Metal Ion Catalysis in Transamination. III. Nickel(II) and Zinc(II) Mixed Complexes Involving Pyruvate and Various Substituted Aliphatic Amino Acids[1]

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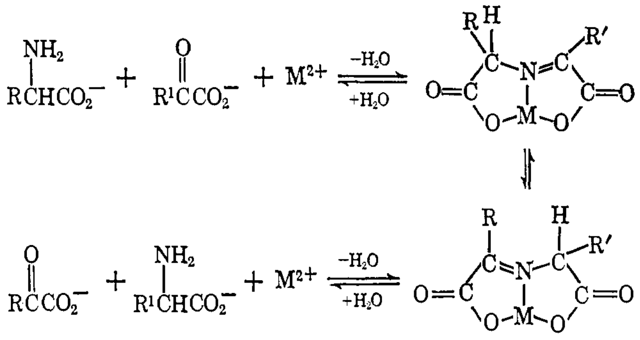
Abstract: Mixed Ni(II) and Zn(II) complexes involving pyruvate and an amino acid anion were studied. With glycinate, α-alanate, isoleucinate, and β-alanate, imine complexes are formed. The chelate ring systems with all ligands but the last appear to be strained. The pyruvate methyl group interferes with alkyl substituents on the α carbon of the amino acid, weakening the mixed complexes. Sarcosinate forms weak "normal" mixed complexes in which the ligands for the most part are independently coordinated; however, some contribution of carbinolamine complexes is indicated by the data.

Enzymatic transamination ordinarily requires pyridoxal phosphate as a cofactor;[2] however, nonenzymatic transamination has been observed in the absence of pyridoxal with glyoxalate[3,4] and pyruvate.[5] The reactions are metal ion catalyzed and the mechanism appears to involve the formation of an intermediary Schiff base according to the scheme



The facility with which enzymatic transamination proceeds is influenced by the nature of the substituent on the carbon of the amino acid; *e.g.*, valine shows relatively little tendency for transamination. To gain further insight as to which are the important factors in transamination, it is of interest to examine the properties of various Schiff base complexes involving amino acids and having simple aliphatic oxo acid anions, such as pyruvate, as coligands. Furthermore, these purely aliphatic Schiff bases have interest in their own right, since it is reasonable to assume that they and their complexes played an important role in the primordial processes.

In these laboratories, potentiometric techniques have been successfully employed to study equilibria in Schiff base–metal ion systems.[6] The data are processed using a high-speed digital computor. Least-square values of the constants β_{ij} are obtained pertaining to the equilibria, $M^{2+} + iO^- + jA^- \rightleftharpoons MO_iA_j^{2-(i+j)}$ (O^- = oxo acid anion, A^- = amino acid anion). In this respect, Schiff base complex equilibria are considered no different from equilibria in ordinary mixed complex systems.[7] From the values of β_{ij} it may be deduced whether or not the complexed ligands are condensed. Owing to the highly dissociated nature of most Schiff bases in aqueous media, this approach is fruitful. Any tendency for a side equilibrium, such as appreciable association of the uncomplexed ligands, is easily included in the algorithm for the system.

In the pyruvate–glycinate studies[6] the mixed species MOA, MOA_2^-, and $MO_2A_2^{2-}$ were found. Their relatively high stabilities suggested that the coordinated ligands are condensed as the Schiff base. This conclusion was verified by an nmr study.[8] The values of the constants indicate that the second amine in the species MOA_2^- is independently bound.

In the present investigation the effects of alkyl substituents on the α carbon of the α-amino acid and on the nitrogen atom were investigated as well as the effect of increasing the chain length of the amino acid.

Experimental Section

Pyruvic acid was purified and used as previously described.[6] DL-α-Alanine (Eastman), sarcosine hydrochloride (Matheson Coleman and Bell), L-isoleucine (allo free, Calbiochem and Sigma), and β-alanine (Matheson Coleman and Bell) were recrystallized from ethanol–water and dried *in vacuo*. Titration with standard sodium hydroxide in the presence of formaldehyde[9] indicated purities for α-alanine, 100.0 ± 0.1%; β-alanine, 99.6 ± 0.1%; and isoleucine, 97.9 ± 0.2%. Titration of sarcosine hydrochloride to the first end point indicated a purity of 99.2± %.

