30% EtOH; t = 25°)

To use the intensity of the CD-signal at 395 nm as a measure of the diastereoselectivity of the ketimine-aldimine isomerization, it was important to show that the observed signal is due only to the presence of the asymmetric C-atom in the amino-acid moiety, and that the disappearance of the signal is the result of racemization. This could be achieved by direct formation of the Cu^{2+}-aldimine species through the reaction of the corresponding amino acid with pyridoxal, as is illustrated for the case of optically active (*S*)-phenylalanine in *Fig. 3.*

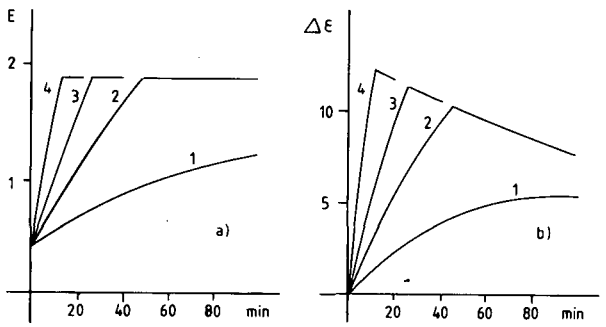


Fig. 3. *Formation and racemization of Cu^{2+}-(S)-phenylalanine-pyridoxylidene from UV* (a) *and CD* (b) *measurements* ($\lambda = 395$ nm; $C_{Cu^{2+}} = 2 \cdot 10^{-3}$; $C_{pyridoxal} = 2 \cdot 10^{-2}$; $C_{phenylalanine} = 2 \cdot 10^{-3}$ (1), $4 \cdot 10^{-3}$ (2); $6 \cdot 10^{-3}$ (3), $1 \cdot 10^{-2}$ (4); pH = 5.0; acetate buffer; $\mu = 0.1$; 30% EtOH; t = 25°)

On the other hand, the observed change in UV and CD absorption with time is in accordance with the proposed reaction mechanism [3], the complex formation between Cu^{2+} and the *Schiff* base being much faster than the *Schiff* base from the two components. The latter is therefore the rate-determining step of the reaction. From the UV absorption limit and by extrapolation of the CD absorption to zero-time, ε- and $\Delta\varepsilon$-values can be calculated. These values, which for phenylalanine are 7600 and 12.5 respectively, allow exact determination of the enantiomeric excess during the isomerization at any moment of the reaction.

Some results for different reaction conditions are presented in *Fig. 4.* The validity of the measurements was verified in several runs by gas chromatographic

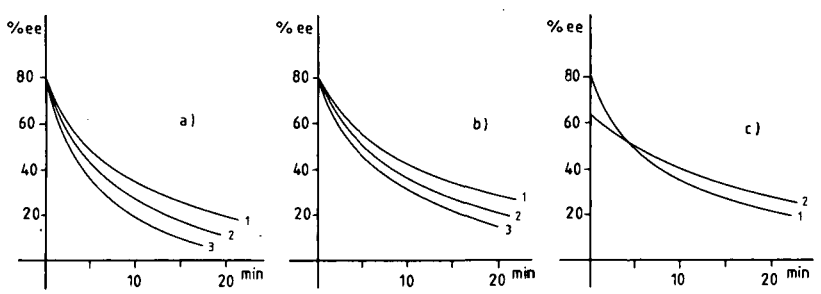


Fig. 4. *Enantiomeric excess in the (R)-phenylalanine formation by transamination between pyridoxamine and phenylpyruvic acid under different reaction conditions* ($C_{Cu^{2+}} = C_{pyridoxamine} = 2 \cdot 10^{-3}$; 30% EtOH; acetate buffer; $\mu = 0.1$; t = 25°; a) pH: 5.00 (1), 5.25 (2), 5.50 (3); $C_{(S,S)^{-1}} = 5.5 \cdot 10^{-3}$; b) ligand concentration: $3.7 \cdot 10^{-3}$ (1), $5.5 \cdot 10^{-3}$ (2), $7.4 \cdot 10^{-3}$ (3); pH = 5.0; c) overall concentration: $C_{Cu^{2+}} = 2 \cdot 10^{-3}$ (1); $C_{Cu^{2+}} = 1.2 \cdot 10^{-3}$ (2), concentration of all other reacting species decreased proportionally)

determination of the enantiomeric excess of the amino acid formed. Details of these determination will be given in the following paper [6]. *Table 2* shows, for some examples, the good agreement between the two modes of determination. This agreement exists although the CD measurement shows only the optically active amino acid contained in the complex, whereas the gas chromatographic determination gives the total amount of the amino acid formed. All the amino acid must be formed by the isomerization reaction and an exchange between free and coordinated amino acid takes place. In this way random distribution of the optically-active amino acid between the free and the coordinated part must occur.

