The Isomers of α-Amino-acids with Copper(II). Part 4.[1] Catalysis of the Racemization of Optically Active Alanine by Copper(II) and Pyruvate in Alkaline Solution

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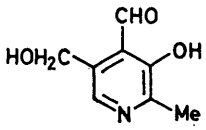
Aqueous solutions containing copper(II) ions, pyruvate, and L(+)-alanine in the molar ratio 1 : 2 : 4 give rapid racemization of alanine. The reaction is first order in the concentration of base, $k_{obs.} = (5.43 \times 10^{-2})[OH^-]\ s^{-1}$ at 60 °C. For the molar ratios $1 : 1 : 4 \leqslant [Cu^{II}] : [pyruvate] : [alanine] \leqslant 1 : 4 : 4$ the reaction is to a very good approximation first order in the concentration of added pyruvate, $k_{obs.} = (2.89 \times 10^{-4})[pyruvate]\ s^{-1}$. We attribute the racemization to the involvement of Schiff-base formation. In similar systems containing Co^{II}, Ni^{II}, or Zn^{II} racemization is also promoted; with Cu^{II} the racemization of threonine and glutamic acid is also promoted.

METAL-CATALYSED racemization of amino-acids co-ordinated as Schiff bases has been well studied,[2-9a] the original work of Snell having stimulated effort on many analogous systems. These studies have generally utilized an aromatic aldehyde deriving either from pyridoxal [2,5] (1) or salicylaldehyde (2),[3,4] Schiff-base complexes of type (3) usually being the reactive species.



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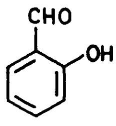

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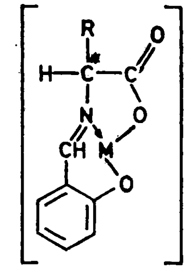
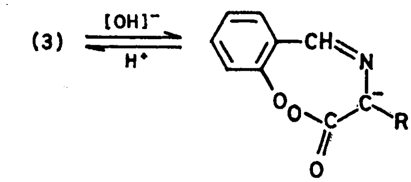


(3)

The racemization is promoted by activation of the system as outlined below [1b] (the metal ion is omitted):



In systems deriving from Cu^{II} and L(+)-alanine (Ala) * little or no racemization was detectable until deamination and pyruvate formation had occurred.[1] We were therefore led to believe that the Schiff base formed from Cu^{II}, pyruvate, and Ala would be an effective catalyst for alanine racemization. The addition of large quantities of pyruvate to Cu^{II}–Ala systems indeed

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* All the amino-acids were used as their L form. For this paper and to simplify naming, L-AlaO$^-$ is A, the pyruvate ion P, and the dianionic Schiff base A–P.

[1] Part 3, R. D. Gillard, P. O'Brien, P. R. Norman, and D. A. Phipps, *J.C.S. Dalton*, 1977, 1988.

[2] J. Olivard, D. E. Metzler, and E. E. Snell, *J. Biol. Chem.*, 1952, **199**, 669.

[3] M. Ando and S. Emoto, *Bull. Chem. Soc. Japan*, 1969, **42**, 2628.

[4] M. Ando and S. Emoto, *Bull. Chem. Soc. Japan*, 1969, **42**, 2624.

[5] M. Ando and S. Emoto, *Bull. Chem. Soc. Japan*, 1975, **48**, 1655.

[6] K. Hirota, K. Miyamota, and Y. Izumi, *Bull. Chem. Soc. Japan*, 1967, **40**, 178.

[7] M. Ikawa and E. E. Snell, *J. Amer. Chem. Soc.*, 1954, **76**, 653.

[8] K. Toi, Y. Izumi, and S. Akabori, *Bull. Chem. Soc. Japan*, 1963, **36**, 734.