Stock solutions of the sodium salts of the amino acids were freshly prepared by weighing the amino acid and then diluting to the desired volume after dissolving in an accurately measured equivalent of a standard sodium hydroxide solution.

The experiments were designed essentially the same as before.[6] Pyruvate dimerization[8] interfered more seriously in the present investigation so concentrations were maintained at somewhat lower levels than used previously, 0.010, 0.020, and 0.030 *M* pyruvate with 0.010 *M* MCl_2.

Although dimerization was severe with sarcosinate, the mixed species were so weak that it was necessary to raise the concentration levels to 0.050, 0.10, and 0.15 *M* pyruvate and 0.050 *M* MCl_2 in order to obtain suitable constants. The data with 0.15 *M* pyruvate were not used in the calculations owing to extensive dimerization.

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(1) Support by the National Science Foundation, GP 1627, is gratefully acknowledged.

(2) E. E. Snell, "Chemical and Biological Apsects of Pyridoxal Catalysis," E. E. Snell, P. M. Fasella, A. Braunstein, and A. Rossi-Fanelli, Ed., The MacMillan Co., New York, N. Y., 1963, p 1.

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(4) H. I. Nakada and S. Weinhouse, *J. Biol. Chem.*, **204**, 831 (1953).

(5) H. I. Mix, *Z. Physiol. Chem.*, **315**, 1 (1959).

(6) (a) D. L. Leussing and D. C. Schulz, *J. Am. Chem. Soc.*, **86**, 4846 (1964); (b) D. L. Leussing, *Talanta*, **11**, 189 (1964).

(7) J. I. Watters, *J. Am. Chem. Soc.*, **81**, 1560 (1959).

(8) D. L. Leussing and C. K. Stanfield, *ibid.*, **86**, 2085 (1964).

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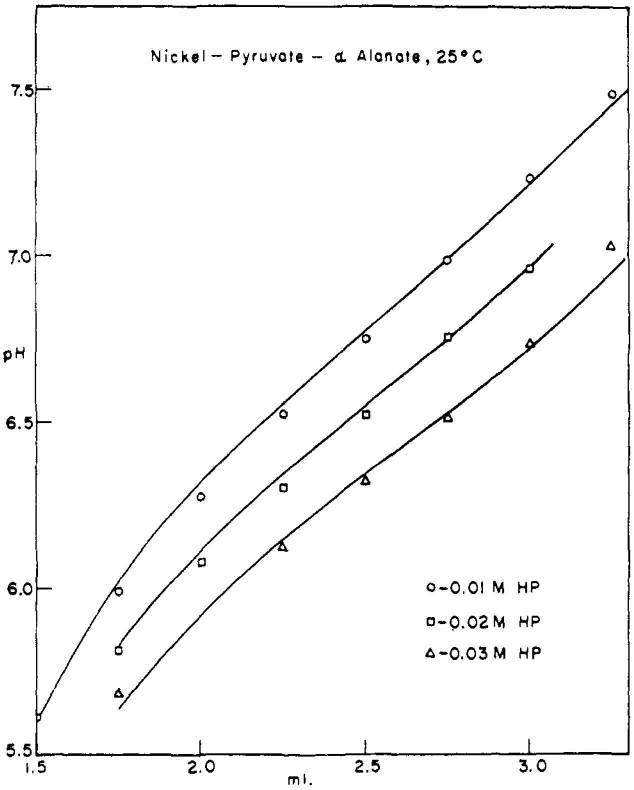

Figure 1. Titration of nickel(II)–pyruvate with 0.2000 *M* sodium alanate. Initial conditions: 0.01000 *M* $NiCl_2$, 25.00 ml; ○, 0.01029 *M* HP; □, 0.02058 *M* HP, 0.01004 *M* NaOH; △, 0.03087 *M* HP, 0.02004 *M* NaOH. The solid lines are the theoretical curves computed using the constants in Table I.

