

Infrared Results.—Previous work⁴ on bis(thietene) complexes has shown that reasonable inferences concerning the qualitative extent of π -bonding between the ligand and the central metal can be drawn from the infrared spectra. The spectral feature of interest is the stretching frequency of the perturbed C=C portion of the chelated thietene rings. In salts these absorptions are readily distinguished from those of the tetraphenylarsonium ion, from which arise the only other bands above 1350 cm^{-1} . The observed frequencies are given in Table VI; these are seen to be

TABLE VI
PERTURBED C=C STRETCHING FREQUENCIES IN
THIETENE COMPLEXES^a

Complex ^b	Frequency, cm^{-1}
[CrS ₆ C ₆ (CF ₃) ₆]	1446
[CrS ₆ C ₆ (CF ₃) ₆] ⁻	1484
[CrS ₆ C ₆ (CF ₃) ₆] ⁻²	1511
[MoS ₆ C ₆ (CF ₃) ₆]	1455
[MoS ₆ C ₆ (CF ₃) ₆] ⁻	1508
[MoS ₆ C ₆ (CF ₃) ₆] ⁻²	1538
[WS ₆ C ₆ (CF ₃) ₆]	1474
[WS ₆ C ₆ (CF ₃) ₆] ⁻	1520
[WS ₆ C ₆ (CF ₃) ₆] ⁻²	1541
(CF ₃) ₂ C ₂ S ₂ (neat)	1621

^a Spectra of fluorolube mulls. ^b Measurements of salts refer to the tetraphenylarsonium salts.

substantially reduced from the free ligand value and to lie in the range 1440–1550 cm^{-1} . The variation of the frequencies is the same as with the bis complexes,

viz., they increase for a given metal as the charge on the complex increases, *i.e.*, as the formal valence state of the metal decreases. The explanations of these variations in the bis and tris complexes are similar, and in the tris complexes are consistent with the electronic configurations proposed above. If in proceeding from the $z = 0$ to $z = -2$ complexes the a_2^* -orbital becomes occupied, the C=C frequencies should increase since this orbital has a node between the carbons and sulfurs and is bonding between the two carbons. That this simple explanation by itself is not completely satisfactory is seen from the observed frequency increase from (Cr)⁻ to (Cr)⁻². However, in the dianion the e_a^* orbital may be occupied, in which case bonding between ligand π -orbitals and the metal would be decreased.²⁹

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(29) It could be reasonably argued that filling the a_1^* and e_a^* orbitals (with nonoccupancy of a_2^*) could cause the observed frequency trends since both of these orbitals are π -bonding with respect to local π -orbitals of the rings. Here we wish simply to point out that the infrared data are consistent with the proposed electronic configurations but are inconclusive in establishing them.

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A Nuclear Magnetic Resonance Study of Aqueous Pyruvate–Glycinate–Zinc(II) and Related Systems^{1a}

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Under the influence of bases (amines, cyanide, hydroxide) pyruvate (P⁻) ions are shown to be dimerized and the dimer formation constant is evaluated. As has been observed with protons, Zn(II) ions also effect the hydration of pyruvate on association. The cause seems to lie in the cooperative operation of field and inductive effects, the former promoting the stability of ZnP⁺_{dio} and the latter destabilizing ZnP⁺_{keto}. Pyruvate and glycinate ions in 1.0 M concentrations react incompletely to give a solution containing pyruvate dimer, the *cis* and *trans* forms of the ketimine, and one other major component, probably the carbinolamine. The rate of the *cis*–*trans* isomerization is acid catalyzed and the separate isomer peaks merge into one when glycine (GH) is added. The presence of Zn(II) or Ca(II) inhibits dimerization by complexing the reactants. The ligands in the "mixed" MPG complexes appear to be condensed as either the Schiff base or the carbinolamine. Over a period of 24 hr., transamination to give alanine is negligible. Alanate and glyoxalate ions react to give a Schiff base which is slow to tautomerize. The reaction is dramatically catalyzed by Zn(II).