[9] (*a*) K. Toi, Y. Izumi, and S. Akabori, *Bull. Chem. Soc. Japan*, 1963, **36**, 829; (*b*) D. E. Metzler, M. Ikawa, and E. E. Snell, *J. Amer. Chem. Soc.*, 1954, **76**, 648.

produces high rates of racemization and a first-order decay of rotation. The rate of racemization is comparable to that for amino-acids in salicylaldehyde complexes.[4]

RESULTS AND DISCUSSION

Nature of the Species in Solution.—We have previously noted[1] the increase in molar rotation measured at 546 nm [α_n (546 nm)] when pyruvate is added to solutions containing Cu^{II} and pyruvate in the alkaline pH region. Before undertaking a study of racemization in the pyruvate–Ala–Cu^{II} system a more thorough study of the dependence of the observed rotation on added pyruvate was undertaken. Over the range [Cu^{2+}] : [P] : [A] = 1 : x : 4 where $1 \leqslant x \leqslant 4$ and [Cu^{2+}] = 1 × 10^{-2} mol dm^{-3} the observed variation of rotation was to a good approximation linear in total pyruvate concentration (Figure 1).

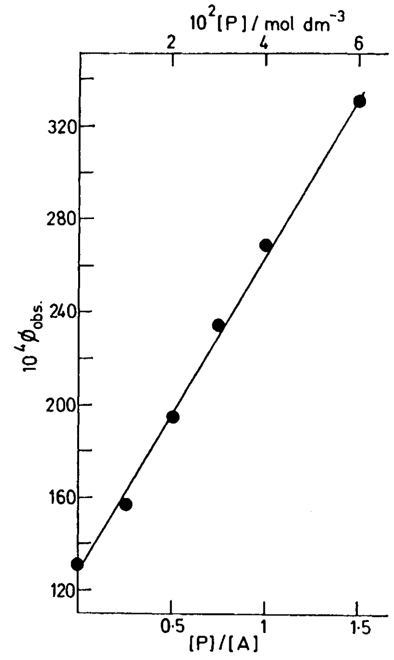


FIGURE 1 Dependence of optical rotation at 546 nm on added pyruvate. Measured in a 1-cm cell at 20 °C. [Cu^{II}] = 1 × 10^{-2} mol dm^{-3}, pH = 9.2

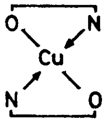
TABLE 1

Stability constants[a] of some copper(II) complexes

Ligand	K_1	K_2	β_2 (or K_{11})
L-Alanine	8.22	6.85	15.07
Glycine	8.59	7.24	15.83
Pyruvate	4.42	2.78	7.20
Salicylaldehyde	5.36	4.75	10.11
(Glycine–salicylaldehyde)[b]			16.15

[a] Given as log$_{10}K$ from 'Stability Constants of Metal-Ion Complexes,' eds. L. G. Sillen and A. E. Martell, *Special Publ.*, The Chemical Society, London, 1971, no. 25. [b] Schiff base.

In order to account for the varying reactivities of these solutions we need to consider the major species present. The stability constants of several possible complexes (Table 1) have been determined, and from these we may derive relative concentrations for some complexes in these solutions. The complexes which the solution may contain (in this pH region) are (4)—(7), where N–O represents AlaO$^-$, O–O pyruvate, and O–N–O the dianionic 1 : 1 Schiff base. For equal concentrations of free pyruvate and alaninate ([P] = [A]), the relative concentrations of bis(alaninato)copper(II) * [CuA$_2$] (4) and bis(pyruvato)copper(II) [CuP$_2$] (5) will be given by $[CuP_2]/[CuA_2] = \beta_{2(P)}/\beta_{2(A)} = 1.35 \times 10^{-8}$. Similarly, we may calculate the concentration of the mixed complex [CuA(P)] (6) at [A] = [P] by using a statistical value for the formation constant of the mixed complex. Thus $2\log K_{(A)(P)} = \log 4 + \log K_{1(A)} + \log K_{2(A)} + \log K_{1(P)} + \log K_{2(P)}$, giving $[CuA(P)]/[CuA_2] = K_{(A)(P)}/\beta_{2(A)} = 2.34 \times 10^{-4}$. Therefore, since we may safely discount (5) and (6) as major species in solution, the dominant species in solution must be (4) and some highly stable complex responsible for the enhanced molar rotations.