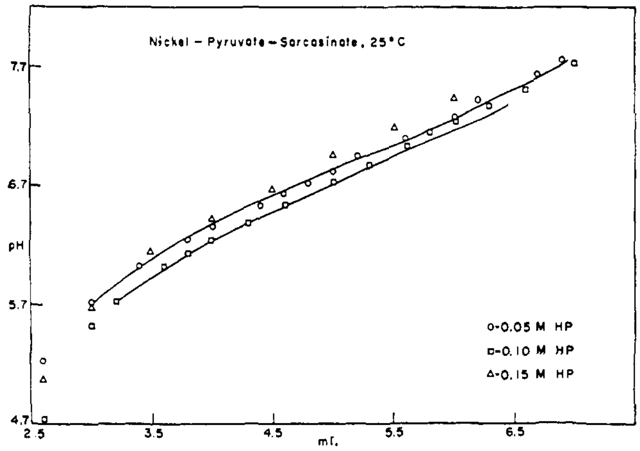

Figure 2. Titration of nickel(II)–pyruvate with 0.5000 *M* sodium sarcosinate illustrating effect of pyruvate dimerization on the titration curves. Initial conditions: 0.04989 *M* $NiCl_2$, 25.0 ml; ○, 0.04977 *M* HP; □, 0.0996 *M* HP, 0.04518 *M* NaOH; △, 0.150 *M* HP, 0.100 *M* NaOH. The solid lines are the theoretical curves computed for the first two experiments.

The more pronounced pyruvate dimerization in these studies no doubt arises from the faster rate of the base-catalyzed reaction. Solutions containing the substituted amines tend to be more alkaline owing to the formation of weaker complexes.

The test solutions, 0.50 *M* in KCl, were titrated at 25° under a blanket of nitrogen with the standard solutions of the sodium salts of the amino acids. The pH measurements were made using a Radiometer pH 27 pH meter. The electrodes were standardized using buffers prepared from NBS standard pH reagents.

The solutions were monitored by recording the pH as a function of time to determine the establishment of equilibrium. The equilibration times with the C-substituted amino acids were appreciably longer than those observed in the glycinate studies.[6] With Zn(II), 0.5–2 hr was required, depending on conditions. The Ni(II) systems required up to 5–7 hr to reach the equilibrium, so with this metal ion batch-wise experiments were run. Each solution represented a single point on the titration curve. The pH was determined after equilibrating 8 hr in a water bath. The sarcosine–pyruvate rates were found to be relatively fast (5–10 min) for both Ni(II) and Zn(II).

The equilibrium constants for the simple amino acid anion–metal ion complexes were determined using standard titration techniques, as were also the Schiff base formation constants, β_{OA}, for the reactions $O^- + A^- \rightleftharpoons OA^{2-}$.[6]

With all amino acids except α-alanate, difficulties were encountered with precipitation of the complexes and/or metal hydroxides. This interference was found to be greater with Zn(II) than with Ni(II). Certain concentration and pH ranges therefore could not be studied, precluding the accurate evaluation of some of the constants. Typical titration curves are illustrated in Figures 1 and 2.

Results and Discussion

The β_{ij} values are given in Table I. First, comparing the series $RCHNH_2CO_2^-$ (R = H, CH_3, $CH_3CH_2CH(CH_3)$–) for the simple complexes it is seen that β_{0j} values decrease with increasing size of R. The effect is somewhat larger with Ni(II) than with Zn(II). This is in agreement with previous observations that steric effects tend to be somewhat smaller with Zn(II) than with Ni(II)[10,11] where the ligand field stabilization energies of the d^8 system tend to enforce a more stringent octahedral configuration. The ability of Zn(II) to easily change from octahedral to tetrahedral and intermediate configurations is of benefit in reducing ligand–ligand repulsions.

In the mixed MOA complexes, the β_{ij} values are also seen to decrease with increasing size of R. The cumulative constants do not have the same units for the mixed and the simple complexes, so a comparison of steric effects between these two types is facilited by considering the stepwise constants for pyruvate addition. The pyruvate addition constants calculated from the data of Table I are presented in Table II along with the stepwise constants for the addition of the second amino acid to the complex of the first.

In the Ni(II) series a very pronounced decrease in the affinity for pyruvate is observed in the order glycinate > α-alanate > isoleucinate. The decrease in stability is greater in the mixed than in the simple complexes. Indeed, with the last ligand the pyruvate addition constant is actually less than that for the aquo Ni(II) ion. This decrease also tends to occur in the Zn(II) series but to a lesser extent. The effect seems to have a steric origin. Models show that in the Schiff base complexes, where the carboxylate groups are coordinated to *trans* octahedral positions, interference will occur between the pyruvate methyl and a substituent on the α carbon of the amino acid. Distortion toward tetrahedral coordination permitted by Zn(II) increases the separation between the interfering groups and reduces the magnitude of the effect.