The role that Schiff bases and their metal ion complexes play in transamination reactions has stimulated much interest in these compounds.^{2–7} While most attention has been focused on the highly significant

pyridoxal systems, metal ions have been shown to catalyze a slow transamination between pyruvate and glycine⁸ and a faster reaction in the reverse direction between glyoxalate and alanine.⁹

At least one possible role of the metal ions in these last systems is to stabilize the Schiff bases by complexing since many Schiff bases, particularly those in the aliphatic series, are extensively dissociated in aqueous solutions. Indeed with pyruvate and glycinate mix-

(1) (a) Supported by Grant GP-1627 from the National Science Foundation; (b) National Science Foundation Summer Scholar, 1963.

(2) A. E. Braunstein and M. G. Kritzmann, *Enzymologia*, **2** (1937).

(3) L. Davis, F. Roddy, and D. E. Metzler, *J. Am. Chem. Soc.*, **83**, 127 (1961).

(4) J. B. Longenecker and E. E. Snell, *ibid.*, **79**, 142 (1957).

(5) D. E. Metzler, *ibid.*, **79**, 485 (1957).

(6) H. Christenson, *ibid.*, **80**, 2305 (1958).

(7) (a) G. L. Eichhorn and J. W. Dawes, *ibid.*, **76**, 5663 (1954); (b) L. J. Nunez and G. L. Eichhorn, *ibid.*, **84**, 901 (1962); (c) B. Witkop and T. W. Beiler, *ibid.*, **76**, 5589 (1954).

(8) H. Mix, *Z. physiol. Chem.*, **315**, 1 (1959); **323**, 173 (1961); **325**, 106 (1961).

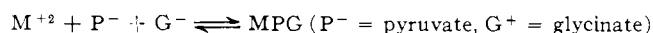
(9) D. E. Metzler, J. Olivard, and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 644 (1954).

TABLE I
POSITIONS OF THE N.M.R. ABSORPTION MAXIMA FOR PYRUVATE, GLYCINATE, AND ALANATE IONS^a

Solution, <i>M</i>	Resonance, c.p.s.	
1.25 pyruvic acid	44.1 (CH ₃ C —)	97.4 (CH ₃ C—)
0.50 pyruvic acid	47.1	97.3
0.50 sodium pyruvate		
1.0 sodium pyruvate	48.3	102 (vw)
0.50 sodium pyruvate	42.6	96.4
0.10 pyruvic acid		
1.0 zinc chloride	-47.6, -40.1, -33.2, -26.2, (CH)	99.0, 106.3 (CH ₃)
0.50 alanine (pH 6.1)		
0.50 alanine	-34.5, -27.2, -20.0, -13.0	108.6, 115.8
0.50 sodium alanate		
1.0 sodium alanate (pH 12.4)	-22.2, -14.7, -7.7, -0.7	113.8, 120.7
0.50 alanine	-43.8, -36.4, -28.9, -21.7	102.1, 109.8
0.50 sodium alanate		
0.50 zinc chloride	-23.5 (-CH ₂ -)	0.0
1.0 glycine (pH 6.2)		
1.0 sodium glycinate (pH 11.0)	0.0	

^a (CH₃)₄N⁺ = 0 c.p.s.

tures so little associated Schiff base exists in solution the principal equilibrium can be expressed in the form for "mixed" complexes¹⁰



A large enhancement in the stabilities of these "mixed" complexes over the stabilities of the species formed with the individual ligands is evidence of strong interligand interactions indicative of Schiff base formation.

Recent n.m.r. studies¹¹ on acetylaceton Schiff bases have demonstrated that various tautomeric forms may be present under certain conditions. In the present investigation this technique was used to study the complexes of zinc(II) with pyruvate and glycinate ions separately and in mixed systems in order to determine which forms are predominant in the complexes. The spectra of the tautomericly related alanate-glyoxalate systems were also obtained. Another of the goals of this study was to determine whether the transamination processes could be followed with this technique.

A 60-Mc. spectrometer was used. Spectral maxima are reported here *vs.* the methyl resonance of internal standard (CH₃)₄NCl taken as 0 c.p.s.

Pyruvate Solutions.—Pyruvic acid molecules and pyruvate ions are partially hydrated in solution.¹² The kinetics of the hydration processes have relaxation times of the order of 1 sec. while the proton-transfer processes are rapid.¹³⁻¹⁵

The positions of the absorption maxima for the pyruvate system under various conditions of pH are listed in Table I. The peak lying in the region 44-48 c.p.s. corresponds closely to the most prominent bands of concentrated and 90% pyruvic acid and accordingly is assigned to the methyl resonance of the keto form.

