Contribution from the Department of Chemistry,
Texas A&M University, College Station, Texas 77843

Aluminum(III) and Gallium(III) Complexes of Schiff Bases of 2-Amino-3-phosphonopropionic Acid and Pyridoxal 5'-Phosphate

BRUNO SZPOGANICZ[†] and ARTHUR E. MARTELL*

Potentiometric equilibrium studies of Al(III) and Ga(III) complexes of Schiff bases (SB) of 2-amino-3-phosphonopropionic acid (APP) and pyridoxal 5'-phosphate (PLP) are described. At pH values above 6.5 and at initial concentrations of Al(III) of 0.05 M, the major Al(III) complex species contain a 2:1 molar ratio of Schiff base ligand to metal ion and exist in the completely deprotonated and the monoprotonated forms. At lower pH values, the 1:1 Al(III)–SB complexes are the major species present in solution. Aldimine and carbinolamine structures are proposed for the completely deprotonated 1:2 Al(III)–SB complex at pH values above 8.80. ^{31}P NMR and ^{1}H NMR spectra show that at pH values between 5.8 and 8.80 the phosphonato group of the 1:2 Al(III)–carbinolamine complex is protonated but that of the aldimine it is not. The monoprotonated form of the 1:2 Al(III)–SB complex is considered to exist in two forms, one with a phosphonate group coordinated to the Al(III) ion and the other with a coordinated carboxylate group. The major species for the Ga(III)–PLP–APP 1:2:2 system are the 1:2 Ga(III)–SB complexes. Only at very low pH values is the 1:1 Ga(III)–SB complex, in triprotonated form, the significant species. Six protonation reactions of the 1:2 Ga(III)–Schiff base complexes are assigned as follows: the first two occur at the phosphonate groups, then two at the phosphates, followed by two protonations at pyridine nitrogens of the two terdentate-coordinated Schiff bases.

Introduction

Metal ions catalyze the reactions of pyridoxal 5'-phosphate (PLP) and pyridoxal with amino acids in nonenzymic systems through the formation of metal chelates of the terdentate Schiff bases in two ways: by increasing the degree of formation of the Schiff bases and by the steric and inductive electronic effects of metal chelate formation.[1] Preliminary studies of Schiff base formation between pyridoxal (PL) and (aminomethyl)phosphonic acid, β-(aminoethyl)phosphonic acid, and 2-amino-3-phosphonopropionic acid (APP) have been reported.[2,3] It has also been demonstrated by spectrophotometric measurements that the PL–APP system undergoes metal ion catalyzed transamination and dephosphonylation.[4–6] As one of the naturally occurring α-aminophosphonic acids,[7–9] the aminophosphonic acid, APP, is of considerable interest. The reaction pathway suggested for its dephosphonylation reaction[4] appears to resemble the mechanism proposed for pyridoxal-catalyzed β-decarboxylation of aspartic acid.[1]

The purpose of this research is to determine the concentrations of the Schiff base species present under varying conditions in the Al(III)–PLP–APP and Ga(III)–PLP–APP systems. The 1:2:2 molar ratio of metal ions to PLP to APP is employed because 1:1:1 systems were found to precipitate over much of the pH range of interest.[1] The results of this equilibrium study will be employed to select the pH ranges and reaction conditions necessary to relate the observed rates of transamination and dephosphonylation to the concentration of specific Schiff base species in solution, to determine specific rate constants for these molecular species, and possibly to relate catalytic activity to chemical constitution for the reactions being studied.

Experimental Section

Materials. Pyridoxal 5'-phosphate (PLP) was obtained from Sigma Chemical Co. 2-Amino-3-phosphonopropionic acid (APP) was purchased from Calbiochem-Behring Corp. The purity of APP and PLP and the extent of hydration of the solid material were determined by potentiometric measurements. Aluminum sulfate, hydrochloric acid, and potassium chloride were obtained from Fisher Scientific Co. Gallium metal was purchased from D. F. Goldsmith Chemical Metal Corp. Potassium deuteroxide (KOD) 40%, deuterium oxide (D$_2$O) (99.8% D), and deuterium chloride (DCl) were obtained from Aldrich Chemical Co., Inc. CO$_2$-free potassium hydroxide was obtained from J. T. Baker Chemical Co.

Potentiometric Equilibrium Determinations. Standard Al(III) solution, having a concentration of about 10^{-2} M, was prepared from reagent grade aluminum(III) sulfate octadecahydrate (Al$_2$(SO$_4$)$_3$·18H$_2$O) and was standardized by titration with the disodium salt of EDTA.[10] Some HCl

was added to avoid hydrolysis. Standard gallium(III) solutions were prepared by dissolving an accurately weighted quantity of 99.99% pure gallium metal in concentrated HCl. The exact amount of excess hydrochloric acid in the Al(III) and Ga(III) solutions was determined by a Gran's plot of $(V_0 + V_{KOH}) \times 10^{-pH}$ vs. V_{KOH}, where V_0 = the initial volume of the Al(III) or Ga(III) solution and V_{KOH} is the volume of added standard KOH. The intercept on the abscissa obtained by extrapolating the straight-line portion of the plot is a direct measure of the excess acid present.[11] The excess acid was also checked by the procedure of Harris and Martell.[12]

Samples of about 0.10 and 0.20 mmol of APP and PLP and 0.10 mmol of Al(III) or Ga(III) were diluted with 50 mL of double-distilled water in a sealed, thermostated (25.0 ± 0.05 °C) potentiometric titration vessel equipped with a Sargent silver–silver chloride glass electrode and a calomel reference electrode, N$_2$ inlet and bubbler outlet, and a graduated (Metrohm) microburet. The test solution, adjusted to 1.00 M with KCl, was titrated with 0.2000 M standard CO$_2$-free KOH containing 0.8000 M KCl while –log [H⁺] was measured with a Corning Model 130 pH meter calibrated with dilute standard hydrochloric acid at 1.00 M (KCl) ionic strength to read –log [H⁺] directly (for the purposes of this research pH is defined at –log [H⁺]) and with dilute standard potassium hydroxide at 1.00 M (KCl) ionic strength. Potentiometric measurements were made on solutions of the following composition: each ligand and Al(III) in 1:1 and 2:1 molar proportions; each ligand and Ga(III) in 1:1 and 2:1 molar proportions; solutions containing Al(III), APP, and PLP having 1:2:2 molar ratio; solutions containing Ga(III), APP, and PLP at a 1:2:2 molar ratio.

NMR Measurements. The proton nuclear magnetic resonance spectra were recorded on a Varian EM 390 NMR spectrometer. The chemical shifts are recorded in ppm with respect to the resonance of 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) as an internal reference. Only enough DSS was used to give a small methyl peak, and the diffuse pattern of the CH$_2$ peaks barely showed on the base line. The temperature of the NMR solution was 35.0 ± 0.05 °C.