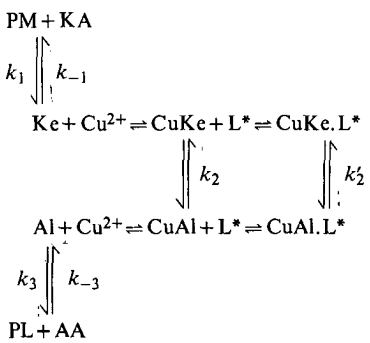
Table 2. *Enantiomeric Excess* (% ee) *of* (R)-*Phenylalanine*[a])

pH	% ee (CD)	% ee (GC)
5.00	40.0	40.9
5.25	42.5	45.5
5.50	41.0	41.2

[a]) Reaction conditions as indicated in *Figure 4a*. The reaction was stopped when the maximum CD intensity was reached.

Discussion. – The system used in the present study of enantioselective synthesis of amino acids is rather complicated; the main equilibria involved are given in the *Scheme*.

Scheme



The optically active amino acid can be formed exclusively through the isomerization reaction of the optically active mixed ligand Cu-ketimine complex. The enantiomeric excess of the reaction is therefore determined by the four following factors: *1.* the relative amount of the mixed species with respect to the free *Schiff*-base complex, *2.* the relative reaction rate characterized by the rate constants k_2 and k_2', *3.* the amount of racemization relative to isomerization, *4.* the stereospecificity of the isomerization in the mixed ligand *Schiff*-base complex. It is the last of these factors which is of special interest because of the way it reflects the real level of transfer of the stereochemical information from the auxiliary ligand to the substrate moiety of the mixed ligand complex.

As the Cu^{2+}-ketimine ligand complex is an intermediary species, formed in an equilibrium reaction, its actual concentration during the reaction is unknown. Approximate determination of this concentration is made even more difficult by the fact that the isomerization rate is approximately twice as fast for the mixed complex as for the unmixed species. On the other hand, as shown in *Fig. 5,* the isomerization rate of CuKe.L* is independent of the pH and excess ligand concentration, whereas the racemization of the Cu^{2+}-aldimine complex, which follows the isomerization, depends on both.

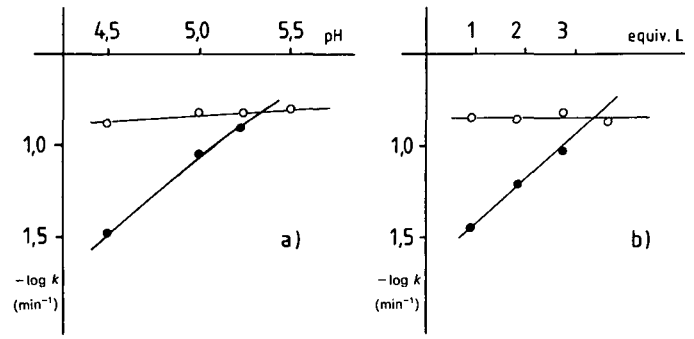


Fig. 5. *First-order rate constants of isomerization* (–O–O–) *and racemization* (–●–●–) *as a function of pH and ligand concentration* (Reaction conditions as indicated in *Fig. 4a* and *4b*)

The real stereoselectivity of the reaction can therefore only be estimated by extrapolation of the observed enantiomeric excess to zero reaction time and for an optimal concentration of the optically active ligand. This extrapolation gave a lower limit of about 80% *(cf. Fig. 4)*, representing a minimum difference of free activation energy $\Delta\Delta G^{+}$ for the two diastereoisomeric transition states of about 5.4 kJ/mol. With respect to this result it seemed interesting to investigate possible relationships which may exist between the structural orientation of the auxiliary ligand in the complex and the configuration of the product formed in excess.

