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.

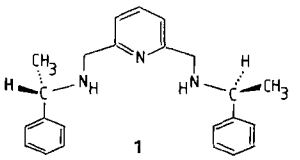
Results. – When an α-keto acid (KA) reacts with pyridoxamine (PM) in the presence of the optically active Cu^{2+}-complex of **1** transamination takes place *(Eqn. 1)*[3]) and an optically active amino acid is obtained.

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

[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].

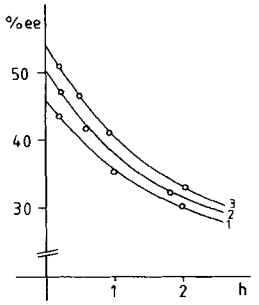


Fig. 1. *Observed % ee for alanine formation as a function of reaction time and reagent concentrations.* $c(Cu^{2+}) = c$(pyridoxamine): c((*S,S*)-**1**): c(pyruvate) = (1:1:5.1:15) · c_0; $c_0 = 2 \cdot 10^{-3}$(1), $3.5 \cdot 10^{-3}$(2), $5 \cdot 10^{-3}$(3); pH = 4.9, acetate buffer ($\mu = 0.1$);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

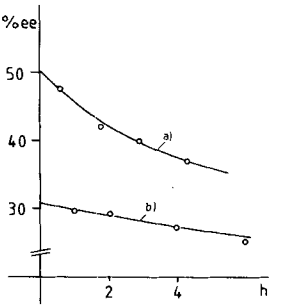


Fig. 2. *Observed % ee for leucine* (a) *and valine* (b) *formation as a function of time.* $c(Cu^{2+}) = c$(pyridoxamine) = $5 \cdot 10^{-3}$; c((*S,S*)-**1**) = $2.6 \cdot 10^{-2}$; leucine: c(keto acid) = $7.5 \cdot 10^{-2}$, t = 5°; valine: c(keto acid) = $1.5 \cdot 10^{-1}$, t = 25°; pH = 4.9; acetate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

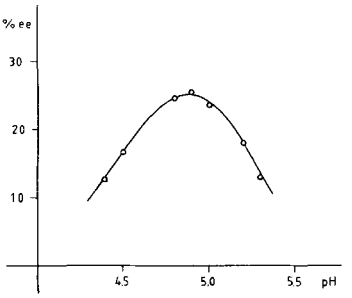


Fig. 3. *Variation of % ee with pH for alanine formation.* $c(Cu^{2+}) = c$(pyridoxamine) $= 2 \cdot 10^{-3}$; c((*S,S*)-**1**) $= 1.02 \cdot 10^{-2}$; c(pyruvate) $= 3 \cdot 10^{-2}$; acetate buffer ($\mu = 0.1$); t = 25°; reaction time = 60 min.

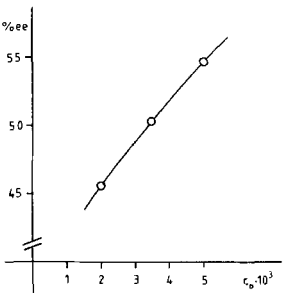


Fig. 4. *Variation of % ee of alanine formation extrapolated to zero reaction time with total concentration* (Conditions as indirected in *Fig. 1*).

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.

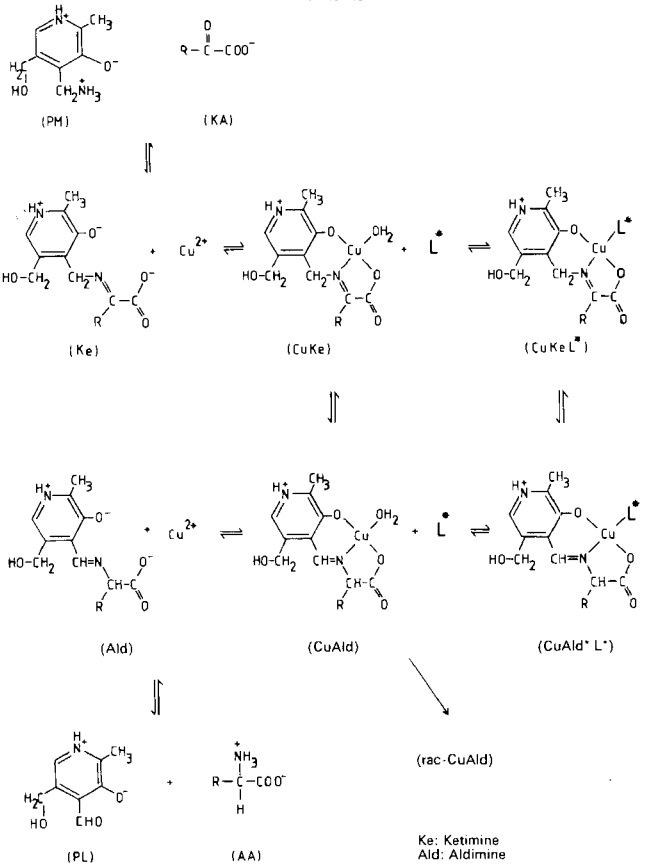
Discussion. – The optical yield of a complex asymmetric reaction depends not only on the diastereoselectivity of the reaction step leading to the desired product, but also on possible additional reaction paths giving the racemic product, as well as on the loss of optical activity through subsequent racemization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