The phosphorus ^{31}P NMR spectra were recorded with a Varian FT 80A NMR spectrometer. The chemical shifts are reported in ppm with respect to the resonance of H$_3$PO$_4$ (0.30 M in D$_2$O). A spectral width

(1) Martell, A. E. *Adv. Enzymol.* **1982**, *53*, 163.
(2) Martell, A. E.; Langohr, M. F. *J. Inorg. Nucl. Chem.* **1978**, *40*, 149.
(3) Szpoganicz, B.; Martell, A. E. *J. Am. Chem. Soc.* **1984**, *106*, 5513.
(4) Martell, A. E.; Langohr, M. F. *J. Chem. Soc., Chem Commun.* **1977**, 343.
(5) Cassaigne, A.; Lacoste, A. M.; Neuzil, E. *Biochim. Biophys. Acta* **1971**, *252*, 506.
(6) Martell, A. E.; Langohr, M. F.; Tatsumoto, K., to be submitted for publication.
(7) Horiguchi, J.; Kandatsu, M. *Nature* **1959**, *184*, 901.
(8) Kittredge, J. S.; Hughes, R. R. *Biochemistry* **1964**, *3*, 991.
(9) Kittredge, J. S.; Roberts, E. *Science (Washington, D.C.)* **1969**, *No. 164*, 37.
(10) Schwarzenbach, G.; Flaschka, H. "Complexometric Titration"; Methuen: London, 1969; p 264.
(11) Rossotti, F. J. C.; Rossotti, H. *J. Chem. Educ.* **1965**, *42*, 375.
(12) Harris, W. R.; Martell, A. E. *Inorg. Chem.* **1976**, *15*, 713.

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Table I. Protonation Constants[a] of Pyridoxal 5'-Phosphate (PLP), 2-Amino-3-phosphonopropionic Acid (APP), and APP–PLP Schiff Base (t = 25 °C, μ = 1.00 M (KCl))

compd	log K_1^{H}	log K_2^{H}	log K_3^{H}	log K_4^{H}
pyridoxal 5'-phosphate	7.915	5.756	3.579	
2-amino-3-phosphonopropionic acid	10.406	5.928	2.317	
APP–PLP Schiff base[b]	11.89	7.54	6.27	5.43

[a]From ref 15. [b]From ref 3 (t = 35.0 °C, μ = 1.00 M (KCl) in D_2O) and corrected for aqueous solution with the relationship pK-(D_2O) – p$K(H_2O)$ = 0.41 + 0.020[p$K(H_2O)$].[15]

of 2000 Hz was generally employed, with a pulse of 10 μs and an acquisition time of 1.0 s. The temperature of the solutions was 35.0 ± 0.5 °C.

The analytical concentrations of Al(III), Ga(III), APP, and PLP employed for NMR measurements were 0.0500, 0.0500, 0.100, and 0.100 M, respectively. The experimental solutions were prepared by direct weighing of APP and PLP and by dilution of stock solutions of Al(III) or Ga(III). Standard Al(III) solution having a concentration of about 0.50 M was prepared from reagent grade aluminum(III) sulfate octadecahydrate in D_2O. The aluminum salt was dissolved in D_2O, and some DCl was added to avoid formation of hydrolysis products. The solution was standardized by the procedure described previously.[10] A Gilmont microburet was employed in the measurement of small volumes. Standard Ga(III) solution was evaporated to dryness, redissolved with a small volume of 20% DCl in D_2O, and then diluted to its final volume with D_2O.

The pD values (–log [D$^+$]) of the D_2O solutions were measured with a Metrohm/Brinkman Model 103 pH meter fitted with a Metrohm miniature combination-glass electrode. The instrument was standardized with dilute HCl at 1.00 M ionic strength (completed with KCl) to read –log [H$^+$]. The pD values were computed by adding 0.40 to the observed reading.[13,14]

Results and Discussion

Ligand Protonation Constants. Protonation constants of APP, PLP, and the AP–PLP Schiff base are given in Table I. These constants are defined by eq 1 where L represents the ligand.
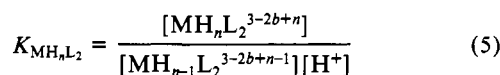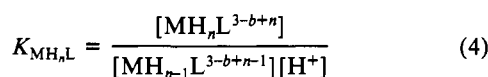
$$H^+ + H_{n-1}L \overset{K_n^{\mathrm{H}}}{\rightleftharpoons} H_nL \qquad K_n^{\mathrm{H}} = \frac{[H_nL]}{[H^+][H_{n-1}L]} \qquad (1)$$
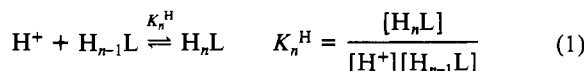
Stability Constants. The normal chelate stability constants of complexes having 1:1 and 1:2 molar ratios of metal ion to ligand are defined by eq 2 and 3, respectively. ML^{3-b} represents the 1:1

$$M^{3+} + L^{-b} \overset{K_{\mathrm{ML}}}{\rightleftharpoons} ML^{3-b} \qquad K_{\mathrm{ML}} = \frac{[ML^{3-b}]}{[M^{3+}][L^{-b}]} \qquad (2)$$

$$M^{3+} + 2L^{-b} \overset{K_{\mathrm{ML_2}}}{\rightleftharpoons} ML_2^{3-2b} \qquad K_{\mathrm{ML_2}} = \frac{[ML_2^{3-2b}]}{[M^{3+}][L^{-b}]^2} \qquad (3)$$

unprotonated, unhydrolyzed chelate; ML_2^{3-2b} represents the 1:2 unprotonated, unhydrolyzed chelate; M^{3+} represents the free unhydrolyzed aquometal ion; L^{-b} represents the uncomplexed, totally deprotonated form of the ligand.

The metal chelate protonation constants are expressed in the same form as the ligand protonation constants, and these parameters, defined by (4) and (5), as well as the stability constants
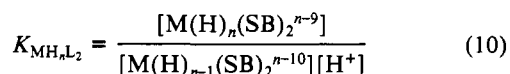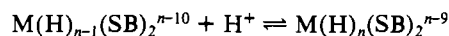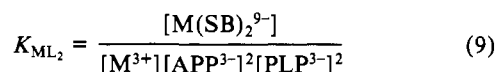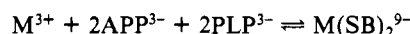
$$K_{\mathrm{MH_nL}} = \frac{[MH_nL^{3-b+n}]}{[MH_{n-1}L^{3-b+n-1}][H^+]} \qquad (4)$$