The very low K_{01}^{11} for Ni(II)–isoleucinate suggests that the ligands are independently bound in the mixed complex. However, the absorption spectra indicate otherwise (see below). Furthermore, the β_{22} values with Ni(II)–isoleucinate–pyruvate are of the order of

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[11] D. L. Leussing, *Inorg. Chem.*, 2, 77 (1963).

Table I. Log Formation Constants of Pyruvate–Amino Acid Anion Complexes at 25° in 0.50 *M* KCl[a]

$$M^{2+} + iO^- + jA^- \rightleftharpoons MO_iA_j; \qquad \beta_{ij} = [MO_iA_j]/([M^{2+}][O^-]^i[A^-]^j)$$

	Log β_{10}[b]	Log β_{20}[b]
Ni^{2+}	1.121	0.46
Zn^{2+}	1.258	1.98

A		β_{01}[d]	β_{02}	β_{03}	β_{11}	β_{12}	β_{22}	No. of points[e]	Σ[e]	β_{OA}[f]
Glycine[c]										
$pK_{1a} = 2.47$	Ni^{2+}	5.653	10.505	13.95	8.068 ± 0.011	12.95 ± 0.04	15.283 ± 0.002	44	0.0257	0.40
$pK_{2a} = 9.70$	Zn^{2+}	4.878	9.009	11.02	7.601 ± 0.019	11.87 ± 0.10	14.352 ± 0.019	33	0.0420	
α-Alanine										
$pK_{1a} = 2.44$	Ni^{2+}	5.312	9.725	12.73	7.140 ± 0.045	11.63 ± 0.14	14.025 ± 0.058	20	0.041	0.32
$pK_{2a} = 9.83$	Zn^{2+}	4.556	8.520	10.51	6.785 ± 0.022	9.36 ± 0.14	13.013 ± 0.028	15	0.0177	
Isoleucine										
$pK_{1a} = 2.66$	Ni^{2+}	5.220	9.45	Pptn	6.21 ± 0.12	11.08 ± 0.10	13.053 ± 0.074	21	0.0208	1.32
$pK_{2a} = 9.70$	Zn^{2+}	4.494	8.487	10.9 (pptn)	6.760 ± 0.016	9.58 ± 0.04	12.340 ± 0.052	15	0.0265	
β-Alanine										
$pK_{1a} = 3.62$	Ni^{2+}	4.459	7.840	9.55	8.338 ± 0.014	11.95 ± 0.01	15.173 ± 0.020	19	0.020	0.91
$pK_{2a} = 10.21$	Zn^{2+}	3.9	Pptn	Pptn	7.08		12.1	20		
Sarcosine										
$pK_{1a} = 2.30$	Ni^{2+}	5.236	9.544	12.4	5.97 ± 0.06	7.78 ± 0.14	8.74 ± 0.11	28	0.038	0.76
$pK_{2a} = 10.14$	Zn^{2+}	4.314	8.3	Pptn	4.60 ± 0.40	9.85 ± 0.06	10.1[a] (pptn)	22	0.026	

[a] O^- = pyruvate, $pK_a = 2.35$. [b] Bis pyruvato complexes have been found using spectrophotometric methods. These new values of β_{10} and β_{20} were calculated from the spectrophotometric data. D. E. Tallman, unpublished results, obtained in these laboratories. [c] The data used in ref 6 were recalculated using the new β_{10} and β_{20} values. [d] The logarithms are those of the numbers actually used in the calculations. No specification of precision is intended. The standard deviations assigned to the mixed complex constants reflect how well the values reported reproduce the observed results. These assigned uncertainties do not represent deviations from the "true" values since the correlations between all the constants applying to the system were not studied. Qualitatively, there is strong correlation between the values of the mixed species and the pK_{2a} values of the amino acids. Moderate correlation exists with the β_{10} values and only slight correlation exists with the β_{01} values. [e] The number of points and the sum of the square of the residuals $(pH_{i,calcd} - pH_{i,obsd})^2$ for the mixed complex experiments. [f] $\beta_{OA} = [OA^{2-}]/([O^-][A^-])$ for $O^- + A^- \rightleftharpoons OA^{2-}$.