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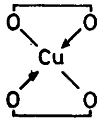

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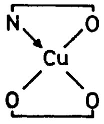

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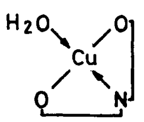

(7)

This last molecule we take to be (7) for the following reasons. Schiff bases of amino-acids generally form very stable complexes.[10a] Few thermodynamic studies have been directed toward copper(II)–Schiff base complexes, and the stability of the [*N*-(1-carboxylatoethylidene)-L-alaninato]copper(II) complex is not yet known, although Leussing has placed values of 0.06 and 0.32 on the equilibrium constant for Schiff-base formation from its components in the presence of various other metals. An approximate value for the stability constant of this copper(II)–Schiff-base species may be predicted as follows. The stability of the Schiff-base complex formed from glycinate, salicylaldehyde, and Cu^{II} is known,[10a] and, in general, glycinato-complexes are approximately an order of magnitude more stable than are the analogous alaninates. So, assuming a roughly equivalent contribution from salicylaldehyde, we have $K_{(A-P)} = 10^{13}$—10^{14} for $Cu^{2+} + A^- + P^- \rightleftharpoons [Cu(A\text{-}P)]$. This stability is comparable to that for [CuA$_2$].

Effectively, the solution will contain only free alaninate (A), [CuA$_2$], free pyruvate (P), and [Cu(A–P)]. The observed rotation may be expressed (in an obvious nomenclature) as in (i) where ϕ_0 is the molar rotation of a similar solution containing no pyruvate and

$$\phi_{obs.} = \phi_A[A] + \phi_{CuA_2}[CuA_2] + \phi_{Cu(A-P)}[Cu(A\text{-}P)] \qquad (i)$$
$$\approx \phi_0 + (\phi_{Cu(A-P)} - \phi_{CuA_2})[Cu(A\text{-}P)]$$

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* We assume that the *trans* isomer only of (4) is present in solution.

[10] (*a*) D. L. Leussing and K. S. Bai, *Analyt. Chem.*, 1968, **40**, 575; (*b*) Y. Nakao, K. Sakurai, and A. Nakahara, *Bull. Chem. Soc. Japan*, 1966, **39**, 1471.

$\phi_{\mathrm{Cu(A-P)}} \gg \phi_{\mathrm{A}}$ and $\phi_{\mathrm{CuA_2}} \gg \phi_{\mathrm{A}}$. Mass balance will simplify as (ii) and equation (iii) follows.

$$[\mathrm{P}]_{\mathrm{T}} = [\mathrm{P}] + [\mathrm{Cu(A-P)}] \qquad \text{(ii)}$$

or

$$[\mathrm{P}] = \frac{[\mathrm{P}]_{\mathrm{T}}}{1 + K_{\mathrm{Cu(A-P)}}[\mathrm{Cu}][\mathrm{A}]}$$

$$\phi_{\mathrm{obs.}} = \phi_0 + \frac{(\phi_{\mathrm{Cu(AP)}} - \phi_{\mathrm{CuA_2}})[\mathrm{P}]_{\mathrm{T}}}{1 + (K_{\mathrm{Cu(A-P)}}[\mathrm{Cu}][\mathrm{A}])^{-1}} \qquad \text{(iii)}$$

Further evidence for the new species in solution comes from the circular dichroism (c.d.) spectrum. The c.d. spectrum (visible region) hardly changes on the addition of pyruvate: a slight decrease in intensity is observed. However, a new c.d. band *is* observed at 360 nm (Figure 2). We attribute this to ligand-to-metal charge transfer, which would occur at lower energy for the more conjugated Schiff-base ligand than for L-alanine. The new and reactive species in solution is almost certainly (7) and possibly its conjugate aqua-base.

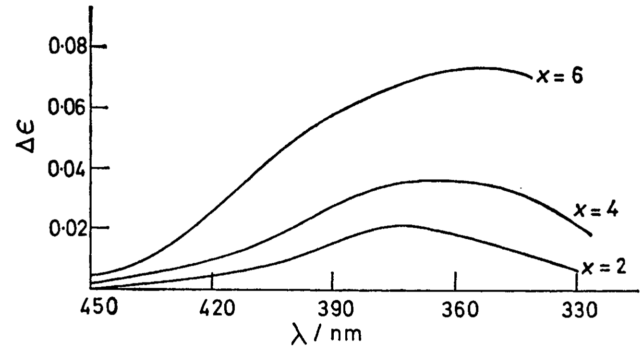


FIGURE 2 C.d. spectrum in the near-u.v. region of solutions having $[\mathrm{Cu^{2+}}] : [\mathrm{AlaO^-}] : [\mathrm{P}] = 1 : 4 : x$ at pH 10.0. The molar circular dichroism is calculated for the total copper content