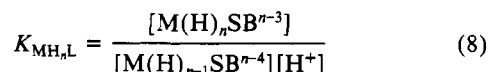$$K_{\mathrm{MH_nL_2}} = \frac{[MH_nL_2^{3-2b+n}]}{[MH_{n-1}L_2^{3-2b+n-1}][H^+]} \qquad (5)$$
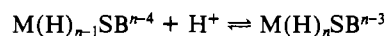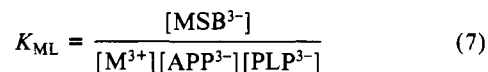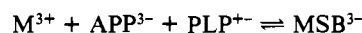
defined by (2) and (3) were determined by iterative nonlinear least-squares fits of the respective potentiometric equilibrium curves with the Fortran computer program BEST.[16] The hydrolysis constants (eq 6) of Al(III) and Ga(III) were taken from the

$$M^{3+} + nOH^- \overset{K_h}{\rightleftharpoons} M(OH)_n^{3-n} \qquad K_h = \frac{[M(OH)_n^{3-n}]}{[M^{3+}][OH^-]^n} \qquad (6)$$
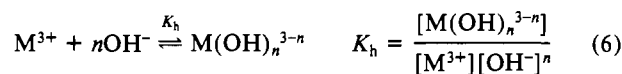
literature.[17] Allowance was made in the computations for the presence of $Al(OH)^{2+}$, $Al(OH)_2^+$, $Al(OH)_3$, $Al_2(OH)_2^{4+}$, Al_3-$(OH)_4^{5+}$, and $Al(OH)_4^-$ for the aluminum(III) systems and $GaOH^{2+}$, $Ga(OH)_2^+$, $Ga(OH)_3$, and $Ga(OH)_4^-$ for the gallium(III) systems. The stability constants determined for the Al–APP, Al–PLP, Ga–APP, and Ga–PLP systems are reported in Table II.

Al(III)–APP–PLP and Ga(III)–APP–PLP Systems. Potentiometric [H$^+$] measurements on 1:1:1 and 1:2:2 molar ratios of Al(III)–APP–PLP and Ga(III)–APP–PLP were carried out, and in both systems formation of precipitates was observed. However, in the 1:2:2 systems the precipitates redissolved at about pH 6 for the gallium system and pH 7 for the aluminum system (Figure 1). The precipitates are probably neutral products of hydrolysis of the cations and/or neutral metal ion Schiff base species. As the pH of the solution with the precipitate increases, deprotonation of the reagents (APP and PLP) favors formation of $M(SB)_2$ (M = Al(III) or Ga(III), SB = APP–PLP Schiff base), which forms anionic species in that pH range. The equilibria for normal and protonated chelates are indicated by eq 7–10, and the stability constants determined are presented in Table II.

$$M^{3+} + APP^{3-} + PLP^{+-} \rightleftharpoons MSB^{3-}$$

$$K_{\mathrm{ML}} = \frac{[MSB^{3-}]}{[M^{3+}][APP^{3-}][PLP^{3-}]} \qquad (7)$$

$$M(H)_{n-1}SB^{n-4} + H^+ \rightleftharpoons M(H)_nSB^{n-3}$$

$$K_{\mathrm{MH_nL}} = \frac{[M(H)_nSB^{n-3}]}{[M(H)_{n-1}SB^{n-4}][H^+]} \qquad (8)$$

$$M^{3+} + 2APP^{3-} + 2PLP^{3-} \rightleftharpoons M(SB)_2^{9-}$$

$$K_{\mathrm{ML_2}} = \frac{[M(SB)_2^{9-}]}{[M^{3+}][APP^{3-}]^2[PLP^{3-}]^2} \qquad (9)$$

$$M(H)_{n-1}(SB)_2^{n-10} + H^+ \rightleftharpoons M(H)_n(SB)_2^{n-9}$$

$$K_{\mathrm{MH_nL_2}} = \frac{[M(H)_n(SB)_2^{n-9}]}{[M(H)_{n-1}(SB)_2^{n-10}][H^+]} \qquad (10)$$

Molecular Species in the Al(III)–SB System. The Fortran computer program SPECIES,[18] which employs the same algorithm as BEST, was used to calculate the species distribution curves shown in Figure 2. Figure 2a shows all species present in the 1:2:2 Al(III)–APP–PLP system. The 1:2 Al(III)–Schiff base appears at pH values above 6.0, and the major species becomes the monoprotonated form, which reaches its maximum concentration at pH 7.9. The 1:1 Al(III)–Schiff base chelate appears at pH values lower than 8.0. The protonation sites of the 1:1 complex are assigned to the various basic functional groups to give the following sequence, starting with the completely deprotonated form: first the phosphonate groups, second at the phosphates, and third at the pyridine nitrogens. At pH values higher than 7.04 either the phosphonate or carboxylate group may be coordinated to the metal ion as indicated by formulas **1** and **2**. Protonation of the phosphate group would be expected to favor carboxylate coordination of the metal ions, as indicated by **3**. The conformations **2** and **3** are similar to that determined for the Zn(II)–APP–PLP–PDA 1:1:1:1 system (where PDA is 2,6-pyridine-

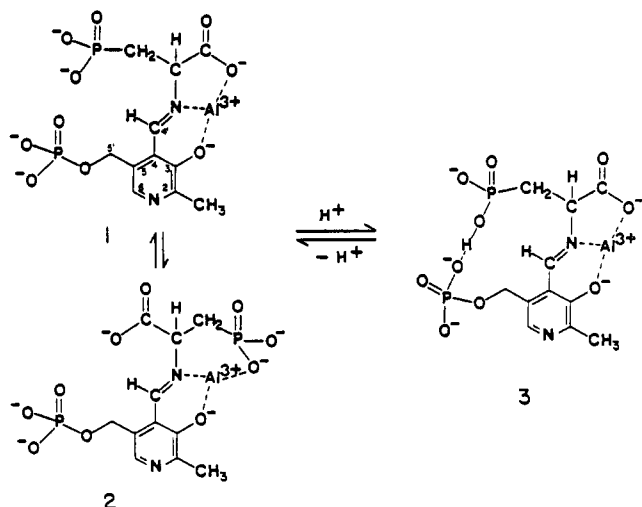(13) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188.
(14) Schowen, K. B. J. "Transition States of Biochemical Processes"; Gandour, R. D., Schowen, R. L., Eds.; Plenum Press: New York, 1970; p 224.
(15) Robertson, R. E.; Laughton, P. M. "Solute Solvent Interactions"; Coetzee, J. F., Ritchie, C. D., Eds., Marcel Dekker: New York, 1969; pp 400–538.

(16) Motekaitis, R. J.; Martell, A. E. *Can. J. Chem.* **1982**, *60*, 168.
(17) Baes, C. F., Jr.; Mesmer, R. E. "The Hydrolysis of Cations"; Wiley: New York, 1976.
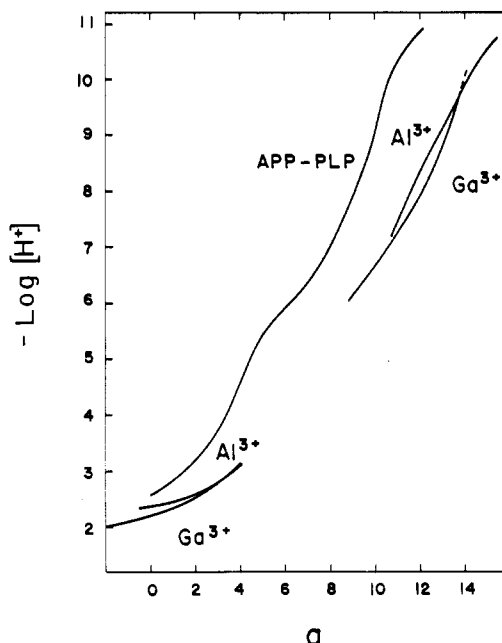(18) Motekaitis, R. J.; Martell, A. E., to be submitted for publication.