Table II. Constants for Pyruvate Addition

$$MA^+ + P^- \xrightleftharpoons{K_{01}{}^{11}} MPA°; \quad MA^+ + A^- \xrightleftharpoons{K_{01}{}^{02}} MA_2$$

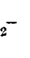
$$\log K_{01}{}^{11} = \log \beta_{11} - \log \beta_{01}$$

A^-	Log $K_{01}{}^{11}$ Ni^{2+}	Log $K_{01}{}^{11}$ Zn^{2+}	Log $K_{01}{}^{02}$ Ni^{2+}	Log $K_{01}{}^{02}$ Zn^{2+}
Glycinate	2.415	2.72	4.85	4.13
α-Alanate	1.83	2.23	4.41	3.96
Isoleucinate	0.99	2.27	4.23	3.99
β-Alanate	3.88	3.2	3.38	?
Sarcosinate	0.73	...	4.31	4.0
H_2O	1.12	1.26	...	...
Pyruvate	−0.66	0.72	...	...

$2\beta_{11}$ which seems to be characteristic of the Schiff base complexes contrasted to other types of mixed complexes, such as those formed with sarcosinate–pyruvate.

The simple β-alanate complexes are seen from the data of Table I to be considerably less stable than those of glycinate. This is a manifestation of the well-known effect of chelate ring size wherein five-membered rings are found to have maximum stability.[12,13] On the other hand, the $K_{01}{}^{11}$ values are much higher with β-alanate than with glycinate. The difference is so great with Ni(II) that β_{11} is actually larger than for glycinate. The $K_{01}{}^{11}$ values for β-alanate occupy a "normal" order with Ni(II) > Zn(II).[14] With the other ligands the differences between Ni(II) and Zn(II) once again suggest that steric factors are involved. Indeed, models show that when the Schiff bases involving the α-amino acids coordinate to octahedral Ni(II) positions the chelate ring system is strained owing to the constraints introduced by the imine nitrogen. With an additional methylene group in the amino acid ring, this strain is not only removed but *cis*-carboxylate coordination can also occur as well as *trans*, thereby contributing statistically toward a higher formation constant.

The presence of an N-methyl group in sarcosine produces very weak mixed species. The log $K_{01}{}^{11}$ value is the lowest of those found in this study and is practically identical with the statistical value 0.74 (*i.e.*, $^5/_{12}\beta_{10}$) expected for no chemical interaction between the ligands. It is interesting that the sarcosinate data yield a small but, nevertheless, significant value of β_{22}. The species $MO_2A_2{}^{2-}$ can form only if at least two of the ligands are monodentately bound or if interligand condensation occurs to give a tridentate ligand. It is expected that in the first case β_{22} would be too small for its effect to be observed under the experimental conditions used, but the second alternative is consistent with the results. Evidence supporting the latter view lies in the surprisingly high value of β_{OA} for pyruvate–sarcosinate, which shows that these ligands interact significantly in the uncomplexed state. Most likely, addition occurs to give the carbinolamine



$$^-O_2C\overset{\overset{H}{|}\\O}{\underset{|\\CH_3}{C}}—\overset{CH_3}{\overset{|}{N}}CH_2CO_2{}^-$$

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(14) P. George and D. S. McClure, *Progr. Inorg. Chem.*, 1, 381 (1959).

and it is this carbinolamine which forms the weak $NiO_2A_2^{2-}$ species. Evidence for carbinolamine complex formation has previously been reported by Christensen and Riggs[15] in pyridoxal–sarcosine–metal ion systems. With the other amino acids β_{22} is roughly equal to β_{11}.[2] This is not observed for sarcosinate, which suggests that the lower complex, MOA, predominantly consists of independently bound pyruvate and sarcosinate.