(10) D. L. Leussing, *J. Am. Chem. Soc.*, **85**, 231 (1963); *Talanta*, in press.

(11) G. O. Dudek and R. H. Holm, *J. Am. Chem. Soc.*, **83**, 2099, 3914 (1961).

(12) M. Becker and H. Strehlow, *Z. Elektrochem.*, **64**, 813 (1960).

(13) M. Bigen, K. Kustin, and H. Strehlow, *Z. physik. Chem. (Frankfurt)*, **31**, 140 (1962).

(14) H. Strehlow, *Z. Elektrochem.*, **66**, 392 (1962).

(15) M. Becker, quoted in ref. 12 and 13, an unpublished n.m.r. study.

The 97-102 c.p.s. band is then attributed to the CH₃ resonance of the *gem*-diol. This latter assignment is consistent with the position of the CH₃C< resonance as exhibited by alanine, also given in Table I. In 1.25 *M* pyruvic acid the relative areas of these two bands have been found by us to be 6.45:7.5 giving proportions of 46% keto and 54% hydrate for undissociated pyruvic acid. In 1.0 *M* sodium pyruvate, however, we find that about 3% of the pyruvate ions are hydrated. These results are in fair agreement with the conclusions reached earlier from considerations of the relative intensities of the optical absorption bands that 70% of the pyruvic acid and less than 2% of the pyruvate ions are hydrated.¹²

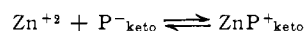
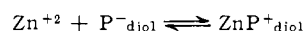
The peak positions given in Table I show that ionization of pyruvic acid produces small upfield shifts in both CH₃ resonances. The magnitudes of the shifts are of the same order as is observed for the CH₃ resonance of betaine when this substance undergoes ionization¹⁶ indicating that neither the CH₃C(O)- nor the CH₃C(OH)₂- groups of pyruvic acid are intimately involved in the ionization process as would be the case if a strong internal hydrogen bond were formed. Although, the degree of hydrolysis of 1.0 *M* pyruvate ions is slight the band arising from the methyl resonance of the keto form continues to shift to slightly higher fields as the solution alkalinity is further increased.

Zinc(II) ions produce a downfield shift in the methyl resonance of both forms of pyruvate. The data of Table I show that the shift for the keto form is slightly greater than that for the diol form, resulting, perhaps, from weak chelate ring formation in the former.

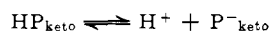
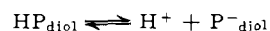
In addition to producing downfield shifts, Zn(II) ions behave as protons toward pyruvate ions by increasing the degree of hydration. In the spectrum of the 1.0 *M* Zn(II)-pyruvate solution described in Table I the relative areas of the methyl bands of the keto and diol forms were found to be 1.00:0.86. Using a formation constant of ZnP⁺ equal to 19,¹⁷ this solu-

(16) D. Chapman, D. R. Lloyd, and R. H. Prince, *J. Chem. Soc.*, 3645 (1963).

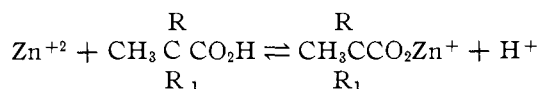
tion is calculated to contain approximately 0.54 M Zn^{+2} , 0.46 M ZnP^+ , and 0.045 M P^- in addition to containing 0.092 M pyruvic acid. After correcting for the amount of hydrate contributed by this latter species it is found that 49% of the complexed P^- ions are hydrated. From this result the constants for the equilibria



are calculated to be 3×10^2 and 10, respectively. Similarly, the microscopic constants for the proton dissociation reactions



are calculated to be 2.5×10^{-4} and 9.5×10^{-3} using the over-all proton dissociation constant of HP equal to 4×10^{-3} . This formation constant of ZnP^+_{diol} is surprisingly large compared with those reported for the lactate and propionate complexes of $Zn(II)$.¹⁸ This can be observed in the values of the replacement constants calculated for the reaction



which are presented in Table II.