Figure 1. Potentiometric equilibrium curves of solutions containing 4.00 $\times$ 10^{-3} M PLP and APP, in the absence and in the presence of 2.00 $\times$ 10^{-3} M Al^{3+} or Ga^{3+}. Conditions: a = moles of base added per mole of metal ion; μ = 1.00 M (KCl); t = 25.0 °C.

carboxylic acid, a secondary ligand).[19] The initial concentrations of the reagents in Figure 2b are 50 times higher than those in Figure 2a. This increase in concentration produces a much higher degree of formation of the Al(III)–SB chelate (SB = Schiff base formed from APP and PLP), relative to the free components and other complexes present in the system. Since the initial concentrations of the components in the metal ion–vitamin B_6–amino acids systems to be employed for kinetic studies by NMR will probably be about 0.10 M, Figure 2b reflects more closely than Figure 2a the distribution of species that will be employed for kinetic studies. The formation of the metal-free Schiff base from APP and PLP was taken into account for the calculations of the species distribution. The value of β_0^{SB} of 0.69 was taken from a previous report.[19]

In order to establish the conformations of the 1:2 Al(III)–SB complex and to determine which donor groups are coordinated with the metal ion, ^1H and ^{31}P NMR spectra were measured. Figure 3 shows the ^1H NMR spectra of the Al(III)–APP–PLP 1:2:2 system at pD values 11.31, 8.00, and 7.16. The resonances appear to broaden as the pH values of the solutions decrease. This effect is due to faster equilibria between the 1:2 Al–SB complex and its components. The resonances of free PLP and APP are known,[18] and those of the Al(III)–SB complexes are comparable to the aluminum complexes of Schiff bases formed between pyridoxal and several amino acids.[20-22] Two resonances are due to the 4′-CH proton as shown in the spectrum at pD 11.31. The resonance at 8.80 ppm corresponds to an aldimine chelate as in **1** or **2**, and that at 6.28 ppm is assigned to the Al(III) complex of a carbinolamine, indicated by **4** or **5**, depending on whether
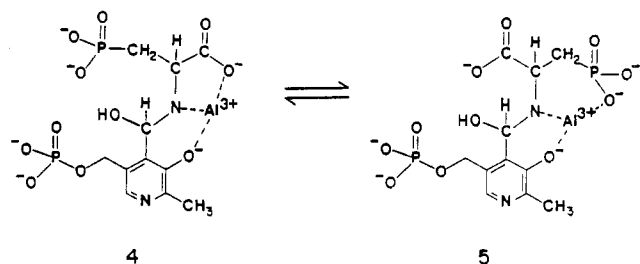
the phosphonate or the carboxylate group is coordinated to the Al(III). This resonance at 6.28 ppm, assigned to a H–C–OH proton, is very similar to one observed for the H–C(OH)$_2$ proton of hydrated PLP.[3] Moreover, this value is close to 5.98 ppm calculated from Shoolery's rule[23] for this proton.

(19) Szpoganicz, B.; Martell, A. E. *Inorg. Chem.* **1984**, *23*, 4442.
(20) Martell, A. E.; Abbott, E. H *J. Am. Chem. Soc.* **1970**, *92*, 5845.
(21) Martell, A. E.; Abbott, E. H. *J. Am. Chem. Soc.* **1969**, *91*, 6866.
(22) Gansow, O. A.; Holm, R. H. *J. Am. Chem. Soc.* **1969**, *91*, 573.
(23) Silverstein, R. M.; Clayton Bassler, G.; Mom'l, T. C. "Spectrometric Identification of Organic Compounds"; Wiley: New York, 1981; p 223.
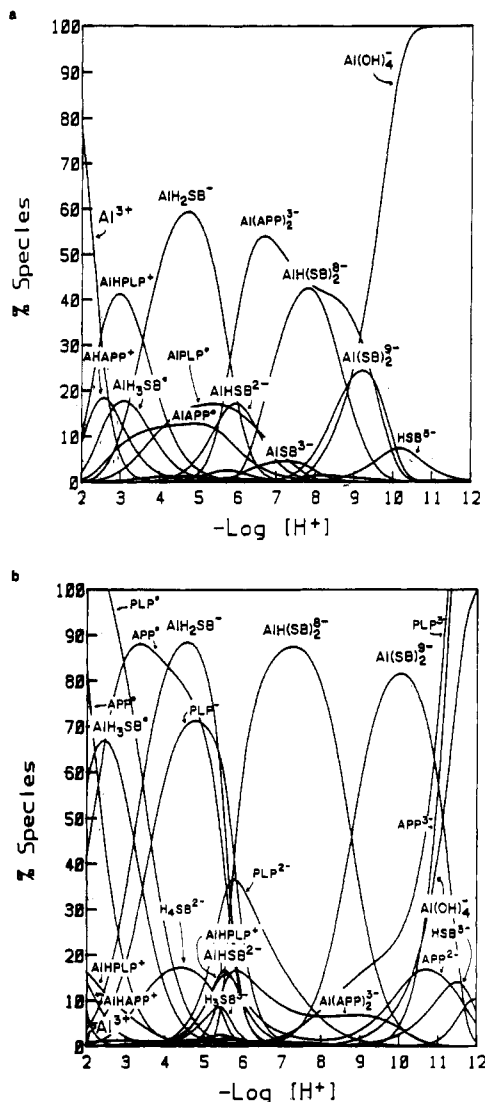
At pD 8.00 three resonances corresponding to the 4′-CH proton appear: the resonance at 8.85 ppm and two broad lines, one at 8.80 ppm and the other at 6.36 ppm. The broad resonance at 8.80 ppm seems to be in equilibrium with the broad resonance at 6.36 ppm of a carbinolamine, which disappears at lower pD values as shown in the ^1H NMR spectrum at pD 7.16. This behavior indicates that at lower pD values only the aldimine form is present. The resonance due to the 6-CH proton of the Al(III)–SB complex appears at 7.60 ppm at pD 11.31, and its position increases to 7.70 ppm as the pD is lowered. This resonance is close to the resonance of the 6-CH proton of free PLP and the Al(III)–PLP complex, and at lower pD values they cannot be distinguished. Two resonances associated with 2′-CH$_3$ protons appear upfield relative to those of the methyl groups of free PLP and of the APP–PLP Schiff base at pD 11.31 (at 1.26 and at 1.47 ppm, respectively). Both resonances broaden at lower pD values. It is of interest to observe that the integrals of the two methyl groups are of the same magnitude. Also those of the 4′-CH proton of aldimine and carbinolamine are of the same magnitude. This suggests that the aldimine and the carbinolamine Schiff base ligands are in the same complex, in which the two terdentate ligand occupy the six octahedral positions in the coordination sphere of the aluminum(III) ion. This interpretation is in agreement with potentiometric results, which show that only two Al(III)–SB species are present in the pH range studied by NMR and that both are 1:2 complexes.