It was first necessary to consider the possible configurations of the two asymmetric N-atoms with respect to the asymmetric C-atoms. A ligand having a given configuration for the latter, for example (*S, S*), allows three such arrangements: $C_{(S,S)}N_{(S,S)}$; $C_{(S,S)}N_{(R,S)}$ and $C_{(S,S)}N_{(R,R)}$. In all the transamination reactions performed so far, in the presence of the ligand exhibiting the $C_{(S,S)}$-configuration, the amino acid formed in excess has the (*R*)-configuration. The same (*R*)-amino acid is also enriched by the retroracemization reaction of the racemic mixture in a basic solution of the mixed ligand complex CuAl.L* [7]. These results indicate that the voluminous substituent lies preferentially in the same position in the transition state as in the product. For the (*R*)-amino acid such a favorable position is provided when the ligand shows the $C_{(S,S)}N_{(S,S)}$-configuration.

In the free ligand, the C–N-bond allows rotation of the asymmetric C-atom; this rotation seems to be strongly hindered in the mixed complex. Study of a model shows that the stable conformation is probably the one pointing the H-atoms in the direction of the *Schiff*-base ligand. In this arrangement the phenyl substituent

lies above the aromatic ring of the *Schiff*-base allowing a hydrophobic interaction between the two ligands, as shown in *Fig. 6.*

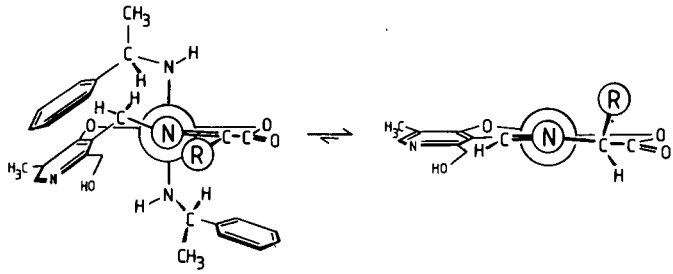
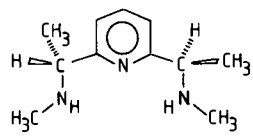


Fig. 6. *Proposed structure of intermediate mixed ligand Cu^{2+}-ketimine complex, and of its product after isomerization*

Some interesting observations seem to support the hypothesis of such a hydrophobic interaction. No stereoselectivity was observed when in identical conditions the optically active ligand 2,6-bis(3-aza-2-butyl)pyridine (**2**) [7] was used, which shows an identical basic structure with respect to **1** but contains no phenyl substituents. On the other hand, both ligands, **1** as well as **2** show retro-racemization in basic media with Cu(II)-salicylidene-aminoacid complexes [7]. The stability of the mixed ligand Cu(II)-ketimine complex with **1** may therefore be enhanced by a hydrophobic interaction between aromatic groups as was also observed with other systems [8].



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Another question worthy of discussion in this context is the relative stability of the mixed complex of Cu(II)-ketimine with respect to that of Cu(II)-aldimine. Zero-time extrapolation of the optical yield shows a value of about 80% which, in the concentration range used, is almost independent of the ligand concentration *(Fig. 4a).* Taking into account the fact that isomerization in the mixed complex is only slightly faster than in the complex without an auxiliary ligand, this means that with Cu(II)-ketimine the mixed complex is almost quantitatively formed. However the mixed complex with Cu(II)-aldimine seems to be much less stable. This is shown firstly by the fact that racemization of the product is almost complete even in the presence of a large excess of the optically active ligand, and secondly as the racemization rate increases linearly with ligand concentration *(Fig. 5b).* This may be due to the presence of a small amount of mixed complex which augments with increasing ligand concentration. The behavior of the system as a function of the overall concentration *(Fig. 4c)* can be explained in the same way. Whereas the dilution affects only slightly the enantiomeric excess of the isomerization, the initial racemization rate is strongly reduced.

The difference in the stability of the two mixed complexes is probably a consequence of the conformational change which occurs during the isomerization *(Fig. 6)*. Whereas in the ketimine compound, the pyridine ring of the *Schiff*-base is considerably drawn out of the coordination plane due to the presence of the CH_2-group in the six-membered chelate ring, the whole ligand is in an almost planar arrangement in its aldimine form. Model considerations suggested that ligand-ligand interactions between aromatic groups were most likely to occur in the ketimine structure with the pyridine group in the puckered six-membered chelate ring.