TABLE II
CONSTANTS FOR THE REACTION

	Log formation constant of Zn complex	pK_a	$\log K_{replace}$
$R = R_1 = H$	1.01	4.70	-3.69
$R = H, R_1 = OH$	1.86	3.79	-1.93
$R = OH, R_1 = OH$	2.5	3.6	-1.1
Pyruvic acid keto form	1.0	2.02	-1.0

Here it is seen that as OH substitution increases, the proton affinity decreases and the $Zn(II)$ affinity increases. These effects combine to give a marked increase in the replacement constant. This is opposite to the behavior usually observed for closely related ligand systems where it is found that complex stabilities often parallel proton affinities. While a chelate effect may play some role in the large difference between propionic and lactic acids for steric reasons this cannot be the source of the additional increase in going the next step to the α, α -dihydroxypropionic acid. The n.m.r. data cited above also indicate the absence of a strong chelate effect with this last ligand. A purely inductive effect, on the other hand, by withdrawing electron density from the carboxylate group would act to decrease both the complex ion stability and the proton affinity. The evidence seems to indicate the operation of a field effect by which the substitution of polar groups on the propionate chain increases the stabilities of both negatively and positively charged ions.

(17) D. L. Leussing and D. C. Shultz, to be reported.

(18) J. Bjerrum, G. Schwarzenbach, and L. G. Sillen, "Stability Constants," The Chemical Society, London, 1957, Part 1.

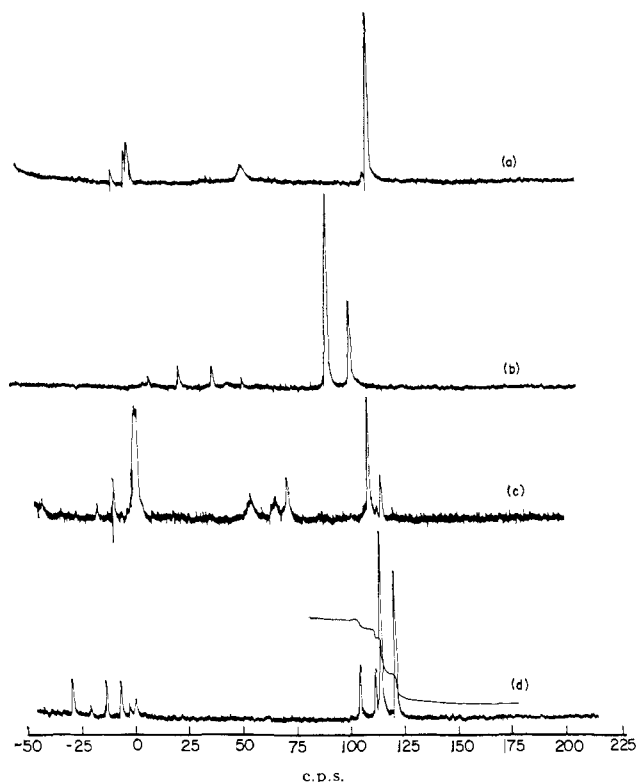


Fig. 1.—Proton magnetic resonance spectra: (a) 2.0 M sodium pyruvate-0.010 M potassium cyanide; (b) 2.0 mmoles of polymeric ZnP_2 dissolved in 1.0 ml. of 4 M HCl (15 min.); (c) 1.0 M sodium pyruvate-1.0 M sodium glycinate; (d) 1.0 M sodium alanate-1.0 M sodium glyoxalate.

In both the keto and diol forms of pyruvate the ease with which $Zn(II)$ ions replace protons seems to be the same. The differences here which are also manifested in the positions of the methyl resonances of these two species must essentially result from the differences in the inductive effects of the oxo and *gem*-diol groups.

Polymerization of Pyruvate in Aqueous Solution.—The slow polymerization of pyruvic acid and pyruvate ions has been known for many years.^{19,20} The process with pyruvate ions is catalyzed by both bases and metal ions.¹⁹ Divalent heavy metal ions such as Ba^{+2} , Zn^{+2} , Hg^{+2} , etc., form highly insoluble polymeric salts having a metal to pyruvate ratio of 1:2. On the basis of isolation of α -ketovalerolactocarbonic acid from solutions of these salts dissolved in strong acid, Wolff¹⁹ has concluded that these compounds are salts of dimeric pyruvate. However, it has been shown,²¹ at least under strongly alkaline conditions at elevated temperature, that pyruvate solutions also contain higher polymerization products and their derivatives. Furthermore, the isolation of trimethyl 2-methoxy-6-methyl-3-dihydropyran-2,4,6-tricarboxylate upon treatment of the polymeric lead salt with HCl and methanol has led to the conclusion²¹ that the pyruvate ions in these salts are trimerized.