The visible region absorption bands arising from the d–d transitions of these spin-free Ni(II) Schiff base complexes show the same interesting behavior noted previously for pyridoxal–glycinate–nickel(II)[15] and salicylaldehyde–glycinate–nickel(II).[16] In these imine complexes the absorbance of ν_1 ($\sim$10,000 cm^{-1}) is considerably enhanced relative to the absorbance of this band in other spin-free Ni(II) complexes. This effect is illustrated in Table III where the ratio $\epsilon_{\nu 1}/\epsilon_{\nu 3}$ ($\nu_3 \sim -15{,}000$ to $-16{,}000$ cm^{-1}) is given for various Ni(II) complexes. In addition, relatively high values of $\epsilon_{\nu 1}$ have also been observed in a series of octahedral salicylaldehyde–ethylenediamine–nickel(II) complexes.[17]

The β_{OA} values in Table I show significant interactions between pyruvate and all the amines studied here. The highest value of β_{OA} is found with isoleucinate which forms the weakest imine complex. It is very likely that uncoordinated pyruvylidene–isoleucinate is predominantly present in the *trans* configuration where steric hindrance is minimized. In the metal complex, the configuration must be *cis*. *cis–trans* isomerism as well as carbinolamine formation obscures attempts at a comparison between β_{OA} values and the complex stabilities. What is needed for comparison purposes are the constants for the reaction: $OA^{2-}{}_{cis} + M^{2+} \rightleftharpoons$ MOA.

Table III. Ratios of $\epsilon_{\nu 1}/\epsilon_{\nu 3}$ for Various Ni(II) Species

Complex	$\epsilon_{\nu 1}/\epsilon_{\nu}$
$Ni(H_2O)_6^{2+}$	$\sim$1.0
$Ni(gly)_3^-$	1.1
$Ni(en)_3^{2+}$	1.1
Ni(*o*-phen)$_3^{2+}$	0.6
NiP^+ [a]	0.8
Ni(1,3-propanediamine)$_2^{2+}$	0.6[b]
NiP_2 (gly)$_2^{2-}$ [a]	4.6
$NiP_2(\alpha$-ala)$_2^{2-}$ [a]	4.1
NiP_2(*i*-leuc)$_2^{2-}$ [a]	3.4
$NiP_2(\beta$-ala)$_2^{2-}$ [a]	2.1[c]
$Ni(sal)_2(gly)_2^{2-}$	1.9

[a] P^- = pyruvate. [b] With six-membered ring chelates $\epsilon_{\nu 3}$ tends to be greater than $\epsilon_{\nu 1}$: R. G. Wilkins, private communication. [c] The value of $\epsilon_{\nu 3}$ is somewhat high (see footnote *b*), but, nevertheless, ν_1 is somewhat less intense than is observed with the other pyruvylidenimines.

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Metal Ion Catalysis in Transamination. IV. Nickel(II)- and Zinc(II)-Glyoxalate-Amino Acid Complexes[1]

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Abstract: Mixed Ni(II) and Zn(II) complexes involving glyoxalate and the amino acids glycine, α-alanine, and α-aminoisobutyric acid have been studied potentiometrically. Glyoxalate reacts rapidly and cleanly in these systems and offers a smaller steric requirement than pyruvate. Spectral data indicate a tendency to form carbinolamine complexes which decreases as the degree of substitution of the α carbon of the amino acid increases. With pyruvate, on the other hand, the imine form predominates. This behavior seems to have a stereochemical basis. The mixed complexes formed in nickel(II)–pyruvate–glycinate mixtures do not have structures identical with those formed with nickel(II)–glyoxalate–α-alanate. No changes in either case are observed for up to 24 hr showing that tautomerization in these species is slow under the reaction conditions employed.

Glyoxalate undergoes rapid nonenzymatic transamination, usually in good yield, to give glycine and the corresponding oxo compound. Metzler, Olivard, and Snell[2] report that pyridoxamine and glyoxalate react rapidly at pH 5, 79°, in the presence of Al(III) to give about a 70% yield of pyridoxal. Amino acids also react facilely in a metal ion catalyzed reaction to give the keto acids.[2,3] Proton magnetic resonance[4] shows that the α-alanate–glyoxalate–zinc(II) system rapidly forms the Schiff base complex, N-glyoxylidene-α-alanatozinc(II), which slowly tautomerizes to give about a 50% equilibrium conversion to the N-pyruvylideneglycinatozinc(II) form after 1 hr. The tendency for the reverse reaction to give glyoxalate is slight[2] but, nevertheless, is detectable.[5] Previous papers[6,7]

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[1] Support by the National Science Foundation, GP 1627, is gratefully acknowledged.

[2] D. E. Metzler, J. Olivard, and E. E. Snell, *J. Am. Chem. Soc.*, 76, 644 (1954).

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