The observed expulsion of the tridentate auxiliary ligand by the only conformational change during the transformation of the substrate to product seems of some interest with respect to analogous mechanisms in enzyme-catalyzed reactions. Nevertheless, the given rationalization of stereoselectivity in terms of a given structure of the reactive intermediate needs further confirmation by the use of a more rigid auxiliary ligand. Synthesis of such ligands is currently in progress. In spite of this, the high stereoselectivity observed can certainly be considered as a consequence of the elimination of geometrical isomerism performed by using a C_2-symmetric metal-ligand system as a source of asymmetric induction.

We thank the *Swiss National Science Foundation* for financial support.

Experimental Part

General. Optical rotations were measured on a *Perkin-Elmer 241* polarimeter, UV and VIS spectra were measured on a *UVIKON 810* spectrophotometer and CD measurements were obtained from a *JASCO J-500C* spectropolarimeter. NMR spectra were recorded on a *Bruker WP-200* in D_2O with DSS as an internal standard.

Materials. (−)-(*S*)-1-Phenylethylamine was prepared according to [10] b.p. 80°/15 Torr, $[\alpha]_D^{22} = -40.03°$ (neat, optical purity: 99.3%). Keto acids, pyridoxamine and pyridoxal were of analytical grade *(Fluka)* and used without further purification.

2,6-Bis[(3S)-3-phenyl-2-azabutyl]pyridine. Pyridine-2,6-bis(carboxaldehyde) (11.7 g, 86.6 mmol) obtained by the method described in [9] is mixed with a solution of 21.0 g (173 mmol) (−)-(*S*)-1-phenylethylamine in 80 ml of dry EtOH. The mixture is hydrogenated in the presence of 10% Pd/C as a catalyst at r.t. and at a H_2 pressure of 3 atm. The mixture is filtered through *Celite*, and the filtrate diluted with 80 ml of H_2O. The pH is brought to 4.5 by dropwise addition of conc. H_2SO_4 and the solution is then evaporated to dryness. By crystallization of the crude salt from EtOH/acetone, 32.6 g (85%) of the sulfate are obtained, $[\alpha]_{436}^{25} = +36.1°$ (H_2O, $c = 0.2$). ^{1}H-NMR (200 MHz): 1.8 (*d*, $J = 7.5$, 6 H); 4.1–4.4 (*dd*, $J = 15$, 4 H); 4.5–4.7 (*q*, $J = 7.5$, 2 H); 7.2–7.4 (*d*, $J = 8.5$–9, 2 H); 7.4–7.6 (*s*, 10 H); 7.7–7.9 (*t*, $J = 8.5$–9, 1 H).

$C_{23}H_{29}N_3O_4S \cdot 2\,H_2O$ (479.23) Calc. C 57.62 H 6.89 N 8.94% Found C 57.63 H 6.30 N 8.66%

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43. Stereoselectivity in Reactions of Metal Complexes VIII[1])

Asymmetric Synthesis of Some Amino Acids by Stereoselective Transamination of Aliphatic Keto Acids in Mixed Ligand Copper(II)-*Schiff*-Base Complexes

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Summary

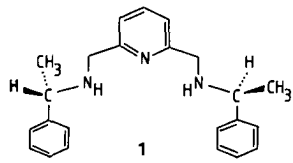
Optically active alanine, valine and leucine were obtained by a transamination reaction between pyridoxamine and the corresponding α-keto acid in the presence of a Cu^{2+}-complex with the tridentate ligand 2,6-bis[(3*S*)-3-phenyl-2-azabutyl]pyridine. In each case the amino acid with (*R*)-configuration was formed preferentially, and the maximum enantiomeric excesses were 54% (alanine), 48% (leucine) and 29% (valine). The stereoselectivity of the reaction is discussed in terms of the possible structure and the stability of the intermediate Cu^{2+}-ketimine-ligand complex.

Introduction. – In the preceeding paper [1] we presented some results on the enantioselective formation of phenylalanine by stereoselective isomerization of a mixed ligand Cu^{2+}-*Schiff*-base complex formed from pyridoxamine, phenylpyruvic acid and the optically active ligand 2,6-bis[(3*S*)-3-phenyl-2-azabutyl]pyridine (**1**). High stereoselectivity with an enantiomeric excess up to 80% was observed. Thus it was of interest to study the analogous formation of other amino acids to explore the stereoselectivity of the reaction as a function of the substituents of the different amino acids. CD spectra were used to determine the stereoselectivity of the formation of phenylalanine [1]. In the case of amino acids with simple aliphatic substituents the intensity of the CD bands is weak and the determination of enantiomeric excess by this technique is inaccurate. For this reason GC analysis was used to separate and determine the relative amount of the antipodes for each of these aliphatic amino acids, namely alanine, leucine and valine.