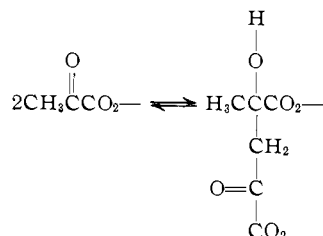
The spectrum of a solution 2.0 M in sodium pyruvate and 0.010 M in KCN is shown in Fig. 1a. The relative intensity of the $CH_3C(O)$ -resonance at 50 c.p.s. is greatly reduced from that of pyruvate in the absence of CN^- and two additional peaks appear, one in the $CH_3C <$

(19) L. Wolff, *Ann. Chem.*, **305**, 154 (1899).

(20) A. W. K. De Jong, *Rec. trav. chim.*, **20**, 81 (1901).

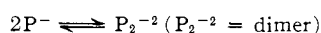
(21) E. Waldman, V. Prey, and F. Jellinek, *Monatsh. Chem.*, **85**, 872 (1953); V. Prey, E. Waldmann, and H. Berbalck, *ibid.*, **86**, 408 (1955).

region at 109 c.p.s. and the other in the methylene region at -8 c.p.s. This latter peak is split owing to non-equivalent protons in a manner similar to that observed in the $-\text{CH}_2-$ resonance of citric acid.²² The ratio of the areas of the $\text{CH}_3\text{C}=\text{O}$ to $-\text{CH}_2-$ bands is 1.52:1.00 indicating that under the present conditions the predominant reaction is the formation of dimer according to the equation



The formation of appreciable amounts of higher polymers would yield lower methyl to methylene ratios.

The 50 and 109 c.p.s. methyl bands of the monomer and polymer were found to have relative areas of 0.45:1.00 in the 2.0 *M* sodium pyruvate-0.01 *M* KCN solution. The spectrum remains essentially unchanged from the time required to obtain the spectrum immediately after mixing up to 48 hr., showing the rapid attainment of equilibrium. The constant for the reaction



is evaluated from these results to be 6.0. A solution 1.0 *M* in NaP and 0.10 *M* in KCN was found to have relative areas of 0.635:1.00, yielding a dimer constant of 6.6. Similar values of the dimer constant were obtained for 0.50 and 3.0 *M* pyruvate solution verifying the observation from the intensity data that the formation of higher polymerization products is negligible in these experiments.

The acidities of the pyruvate and dimerate ions apparently are low since the spectrum in Fig. 1a remains essentially unchanged even when the solution is made 1.0 *M* in NaOH.

The n.m.r. spectrum of a solution obtained by dissolving 2.0 mmoles of the polymeric Zn(II) salt in 1.0 ml. of 4 *M* HCl is shown in Fig. 1b. The spectrum obtained as soon as possible after dissolving the salt shows an intense 98.5 c.p.s. peak and a slightly less intense 88.0 c.p.s. peak. The relative intensities of these two methyl peaks rapidly change until an equilibrium condition is reached in about 15 min. The upfield peak is attributed to the methyl resonance of the acid form of the dimer while the downfield peak apparently results from cyclization giving the ketovalerolactone reported by Wolff.¹⁹ The relative areas of these peaks gives a lactone formation constant of 2.2.

The $-\text{CH}_2-$ resonance of the acid form of the dimer lies at -7 c.p.s. while that of the lactone appears as a quartet typical of an AB spectrum²³ with $J = 13.5$ c.p.s., $C = 18.0$ c.p.s., and having a center of gravity at 28.5 c.p.s. In aqueous solutions the lactone is very likely hydrated, thereby reducing strain. This would account for the high position of the $-\text{CH}_2-$ band and the inability of this substance to give a phenylhydrazone.¹⁹

(22) J. D. Roberts and A. Lowenstein, *J. Am. Chem. Soc.*, **82**, 2705 (1960).

(23) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., New York, N. Y., 1959.

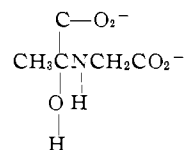
Glycine and Alanine Spectra.—The positions of the glycine $-\text{CH}_2-$ singlet and the alanine CH_3- doublet and $\text{HC}=\text{O}$ quartet in solutions of various pH are also given in Table I. The shifts which these peaks exhibit when the parent compounds undergo ionization are fairly typical of those exhibited for protons α to ionizing carboxylic acid or ammonium groups.^{16,24} On complexing with Zn(II) the CH_3- resonance of alanine undergoes a downfield shift of about 6 c.p.s. while the $\text{CH}=\text{O}$ resonance is shifted in the same direction by 12 c.p.s.