Complex (7) has been prepared.[10b] Unfortunately, we encountered difficulty in repeating the preparation: our products were rather unstable. The physical properties of the solutions used for kinetic studies (c.d., optical rotation, and electronic spectra) were all independent of pH. The stability of 1 : 1 : 1 solutions of Cu$^{\mathrm{II}}$–pyruvate–Ala [*i.e.* those containing high concentrations of (7)] were low with respect both to pH and temperature.[9a] An excess of ligand was therefore employed in all the studies reported. The usual decomposition products were copper(I) or copper(II) oxides and ammonia; exceptionally, reduction as far as copper has been observed. Identical decomposition problems were encountered when crude samples of the complex [10b] were dissolved and racemization studies attempted.

Kinetics of Racemization.—The system contains the following dominant species; alaninate (A) (L-AlaO$^-$ or D-AlaO$^-$), [CuA$_2$], and [Cu(A–P)]. Hence the observed rotation of the solution will be given by equation (i). We know that, under the present mild conditions, alanine and [CuA$_2$] themselves do not undergo rapid racemization.[5,10a] When $k_{\mathrm{obs.}}$ is the apparent rate constant for the interconversion of L- and D-alanine in the system (iv), integrating and expressing in terms of $\phi_{\mathrm{obs.}}$ gives (v). Hence we may measure pseudo-first-order rate constants for the interchange of D- and L-alanine in the system and the results are summarized

$$\text{L(+)-alanine} \underset{-k_{\mathrm{inv.}}}{\overset{k_{\mathrm{inv.}}}{\rightleftharpoons}} \text{D(−)-alanine} \qquad \text{(iv)}$$

$$\ln(\phi_t/\phi_0) = -2k_{\mathrm{inv.}}t \qquad \text{(v)}$$

in Table 2. We attribute racemization to the Schiff base (7): the concentration of this species will determine $k_{\mathrm{obs.}}$, and since the latter is approximately linearly dependent on total pyruvate concentration (in the region studied) we see the observed dependence on total pyruvate (Table 2).

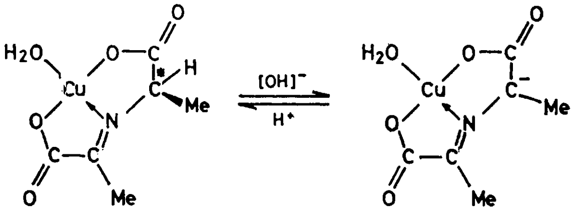
TABLE 2

Summary of kinetic results at 60 °C, with $[\mathrm{AlaO^-}] = 4 \times 10^{-2}$ and $[\mathrm{Cu^{II}}] = 1 \times 10^{-2}$ mol dm^{-3}

10^5[OH]	10^2[P]	$10^6 k_{\mathrm{obs.}}$	Rate law *
mol dm^{-3}		s^{-1}	
3.09	2	2.29	$k = (5.43 \times 10^{-2})[\mathrm{OH^-}]$ s^{-1}
6.03	2	4.03	
11.20	2	5.64	
37.20	2	20.76	
67.61	2	37.10	
4.89	1	2.89	$k = (2.89 \times 10^{-4})[\mathrm{P}]$ s^{-1}
4.89	2	5.58	
4.89	3	8.27	
4.89	4	11.57	

* $k = k_{\mathrm{rac.}}/2$.

We postulate that the racemization involves carbanion formation (vi) at the Schiff base (7) (under these mild conditions). The rate-determining step of the reaction is probably the abstraction of a proton from the α-carbon atom of the co-ordinated alaninate. This being the case, we would expect the reaction to be catalysed by base, and this was indeed found (Table 2). That tautomeric equilibria are significant and rapid in similar systems has been elegantly demonstrated by Yoneda *et al.* for Schiff-base complexes derived from palladium, glyoxylic acid, and alanine.[11] It should be noted that in the present alanine system (unlike other amino-acids) deamination is hindered by the presence of pyruvate since this would be the product of deamination.