Results. – When an α-keto acid (KA) reacts with pyridoxamine (PM) in the presence of the optically active Cu^{2+}-complex of **1** transamination takes place *(Eqn. 1)*[3]) and an optically active amino acid is obtained.

$$CuKe \cdot L^* \rightleftharpoons CuAld^* \cdot L^* \qquad (1)$$

[1]) Part VII: [1].
[2]) Part of Ph.D. thesis of *R.D.*, Université de Neuchâtel (1983).
[3]) For abbreviations see the *Scheme*.



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For the formation of alanine from pyruvic acid, the variation of enantiomeric excess with time is shown in *Fig. 1*. *Fig. 2* gives the corresponding results for the formation of valine from 3-methyl-2-oxobutyric acid and of leucine from 4-methyl-2-oxovaleric acid. In all these reactions the (*R*)-amino acid is obtained in excess, when (*S,S*)-(**1**) is used as the optically active ligand. The same preferential configuration of the reactive intermediate may therefore be proposed as for the formation of phenylalanine [1].

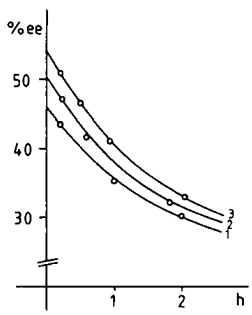


Fig. 1. *Observed % ee for alanine formation as a function of reaction time and reagent concentrations.* $c(Cu^{2+}) = c$(pyridoxamine): $c((S,S)$-**1**$)$: c(pyruvate) $= (1:1:5.1:15) \cdot c_0$; $c_0 = 2 \cdot 10^{-3}$(1), $3.5 \cdot 10^{-3}$(2), $5 \cdot 10^{-3}$(3); pH = 4.9, acetate buffer ($\mu = 0.1$); t = 5°.

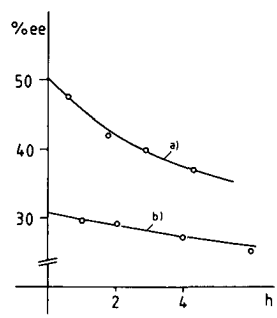


Fig. 2. *Observed % ee for leucine* (a) *and valine* (b) *formation as a function of time.* $c(Cu^{2+}) = c$(pyridoxamine) $= 5 \cdot 10^{-3}$; $c((S,S)$-**1**$) = 2.6 \cdot 10^{-2}$; leucine: c(keto acid) $= 7.5 \cdot 10^{-2}$, t = 5°; valine: c(keto acid) $= 1.5 \cdot 10^{-1}$, t = 25°; pH = 4.9; acetate buffer ($\mu = 0.1$).

A net increase in optical yield is observed, when the temperature of the reaction medium is lowered *(Table 1)*. The interpretation of this result is equivocal because the change of % ee with temperature is not only determined by the stability of the reacting mixed ligand complex and the stereoselectivity of the isomerization, but also by the rate of racemization relative to the rate of isomerization.

Table 1. *% Enantiomeric Excess for Alanine Formation, as a Function of Temperature*[a]) ($c(Cu^{2+}) = c$(pyridoxamine) $= 2 \cdot 10^{-3}$; $c((S,S)$-**1**$) = 1.02 \cdot 10^{-2}$; c(pyruvate) $= 3 \cdot 10^{-2}$; pH = 4.9; acetate buffer ($\mu = 0.1$))

t [°C]	5	15	25
% ee	35	31.5	26

[a]) The reaction was stopped after a reaction time corresponding to four half-lives.

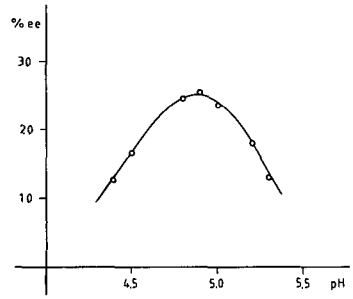


Fig. 3. *Variation of % ee with pH for alanine formation.* $c(Cu^{2+}) = c$(pyridoxamine) $= 2 \cdot 10^{-3}$; c((*S,S*)-1) $= 1.02 \cdot 10^{-2}$; c(pyruvate) $= 3 \cdot 10^{-2}$; acetate buffer ($\mu = 0.1$); t = 25°; reaction time = 60 min.