Schiff Base Spectra.—The spectrum of a solution 1.0 *M* each in sodium pyruvate and sodium glycinate is shown in Fig. 1c. From the value 2.5²⁵ for the formation constant of N-pyruvylidene glycinate the solution is calculated to contain 0.50 *M* P^- , 0.50 *M* G^- , and 0.50 *M* PG^{-2} neglecting dimer formation. Taking into account dimer formation the results give theoretical values of 0.19 *M* P^- , 0.68 *M* G^- , 0.32 *M* PG^{-2} , and 0.24 *M* P_2^{-2} at equilibrium.

The relatively prominent dimer peak at 108.5 c.p.s. shows that the actual solution composition conforms more closely to the results of the latter calculation although the spectrum shows some apparent complications. Three resonances are observed in the $\text{CH}_3\text{C}=\text{O}$ region. The lower one of these at 55.5 c.p.s. is more likely that of free pyruvate. The other two are attributed to the *cis* and *trans* stereoisomers of the ketimine. The behavior is analogous to that found by Lustig²⁶ for oximes with the separation of 4.5 c.p.s. observed here being slightly smaller than the difference 6.2 c.p.s. reported for the two methyl resonances of *syn*- and *anti*-2-butanone oxime.

The methylene resonance of uncombined glycinate is seen in Fig. 1c to overlap one or more similar resonances from other species giving rise to an irregular band at 0 c.p.s. Possibly the $-\text{CH}_2-$ resonance of one of the ketimine isomers lies in this region with the other located at -10.5 c.p.s. The proximity of the glycinate $-\text{CH}_2-$ to the pyruvate $-\text{CO}_2-$ in the *cis* isomer might account for the fairly large difference in the resonances, the *cis* lying at higher fields.

The 114 c.p.s. absorption lies close to the position of one of the alanate doublet peaks; however, the downfield twin of this peak which should be resolvable at 107 c.p.s. is not observed. Furthermore no evidence of the downfield quartet is given. Thus, tautomerization to give an appreciable fraction of alanine is ruled out. Of the remaining possibilities, the most likely species giving rise to the 114 c.p.s. band is the carbinolamine



Compounds of this type occur as intermediates in the formation of Schiff bases^{27,28} and appreciable quantities

(24) R. J. Kula, D. T. Sawyer, S. J. Chan, and C. M. Finley, *J. Am. Chem. Soc.*, **85**, 2930 (1963).

(25) Found in this laboratory using potentiometric means. For somewhat different conditions, Zuman [*Collection Czech. Chem. Commun.*, **15**, 839 (1950)] reports a polarographic value also of 2.5.

(26) E. Lustig, *J. Phys. Chem.*, **65**, 491 (1961).

(27) E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, **84**, 832 (1962).

(28) R. L. Reeves, *ibid.*, **84**, 3332 (1962).

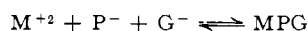
(16%) of the hydrated form have been observed at equilibrium in another system.²⁹

The rate of the ketimine *cis-trans* isomerization is acid catalyzed. The presence of 0.10 *M* glycine, HG, is sufficient to cause the methyl resonances of the *cis* and *trans* isomers to merge into one broad band. In 0.60 *M* NaP, 1.10 *M* NaG, and 0.60 *M* HG this band shows only slight broadening. In this last solution, using the constants reported above, it is calculated that the theoretical ratio of P_2^{-2} to P^- is 0.87:1 and PG^{-2} to P^- is 2.1:1. The corresponding observed areas are in good agreement with these values having ratios 0.85:1 and (2.0-2.5):1. The figure 2.5 was obtained by including the area of the 114 c. p. s. band in the total amount of PG^{-2} .

The aldimine form of the Schiff base can be prepared by combining sodium alanate and sodium glyoxalate. The spectrum of a solution 1.0 *M* in each of these salts is shown in Fig. 1d. Two CH_3- doublets are observed. The higher field doublet corresponds to that for uncombined alanate ions and the less intense pair at a lower field is, therefore, assigned to the aldimine.