In the 1:2 Al(III)–SB complex there are four possibilities for coordination to the metal ion: one conformation with two carboxylate groups coordinated to the Al(III) ion, two conformations with one carboxylate and one phosphonate group coordinated, and one with two phosphonate groups coordinated. The distinction was made by ^{31}P NMR.

The ^{31}P NMR spectra of the Al(III)–APP–PLP system, shown in Figure 4, are characterized by broadening of the resonances with decreasing pD. Of the five signal peaks (A–D and R) at pD 10.94, resonance A at 17.76 ppm is due to the phosphonate group of free APP and the weak resonance B at 15.85 ppm is assigned to the monoprotonated SB. The 1:2 Al(III)–SB complex is 48% formed, as indicated by the resonance C at 12.60 ppm, which is assignable to the phosphonate group of the complex. At this pD about 50% of the total Al(III) ion is in the form of the aluminate(III) anion, as indicated in Figure 2b. The phosphate groups of the 1:2 Al(III)–SB complex and of free PLP are responsible for the resonance D at 3.77 ppm. The fifth peak (R)

Figure 3. Proton magnetic resonance spectra (90 MHz) of a D_2O solution containing a 1:2 molar ratio of Al(III) to PLP–APP Schiff base at pD 11.31, 8.00, and 7.16. The initial concentration of Al(III) ion was 0.050 M, and those of PLP and APP were 0.10 M ($t = 35.0$ °C; $\mu = 1.00$ M (adjusted with KCl)).

Figure 2. (a) Species distribution curves for the system Al(III)–PLP–APP at a 1:2:2 molar ratio, supersaturated, where $Al(SB)_2$ and $AlH(SB)_2$ are the nonprotonated and monoprotonated forms, respectively, of the 1:2 Al(III)–SB complexes. AlSB, AlHSB, AlH_2SB, and AlH_3SB are nonprotonated, monoprotonated, diprotonated, and triprotonated forms, respectively, of the 1:1 Al(III)–SB complexes. AlPLP and AlHPLP are nonprotonated and monoprotonated 1:1 Al(III)–PLP complexes. AlAPP and AlHAPP are 1:1 complexes of Al(III) and APP, and $Al(APP)_2$ and $AlH(APP)_2$ are 1:2 complexes. Al^{3+} is the free (aquo) aluminum(III) ion, and $Al(OH)_4^-$ is the aluminate anion. HSB is the monoprotonated Schiff base of PLP and APP. The PLP and APP species are not shown for simplicity. Initial concentrations of PLP and APP are 4.00×10^{-3} M, and that of Al(III) is 2.00×10^{-3} M ($t = 25.0$ °C; $\mu = 1.00$ M (adjusted with KCl)). (b) Species distribution curves for the 1:2:2 Al(III)–PLP–APP system with initial concentrations of Al(III) ion, PLP, and APP 50.0 times their concentrations in (a). PLP^0, PLP^-, PLP^{2-}, and PLP^{3-} represent neutral and ionized species of PLP, and APP^0, APP^-, APP^{2-}, and APP^{3-} are the corresponding APP species.

is the reference peak set up at 0 ppm (phosphoric acid in D_2O). As the pD value of the solution decreases, a new peak appears at 17.86 ppm, and at pD values of about 9 the integral of this peak is one-fourth that of the peak at 12.60 ppm. It reaches a maximum area at lower pD values where it has integrated intensity comparable to the integral of the peak at 12.60 ppm. This observation is an indication that only one of the two phosphonate groups of the 1:2 Al(III)–SB complex becomes protonated. Moreover, potentiometric equilibrium measurements demand only one protonation constant, with a log value of 8.80. Finally, the 4'-CH proton resonance of the carbinolamine (Figure 3) disappears as the pH decreases from 11.31 to 7.16. These observations strongly support the protonation of the phosphonate group of the carbi-
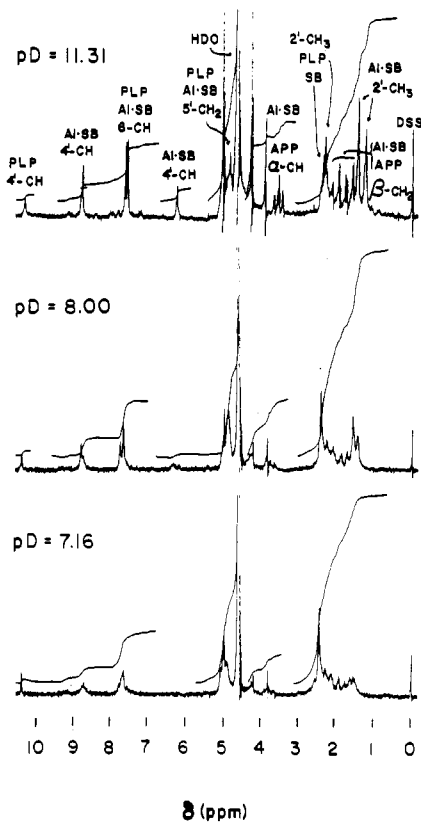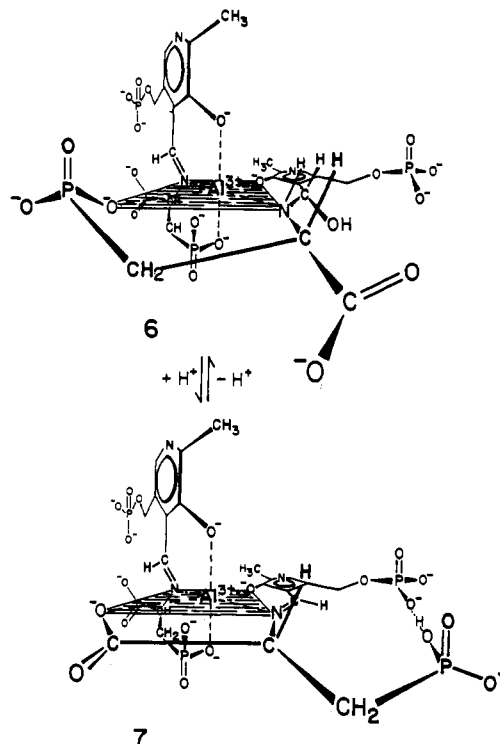
nolamine species derived from the 1:2 Al(III)–SB complex.