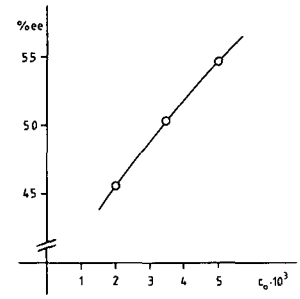


Fig. 4. *Variation of % ee of alanine formation extrapolated to zero reaction time with total concentration* (Conditions as indirected in *Fig. 1*).

The behaviour of the system at different pH-values is shown in *Fig. 3*. When the reaction is stopped after four half-lives of isomerization – the latter being independent of pH in the pH-range studied – the enantiomeric excess shows a maximum value around pH = 5. This behaviour may be explained by assuming two competing factors, each affecting the optical yield in opposite ways when the pH of the solution varies – an increase of the relative amount of mixed ligand complex, as opposed to an acceleration of the racemization.

Taking into account the results obtained for phenylalanine [1], the following series of relative rates of racemization is established: phen > ala > leu >> val. This series is the same as that observed for analogous reactions without auxiliary ligands [2], and it reflects steric as well as electronic influence of the amino acid substituents on the rate of configurational inversion at the asymmetric C-atom.

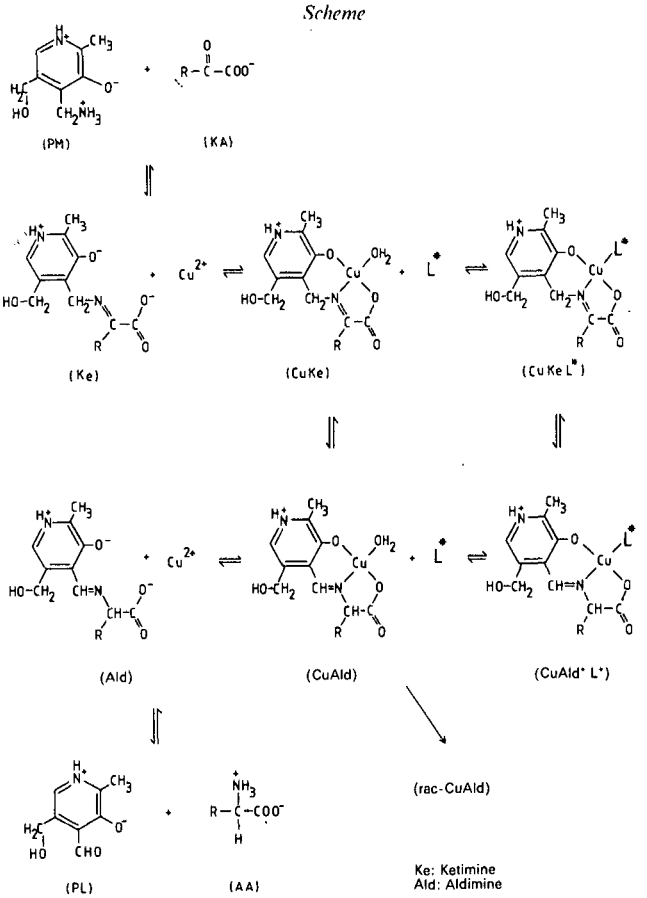
Discussion. – The optical yield of a complex asymmetric reaction depends not only on the diastereoselectivity of the reaction step leading to the desired product, but also on possible additional reaction paths giving the racemic product, as well as on the loss of optical activity through subsequent racemization of the product.

As it is seen from the *Scheme*, the optically active amino acid can be produced by the reaction of the mixed ligand complex CuKeL*. The exact amount of this intermediate species is not known. Furthermore, it is not possible to control this amount by application of limiting conditions, *i.e.* by increasing the ligand concentration, for two reasons: *a*) the unmixed species can only be eliminated by increasing ligand concentration, when the dismutation equilibrium *(Eqn. 2)* is strongly in favour

$$Cu^{2+} + CuKeL* \rightleftharpoons CuL^{*2+} + CuKe \qquad (2)$$

of the mixed species, and *b*) an increasing ligand concentration strongly catalyzes the racemization, as was shown for phenylalanine [1]. Nevertheless, a rough estimation of the amount of amino acid formed by the mixed ligand complex can be made in the following way. Keeping the proportions of the reactants constant and increasing the individual concentrations in the system, the mixed complex is favoured with respect to the unmixed species. On the other hand, the relative increase of the concentration of