A significant feature is the absence of any $CH_3C=$ resonance. This shows, as was suggested by the results of the pyruvate-glycinate study, that the tautomerization rate in these systems is slow.

The presence of Ca^{+2} or Zn^{+2} ions brings about interesting changes in the Schiff base spectra (see Fig. 2). The formation constants for the reactions



have been found to be 9×10^3 when $M^{+2} = Ca^{+2}$ and 3.5×10^7 when $M^{+2} = Zn^{+2}$. Both of these constants are of sufficient magnitude so that the complex MPG can be considered to be quantitatively formed in solutions made up to be initially 1.0 *M* in all reactants.

Only one $CH_3C=$ resonance is observed in these solutions. This could result from a rapid metal ion catalyzed rate of isomerization but more likely shows that the energetics of coordination favors one configuration, the *cis*, in the complexes. In CaPG the absorption is shifted downfield by ~ 2 c.p.s. from that of uncomplexed *cis* PG^{-2} whereas with Zn(II) a shift of 11 c.p.s. is obtained. Ca(II) is an oxygen rather than nitrogen binder in most complexes and likewise for the present system does not appear to interact strongly with the azomethine group. The complex owes its stability to Ca(II)-carboxylate interactions. With Zn(II), however, the metal-nitrogen interaction appears to be greater than is found in the zinc-alanate complexes. This is not an unexpected result considering the increase in Zn(II)-ligand interaction which is observed as the polarizability of the donor group increases.^{30,31}

The methyl resonance in ZnPG is actually a triplet having a splitting of 1.55 c.p.s. through coupling with the N^{14} nuclei. The location of the band and the nitrogen fine structure are definite proof that in the complex the coordinated ligands are condensed as the ketimine. For CaPG only a broadened band is observed and the fine structure is not resolved.

(29) B. Kastening, *Z. Elektrochem.* **60**, 130 (1956).

(30) D. L. Leussing, *Inorg. Chem.*, **2**, 77 (1963).

(31) R. G. Pearson, *J. Am. Chem. Soc.*, **85**, 3533 (1953).

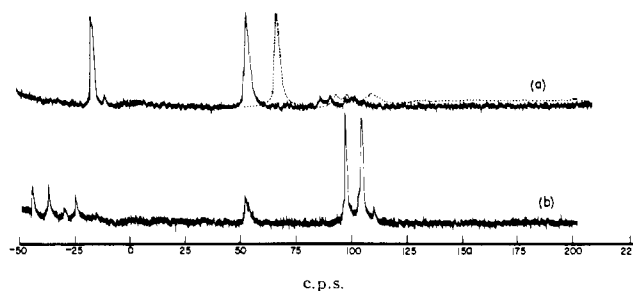


Fig. 2.—Proton magnetic resonance spectra: (a) 1.0 *M* sodium pyruvate-1.0 *M* sodium glycinate-1.0 *M* MCl_2 : ——— $M = Zn(II)$, ···· $M = Ca(II)$; (b) 1.0 *M* sodium glyoxalate-1.0 *M* sodium alanate-1.0 *M* $ZnCl_2$.

In addition to the $CH_3C=N-$ resonance, in both complexes two low intensity broad bands are observed in the $CH_3C<$ region. The integral area under these peaks shows that a substantial fraction (30-40%) of the forms giving rise to these bands is present. The peak positions are dependent on the metal ion showing that these resonances arise in the metal ion complexes. The magnitude of the difference in the positions between the Ca(II) and Zn(II) complexes (~ 8 c.p.s.) is evidence that nitrogen-metal ion bonding is involved. Possibly these bands arise from the diastereoisomeric complexes of the asymmetric carbinolamine. On standing 24 hr., the 102 c.p.s. band of ZnPG increases in intensity as the 90 c.p.s. band decreases. The resonance appears actually to consist of several overlapping peaks; however, it is not possible to identify positively the alanate doublet in these absorptions. At least it seems that Zn(II) ions tend to favor the hydration of the complexed Schiff base as is observed with complexed pyruvate.