The two possible 1:2 Al(III)–SB complex conformations are represented by **6** and **7**. In the pD range studied, protonation

of the second phosphonate group does not occur, and there is no variation in the chemical shift of the phosphonate group resonance in the Schiff base (Figure 5).
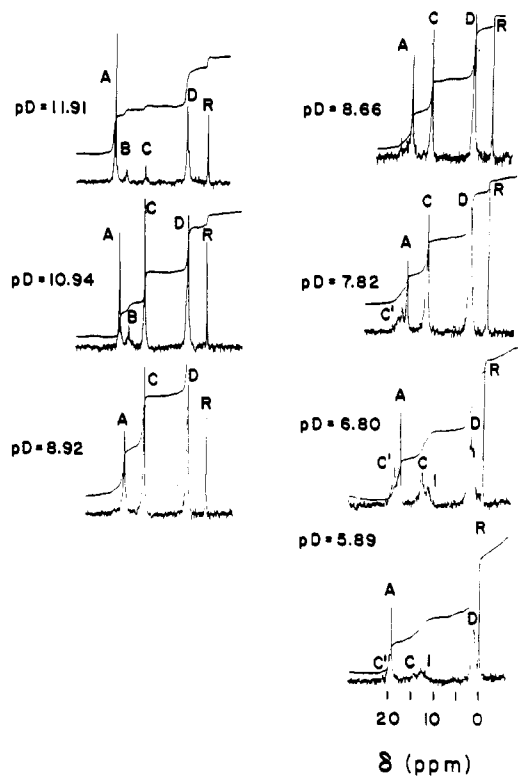
Figure 4. Decoupled ^{31}P magnetic resonance spectra of a D_2O solution containing a 1:2:2 molar ratio of Al(III), APP, and PLP at pD values 11.91, 10.94, 8.92, 8.66, 7.82, 6.80, and 5.89. Key: A = phosphonate group of free APP; B = phosphonate of the APP–PLP Schiff base; C and C' = resonances assigned to the phosphonate groups of the 1:2 Al(III)–SB complex; I = an intermediate; D and D' = resonances due to phosphate groups of the complex; R = the reference (0.300 M phosphoric acid in D_2O). Conditions: $T = 35.0$ °C; $\mu = 1.00$ M (KCl).
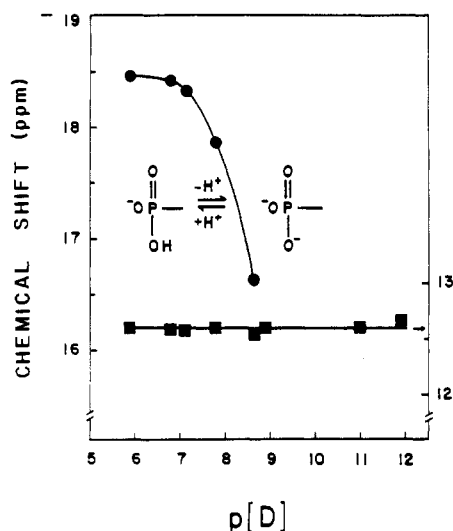

Figure 5. Variation of ^{31}P chemical shifts of the phosphonate group of the Al(III)–SB complex as a function of pD in D_2O: ■, phosphonate group of the carbinolamine and Schiff base part of the 1:2 complex (after protonation of this group the carbinolamine is converted to the Schiff base); ●, phosphonate group of the Schiff base part of the 1:2 complex which before protonation was a carbinolamine. Conditions: $t = 35.0$ °C; $\mu = 1.00$ M (KCl).

Schiff base formation is generally considered[24] to take place in two distinct steps. The amine adds to the carbonyl group, yielding an intermediate carbinolamine, which subsequently is
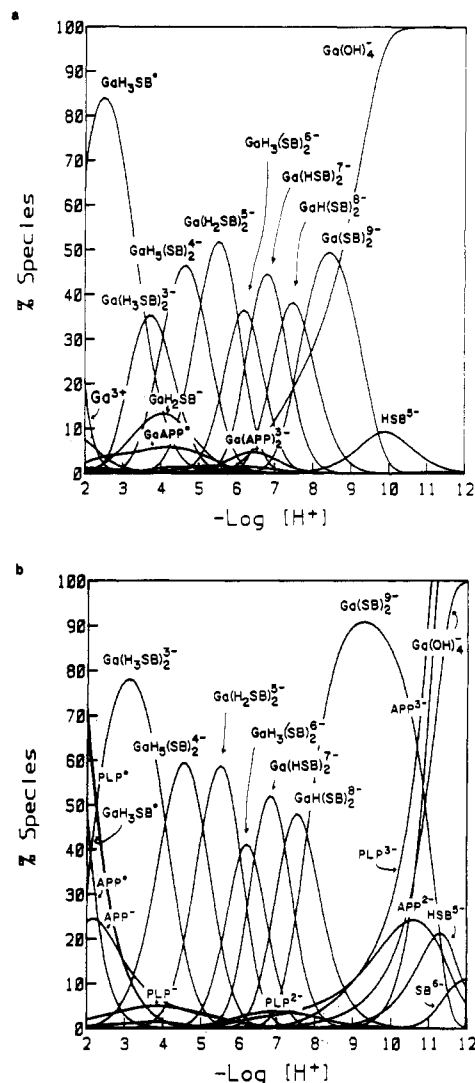

Figure 6. (a) Species distribution curves for a 1:2:2 molar ratio of Ga-(III)–PLP–APP where Ga(SB)$_2$, GaH(SB)$_2$, Ga(HSB)$_2$, GaH$_3$(SB)$_2$, Ga(H$_2$SB)$_2$, GaH$_5$(SB)$_2$, and Ga(H$_3$SB)$_2$ are respectively non-, mono-, di-, tri-, tetra-, penta-, and hexaprotonated forms of the SB complexes and GaH$_2$SB and GaH$_3$SB are respectively diprotonated and tri-protonated forms of the 1:1 Ga(III)–SB complexes. GaAPP and Ga-(APP)$_2$ are 1:1 and 1:2 complexes of Ga(III) and APP. Ga^{3+} is the free (aquo) gallium(III) ion, and Ga(OH)$_4^-$ is the gallate anion. HSB is the monoprotonated Schiff base of PLP and APP. The PLP and APP species are not shown to maintain clarity. Initial concentrations of PLP and APP were 4.02×10^{-3} and 4.01×10^{-3} M, respectively, and that of Ga(III) was 1.98×10^{-3} M ($t = 25.0$ °C; $\mu = 1.00$ M (adjusted with KCl)). (b) Species distribution curves for the 1:2:2 Ga(III)–PLP–APP system with initial concentrations of Ga(III) ion, PLP, and APP 50.0 times their concentrations in (a). PLP^0, PLP$^-$, PLP^{2-}, and PLP^{3-} represent neutral and ionized species of PLP, and APP^0, APP$^-$, APP^{2-}, and APP^{3-} are the corresponding APP species.

converted to an imine through dehydration. An intermediate carbinolamine of PLP and α-alanine was trapped by Cu^{2+} ion and was detected by stopped-flow spectrophotometric techniques.[25] However, in all previous NMR studies of metal ion–vitamin B$_6$–amino acid complexes, the NMR failed to confirm the presence of the carbinolamine intermediates. In the present case, however, it appears that the strong electron-withdrawal effect of Al(III) on the deprotonated nitrogen attached to the 4'-CH position (**4** and **5**) sufficiently stabilizes the carbinolamine in the Al(III)–SB complex.