Scheme



the mixed complex varies with the amount of the mixed complex already formed. If it is assumed that the enantiomeric excess observed depends in a linear way on the amount of mixed complex, the relative change of enantiomeric excess allows an estimation of the concentration of the mixed complex. *Fig. 4* shows the enantiomeric excess as a function of the total concentration for the reaction with pyruvic acid. The values are obtained extrapolating the %ee-data from *Fig. 1* to zero reaction time. From the slope of this curve it can be calculated that the relative amount of the mixed ligand Cu^{2+}-ketimine-complex lies between 65 and 70%. With respect to these values the stereoselectivity of the isomerization of the Cu^{2+}-ketimine-(*S,S*)-(**1**)-complex into the corresponding Cu^{2+}-(*R*)-aldimine-(*S,S*)-(**1**)-complex is in the order of 75 to 80%. For the enantioselective synthesis of alanine this value is amongst the highest ever observed.

Whereas the behaviour of leucine, obtained from the corresponding keto acid, seems very similar to alanine, the case of valine needs special consideration. As mentioned earlier [1], the isomerization of the Cu^{2+}-ketimine-complex from 3-methyl-2-oxobutyric acid is much faster than its formation. During the whole transamination reaction the Cu^{2+}-ketimine complex is therefore present only in very low concentration, corresponding to a steady state situation. This favours the formation of the mixed

complex. Nevertheless, the observed optical yield is lower in this case, even when one considers that the formation of valine was performed at a higher temperature (25 °C). As the stereoselectivity of reactions concerning valine are in general the most significant among all the amino acids [3], we believe that the lowering of the optical yield is a consequence of destabilization of the mixed Cu^{2+}-ketimine-complex, rather than a loss of its stereoselectivity.

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Experimental Part

Analysis. An aliquot of the reaction solution is acidified to pH = 2 with conc. HCl and introduced into an exchange column *Dowex 50* (length: 30 cm, diam.: 1.5 cm) in its H^+-form. The amino acid is eluted with 0.5 N HCl (alanine) or 0.1 N NH_3 (valine, leucine and phenylalanine), and the effluent solution is tested by TLC. The fractions containing the amino acid are collected and evaporated to dryness.

GC analyses of the isopropyl esters of *N*-(trifluoroacetyl)amino acids were performed using an optically active column *(Supelco): SP-300* 10% *Supelcoport 100–120* mesh. The derivatives of the amino acids were prepared as indicated in [4]: 10 ml 2.5 M HCl in 2-propanol are added to the dried sample and heated to 100° during 4 h in a sealed flask. After cooling, the mixture is evaporated to dryness at r.t. and then dissolved in 3 ml CH_2Cl_2. After cooling to −20° 3 ml trifluoroacetic anhydride is added and the mixture stirred at r.t. for 1 h. After evaporation to dryness, the obtained derivative for GC analysis is dissolved in 0.5 ml of $CHCl_3$.

Table 2. *Retention Time* (min) *of the Isopropyl Esters of* (R)- *and* (S)-N-*(Trifluoroacetyl)amino-Acids*

Amino acid	(*R*)	(*S*)	(*S*)/(*R*)
Alanine	22	26	1.18
Valine	34	40	1.18
Leucine	78	99	1.27
Phenylalanine[a])	290	345	1.19

[a]) t_{column} = 130°; flow rate: 30 ml N_2/min.

Chromatography: column length: 4 m, internal diameter: 2 mm, t_{column} = 120°, flow rate: 20 ml N_2/min. Retention times for amino acids are given in *Table 2*. The relative amounts of the antipodes were calculated by integration of the corresponding peaks of the chromatogram. For each of the amino acids used the separation of the antipodes was complete.

Materials. The synthesis of 2,6-bis[(3*S*)-3-phenyl-2-azabutyl]pyridine (**1**) was described in [1]. Keto acids and pyridoxamine were of analytical grade *(Fluka)* and used without further purification.

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LISTE DES PUBLICATIONS

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"Helvetica Chimica Acta, Vol. 66, Fasc. 7, p. 2049-2058 (1983)"

Stereoselectivity in Reactions of Metal Complexes VIII[1].

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