(vi)

Other Metals. A brief survey was made of other first-row transition elements to see whether they were also effective in prompting alanine racemization. At pH >9.0 (60 °C), Zn$^{\mathrm{II}}$, Co$^{\mathrm{II}}$, and Ni$^{\mathrm{II}}$ all promoted racemization in the metal–pyruvate–amino-acid (1 : 2 : 4) system. However, in both the cobalt(II) and nickel(II) systems there was a problem of pre-equilibration, presumably involving Schiff-base formation. However,

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[11] H. Yoneda, Y. Morimoto, Y. Nakao, and A. Nakahara, *Bull. Chem. Soc. Japan*, 1968, **41**, 255.

Zn^{II} behaved in a similar way to Cu^{II}. Cobalt(II) and Ni^{II} were much less effective in promoting the racemization than Zn^{II} which in turn was slightly less effective than Cu^{II}; the Irving–Williams order alone ($Co < Ni < Cu > Zn$) suffices to explain this.

Other Amino-acids. Various other amino-acids were studied in the Cu^{II}–amino-acid–pyruvate system. Of these, L-glutamic acid and L-threonine were effectively racemized, whereas L-valine and L-proline were not. This is the expected result for proline (where Schiff-base formation is unlikely) but not for valine where we would expect Schiff-base formation to be possible. An attempt to study the amino-acids L-tyrosine, -phenylalanine, -glutamine, and -leucine failed because of the low solubility of their complexes in water.[12] Studies of L-aspartic acid and -asparagine were made difficult by their extremely low molar rotations at 546 nm.

It seems likely that ready racemization of amino-acids in the presence of Cu^{II}, when it occurs, involves the formation of α-keto-acids which yield reactive Schiff bases. For example, Guha and Saha [13] used the method of Taurins [14] to make dichlorobis(ornithine)copper(II) dihydrate from boiling solutions of L-ornithine. However, their product had the DL configuration, so, as was pointed out by Stephens *et al.*,[15] racemization had occurred. In the same way, Taurins pointed out [14] that the same lysine complex $[Cu(Lys)_2][HgI_3]_2$ is obtained from heated solutions of D- or L-lysine. This presumably indicates either spontaneous resolution of the '*racemic*' solid, or (more likely judging from the reported yield) racemization of the L-lysine.

EXPERIMENTAL

AnalaR copper(II) sulphate (B.D.H.) was used as the source of Cu^{II} throughout. AnalaR (B.D.H.) reagents were also used as the sources of other metal ions. All the amino-acids were B.D.H. (chromatographically homogeneous) products. Sodium pyruvate [B.D.H. or Aldrich (dimer free)] was used as the source of pyruvate; good reproducibility (better than 5%) was found between different batches of pyruvate: refrigerated stock solutions kept for up to 10 d. Potassium hydroxide (B.D.H. AnalaR) 1M factors were used to adjust the pH in all except buffered runs which utilized standard borax buffers (pH 9.24, 20 °C).

Kinetics.—Volumetrically prepared solutions of amino-acid, $Cu[SO_4]$, and pyruvate were mixed and the mixture adjusted to an appropriate pH with 1 mol dm^{-3} K[OH]. All the kinetic runs were performed at 60 °C, solutions being quenched on ice and the optical rotation recorded. At least three readings of the rotation of each sample were taken and a mean used in the calculation of rate constants. The pH was measured during the kinetic run; the calibration of the meter was checked in buffer (EIL) at 60 °C. Correlation coefficients for the rate constants thus determined were usually better than 0.98. The absorption spectrum remained constant during a run, which together with the good first-order decay indicates the dominance of racemization.

Instrumentation.—Rotations were measured with a N.P.L. type 246 polarimeter at 546 nm (the mercury green line), pH with a Jenway 300 pH meter, circular dichroism with a Roussel Jouan Dichrographe B, and visible spectra with a Pye–Unicam SP 8000 spectrophotometer.

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[12] D. P. Graddon and L. Munday, *Chem. and Ind.*, 1959, 122.

[13] S. Guha and N. N. Saha, *Acta Cryst.*, 1970, **B26**, 2073.

[14] A. Taurins, *Canad. J. Res.*, 1950, **B28**, 762.

[15] F. S. Stephens, R. S. Vagg, and P. A. Williams, *Acta Cryst.*, 1977, **B33**, 438.