Since the species distribution is known as a function of concentration and pH, the conditions needed for relating the rates

(24) Jencks, W. P. "Catalysis in Chemistry and Enzymology"; McGraw-Hill: New York, 1969.

(25) Hershey, S. A.; Leussing, D. L. *J. Am. Chem. Soc.* **1977,** *99,* 1992.

Table II. Stability Constants[a] and Protonation Constants[a] of Al(III) and Ga(III) Chelates of 2-Amino-3-phosphonopropionic Acid (APP), Pyridoxal 5'-Phosphate (PLP), and the PLP–APP Schiff Base in 1:1 and 1:2 Complexes (t = 25.0 °C; μ = 1.00 M (KCl))

		log quotient					
ligand	metal ion	$\dfrac{[ML]}{[M][L]}$	$\dfrac{[MHL]}{[ML][H^+]}$	$\dfrac{[MH_2L]}{[MHL][H^+]}$	$\dfrac{[MH_3L]}{[MH_2L][H^+]}$	$\dfrac{[ML_2]}{[M][L]_2}$	$\dfrac{[MHL_2]}{[ML_2][H^+]}$
APP	Al(III)	13.12	3.22			21.40	4.80
	Ga(III)	14.55	2.52			23.50	5.48
PLP	Al(III)	10.48	4.23				
	Ga(III)	14.61	3.43				
PLP–APP–SB[b]	Al(III)	17.12	7.04	6.01	3.23	26.52	8.80
	Ga(III)	18.98	6.77	6.00	4.08	31.92	7.78
							8.15 (5)[c]

[a] The standard deviations are less than 0.02 pH unit overall for data points on the potentiometric equilibrium curves. The numbers in parentheses express the error in the last significant figure. [b] Protonation constants of Ga(III)–SB 1:2 species and their definitions: $[MH_2L_2]/[MHL_2][H^+]$ = $10^{7.21}$; $[MH_3L_2]/[MH_2L_2][H^+]$ = $10^{6.40}$; $[MH_4L_2]/[MH_3L_2][H^+]$ = $10^{6.05}$; $[MH_5L_2]/[MH_4L_2][H^+]$ = $10^{5.05}$; $[MH_6L_2]/[MH_5L_2][H^+]$ = $10^{4.06}$. [c] Determined by ^{31}P NMR.

of Al(III)–Pyridoxal 5'-phosphate catalyzed transamination and dephosphonylation reactions of APP to the individual molecular species present in solution can not be selected. The pH values at which the Schiff base complexes of interest have their maximum concentrations will be employed for kinetic studies of the these reactions.

Ga(III)–SB Complexes. The species distribution curves for the 1:2:2 Ga(III)–APP–PLP system were calcualted from potentiometric data in the same manner as described above for the 1:2:2 Al(III)–APP–PLP system, and they are shown in Figure 6a, and b. The 1:2 Ga(III)–SB complexes are the major species formed with Ga(III). Only at very low pH values is the 1:1 Ga(III)–SB complex a signficant species present in solution. The protonation reactions of the 1:1 complex are considered to occur first at the phosphonate group, then at the phosphate, and finally at the pyridine nitrogen. The analytical concentrations of reagents in Figure 6b are 50 times in excess of those in Figure 6a. The 1:2 Ga(III)–SB complexes predominate over the 1:1 complexes at pH values higher than 2.4, and the major species formed is the completely deprotonated complex ($Ga(SB)_2{}^{9-}$), which reaches its maximum concentration at pH 9.3.

The conformations of the 1:2 Ga(III)–SB complexes were likewise investigated with the aid of ^{1}H and ^{31}P NMR techniques. Figure 7 shows the ^{1}H NMR spectra of the 1:2:2 Ga(III)–APP–PLP system at pD values of 8.77, 7.69, and 5.91. There is only one resonance due to the two methyl groups of the complex at values 7.69 and 8.77. At lower pD, as is shown for pD 5.91, two methyl resonances and two 6-CH proton resonances appear. The aldimine (4'-CH) resonance is broad, indicating two nonequivalent 4'-CH protons and at least one 4'-CH proton of the intermediate. The doublet at 5.1 ppm is not clearly defined because of two nonequivalent 5'-CH$_2$ groups.

Through NMR investigations, both Abbott and Martell[21] and Gansow and Holm[22] found the various isomeric forms of the 1:2 Al(III)–Schiff base chelates involving pyridoxal and several amino acids. It was shown by Abbott and Martell[21] that different sets of resonances are obtained for bis(pyridoxylidenevalinato)aluminum(III) depending on whether the complex is prepared from the racemic amino acid or from the pure L isomer. Since the Ga(III)–SB complexes were prepared from racemic APP, the doublet resonances observed for all protons for these complexes as the pD is lowered as consistent with the formation of DD, LL, and DL isomers and with the DD and LL isomers having the same resonances.

The formation of a reaction intermediate is indicated by the appearance of new NMR signals at 1.65 and 2.06 ppm for the two nonequivalent methyl groups and to resonances at 7.69 and 7.87 ppm associated with two nonequivalent 6-CH protons. The small doublet at 4.84 ppm is the 5'-CH$_2$ resonance peak of a reaction product. The characterization of the intermediate and the reaction products is under investigation and will be described in a subsequent paper, along with related kinetic data.

^{31}P NMR spectra of the 1:2:2 Ga(III)–APP–PLP system are shown in Figure 8. The two resonances at pD 11.51 are due to phosphonate groups (A and C). The one at 18.59 ppm is free
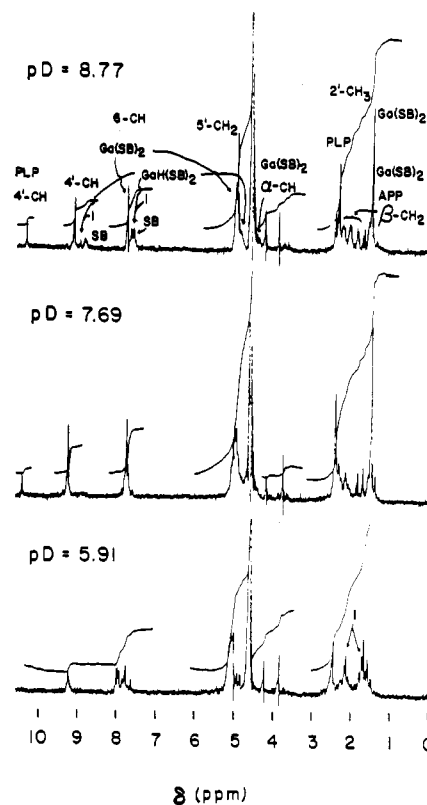

Figure 7. Proton magnetic resonance spectra (90 MHz) of D$_2$O solutions containing a 1:2 molar ratio of Ga(III) to the PLP–APP Schiff base at pD values 8.77, 7.69, and 5.91. The initial concentration of Ga(III) ion was 0.050 M, and those of PLP and APP were 0.10 M (t = 35.0 °C; μ = 1.00 M (adjusted with KCl)).