Zn(II) ions have also been found to produce interesting effects on the alanate-glyoxalate spectra. The spectrum given in Fig. 2b shows that the two CH_3- doublets observed in the absence of the metal ions (Fig. 1d) coalesce and shift downfield. The spectrum obtained immediately after mixing the solution shows only a faint semblance of absorption in the region of the $CH_3C=$ band; however, the intensity in this region increases at a moderate rate until the equilibrium intensities shown in Fig. 2b are reached in about 1 hr. Practically no further change is observed on standing for an additional 24 hr. In view of the relatively high conversion of alanine to pyruvate reported earlier⁹ this low equilibrium concentration of pyruvate is puzzling. By lowering the pH, however, the yield can be increased. When a solution is prepared to have the same composition as that of Fig. 2b but also contains 0.10 *M* alanine, the spectrum shows equimolar concentrations of pyruvate and alanate after 1 hr.

The results of this study show that tautomerization is the rate-determining step in transamination. In addition, the slow rate of appearance of alanine in the glycinate-pyruvate system seems to result from an unfavorable equilibrium position, further verifying the earlier conclusions of Metzler.⁹

While the status of catalysis by metal ions in biological transamination processes is uncertain at the present time,³² the catalytic effect of Zn(II) ions in the present

(32) See, for example, E. M. Kosower, "Molecular Biochemistry," McGraw-Hill Book Co., New York, N. Y., 1962.

systems has been clearly demonstrated. Certainly, it is seen that one of the roles of the metal ion in these systems is to enhance the fraction of the ligands present as Schiff base. However, this reason is not sufficient to explain all the observations. With the glyoxalate-alanate system, for example, a substantial amount of the Schiff base appears to be formed without producing pyruvate. The electron-withdrawing ability of the metal ion would seem to be an additional factor in promoting tautomerization. Thirdly, by reducing the amine activity in solution, complexing metal ions are able to exert a favorable effect by inhibiting side reactions such as the base-catalyzed dimerization of pyruvate. This latter effect may not be as important in biological systems where only trace amounts of metal ions are present.

Experimental

Matheson Coleman and Bell pyruvic acid was vacuum distilled and twice recrystallized by freezing. The purified reagent was stored at 4°.

Solutions of sodium pyruvate at concentrations 1 *M* or greater when prepared by neutralizing pyruvic acid with strong NaOH solutions yielded n.m.r. spectra which exhibited dimer resonances. Dimer formation evidently was promoted by the action of local excesses of base. Sodium pyruvate (pfs) from the Sigma Chemical Co. was found to be spectroscopically free of this contamination and was used throughout.

Glycine (Matheson Coleman and Bell reagent) was recrystallized from ethanol-water mixtures and dried over Al₂O₃ *in vacuo*.

Stock solutions of the metal ions, 3.0 *M*, were prepared by dissolving reagent grade ZnO in an equivalent amount of HCl, and reagent grade anhydrous CaCl₂ in water.

Unless otherwise stated all solutions were prepared immediately before their n.m.r. spectra were determined. The pyruvate-glycinate solutions developed a yellow color over the course of an hour or so. The appearance of this color did not reflect the production of species in concentrations high enough to give detectable changes in the n.m.r. spectra. On standing overnight, however, noticeable changes did occur. The CH₃C= peaks in the absence of metal ions became considerably reduced in intensity while the spectra in the region of 0 and 100 c.p.s. became quite complicated owing to a mixture of species.

Polymeric zinc pyruvate was obtained by allowing a solution of 1.0 *M* ZnCl₂, 1.0 *M* NaOH, and 1.1 *M* HP to stand for 24 hr. The precipitate was filtered off. The solid was analyzed for Zn by titration with EDTA. Carbon and hydrogen analyses were performed by Galbraith Laboratories, Knoxville, Tenn. *Anal.* Calcd. for Zn(CH₃COCO₂)₂·3H₂O: Zn, 22.3; C, 24.5; H, 4.1. Found: Zn, 22.1; C, 23.96; H, 4.4.

The barium salt was prepared following the directions of Wolff.¹⁹ In the region 3000 to 700 cm.⁻¹ the infrared spectra of these two salts were found to be practically identical. These spectra are unmistakably different from the spectra of the sodium salt (keto form) and the lithium salt (*gem*-diol).²³

A Varian A-60 nuclear magnetic resonance spectrometer was used.

Acknowledgment.—The authors are indebted to Prof. G. Fraenkel for his patient instruction and kind advice during this study.

(33) D. A. Long and W. O. George, *Trans. Faraday Soc.*, **56**, 1570 (1960)