APP, and that at 16.20 ppm is on the completely deprotonated 1:2 Ga(III)–SB complex. At that pD value most of the gallium forms the gallate complex ($Ga(OH)_4{}^-$) as shown in Figure 6b. The resonance at 15.92 ppm shifts upfield as the pD is lowered. This behavior is attributed to protonation of one of the phosphonate groups which results in the variation of the chemical shift vs. pD for this resonance as is shown in Figure 9. The protonation constant determined from this change is found to 8.15 ± 0.05 in D$_2$O at 35.0 °C, which corresponds to the value of 7.78 in water at 25.0 °C determined by potentiometric measurements. Variation of chemical shifts at low pD values is a result of the protonation of one of the two pyridine nitrogens of the 1:2 complex. At pK 7.49 a new resonance (C') appears at 10.92 ppm and increases in intensity as the pD decreases. The resonance (C) at 17.94 ppm decreases until it has the same magnitude as that at 10.92 ppm (C'). This behavior corresponds to the change in the configuration on the second phosphonate group, occurring in the same pH range as the second protonation (7.21 in water at 25.0 °C, determined by potentiometric measurements).
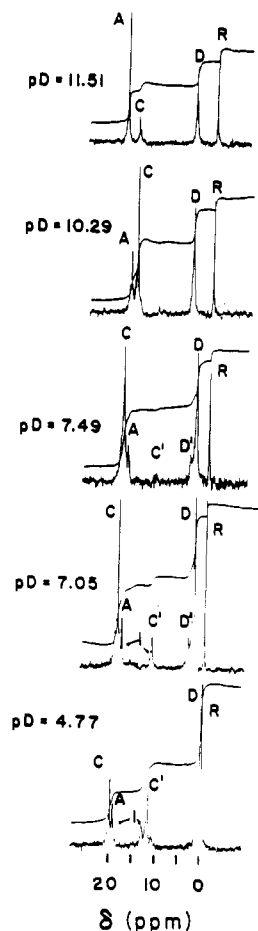
Figure 8. Decoupled ^{31}P magnetic resonance spectra of a D_2O solution containing a 1:2:2 molar ratio of Ga(III), PLP, and APP at pD values 11.51, 10.29, 7.49, 7.05, and 4.77: A = the phosphonate group of free APP; C and C′ = resonances due to the two phosphonate groups of the 1:2 Ga(III)–SB complex; I = an intermediate; D and D′ = resonances due to the two phosphates of the Ga(III)–SB 1:2 complexes; R = the reference (0.300 M phosphoric acid in D_2O).
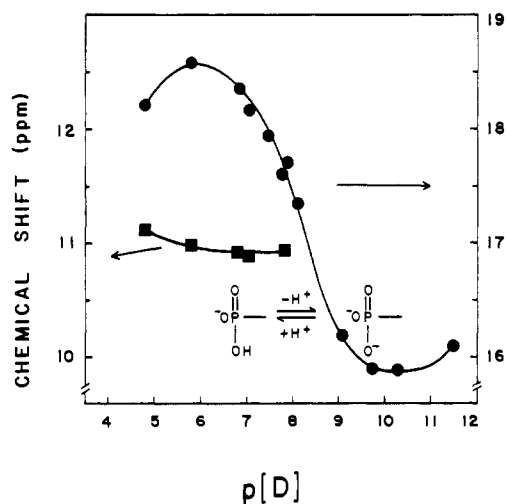

Figure 9. Variation of ^{31}P chemical shifts of the two phosphonate groups of the 1:2 Ga(III)–SB complex as a function of pD in D_2O. At lower pD values the two phosphonate groups have different resonances: ● and ■.

The three possible conformations for the complexes are indicated by **8–10**. Two phosphonate groups are coordinated to the metal ion in **8**, one phosphonate and one carboxylate are coordinated to the metal center in **9**, and a coordination compound is formed in which both carboxylate groups are coordination in **10**. The
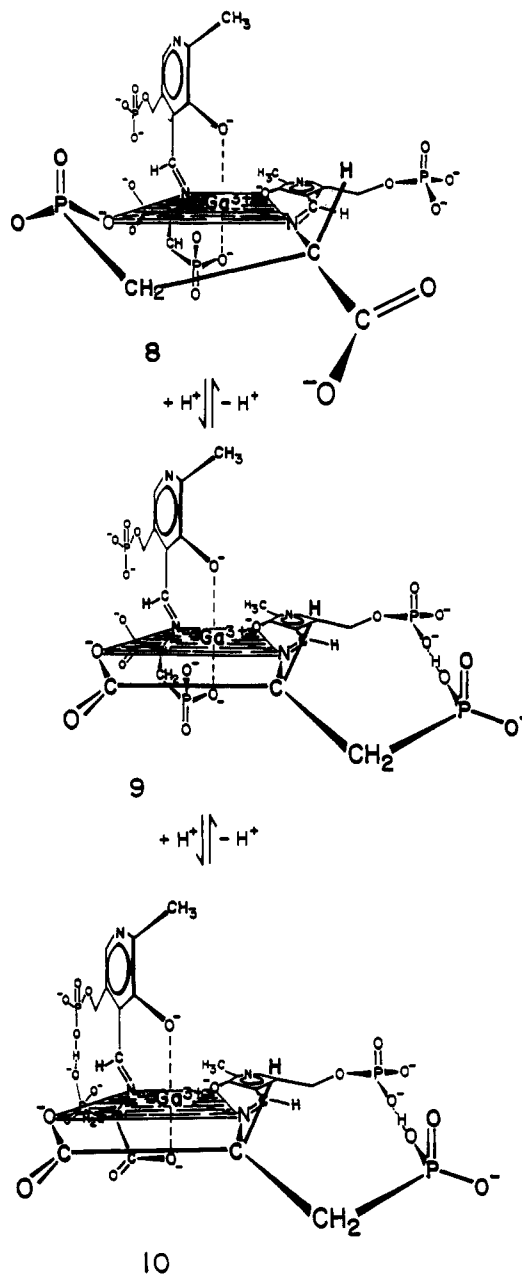
conformation proposed for the complex at pH values higher than 7.78 is **8**, which has the two phosphonate groups of the amino acid part rather than the two carboxylates of the Schiff bases coordinated to the metal ion (as may be seen by comparing K_{ML} for (2-aminoethyl)phosphonic acid (2-AEP) (13.85) and glycine (7.6)). Thus, it is concluded that the complex assumes the arrangement in **10** at pH values lower than 7.21, where the two carboxylate groups of the Schiff bases are coordinated to the metal ion. Protonation of the phosphonate groups results in their displacement from the metal ion, making it possible for the carboxylate groups to coordinate the Ga(III) ion. Conformation **9** is suggested as the major configuration of the complex in the pH range 7.21–7.78.

Kinetic studies are now in progress in order to determine the reactivity of each species toward transamination and dephosphonylation processes and to identify the reaction intermediates formed, if any, in this system.

Registry No. APP, 5652-28-8; PLP, 54-47-7; PLP-APP, 91239-60-0.