

Carbonic Anhydrase Models. 4. [Tris[(4,5-dimethyl-2-imidazolyl)methyl]phosphine oxide]cobalt(2+); A Small-Molecule Mimic of the Spectroscopic Properties of Co(II) Carbonic Anhydrase

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Abstract: The title compound when bound to Zn(II) or Co(II) displays many properties in common with carbonic anhydrase. These include a weak ability of the Zn(II) complex to catalyze the interconversion of CO₂ and HCO₃⁻ and anion-dependent visible absorption spectra of the Co(II) complex. Titration data indicate both the Zn(II) and Co(II) complexes labilize an additional group associated with the complex which appears to be M⁺-OH₂. In the presence of anions, the spectrophotometric pK_a for the Co(II) complex shifts to higher values as it does in Co(II)CA. An explanation for the anion-dependent properties of CA is offered on the basis of the observations with this small-molecule complex.

Carbonic anhydrase (CA) contains an active site in which the catalytically essential Zn²⁺ is bound pseudotetrahedrally to three histidine imidazoles and a water or hydroxide ion.¹ The ability of the enzyme to catalyze the interconversion of CO₂ and HCO₃⁻ is controlled by the ionization of at least one group associated with the active site having a pK_a near 7, although this value appears to be dependent on the presence of anions present in solution. The Co(II) enzyme also displays catalytic activity controlled by a similar ionization,^{1,2} as are its visible absorption spectral properties.³ Bertini and co-workers, in a study of the Co(II) spectral properties of the enzyme as a function of pH in a medium devoid of inhibitory anions or buffers, showed that the spectral variations cannot be explained on the basis of a single ionization but require two or more ionizing groups.⁴ Most recently, work from Koenig's group⁵ seems to indicate that the spectroscopic pK_a of Co(II) CA may be as low as ~5.6 in the absence of monovalent anions and is markedly shifted upward in their presence. The origins of these ionizations have been attributed to M²⁺-coordinated H₂O³, M²⁺-coordinated imidazole,⁶ or other groups in the active site such as histidine⁷ or perhaps Glu-106 in a somewhat hydrophobic environment.⁸ Although the catalytic role of each of the above groups may be questioned,^{7a,9} most workers in the field favor the

Table I. pK_a and pK_{M²⁺} Values for Ligands 1 and 2b^a

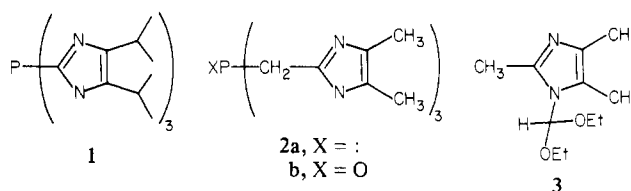
	1 ^b	2b ^b	2b ^c
pK _a ³	6.67 ± 0.07	7.18 ± 0.04	7.50 ± 0.08
pK _a ²	4.36 ± 0.01	5.72 ± 0.02	6.18 ± 0.03
pK _a ¹	2.55 ± 0.03	4.53 ± 0.03	4.96 ± 0.04
pK _{Zn²⁺}	6.00 ± 0.04	6.67 ± 0.03	6.19 ± 0.05
pK _{Co²⁺}	3.48 ± 0.08	3.70 ± 0.03	3.94 ± 0.05

^a pK_a and pK_{M²⁺} values determined by potentiometric titration methods as outlined in ref 16 and 21b. The titrimetric data were analyzed according to the Simms method (Simms, H. S. *J. Am. Chem. Soc.* 1920, 48, 1239), and the error limits represent the maximum deviation in at least three averaged determinations.

^b 80% ethanol; 0.2 M NaClO₄. ^c 20% ethanol; 0.2 M NaClO₄.

involvement of the M²⁺-OH₂ group^{1,8,10a} originally suggested by Davis,¹¹ although only sparse precedence for such a low pK_a exists for that group.^{10b,c,12}

Recently we reported a tris(imidazole)phosphine tridentate ligand (1), which when bound to Zn²⁺ or Co²⁺ shows similar



structural, spectroscopic, and catalytic properties to those of CA.¹³ While 1 shows a definite distorted tetrahedral coordination to Zn²⁺ from its X-ray crystal structure,¹⁴ a well-defined pK_a arising from the M²⁺-coordinated species in solution could not be obtained,¹³ nor could we provide conclusive proof for the presence of

(1) For recent reviews of the properties of CA, see: (a) Prince, R. H. *Adv. Inorg. Chem. Radiochem.* 1979, 22, 349. (b) Golding, B. T.; Leigh, G. J. *J. Inorg. Biochem.* 1979, 11, 50. (c) Lindskog, S.; Henderson, L. C.; Kannan, K. K.; Liljas, A.; Nyman, P. O.; Strandberg, B. *Enzymes 3rd Ed.* 1971, 5, 587. (d) Hay, R. W. *Inorg. Chim. Acta* 1980, 46, L115-L117. (e) Wyeth, P.; Prince, R. H. *Inorg. Perspect. Biol. Med.* 1977, 1, 37-71.

(2) (a) Coleman, J. E. In "Inorganic Biochemistry"; Eichorn, G. L., Ed.; Elsevier: New York, 1973; Vol. 1, p 488. (b) Lindskog, S.; Coleman, J. E. *Proc. Natl. Acad. Sci. U.S.A.* 1973, 70, 2505. (c) Pocker, Y.; Sarkanen, S. *Adv. Enzymol.* 1978, 47, 149. (d) Lindskog, S. *Biochim. Biophys. Acta* 1960, 39, 218.

(3) (a) Lindskog, S. *J. Biol. Chem.* 1963, 238, 945. (b) Lindskog, S.; Nyman, P. O. *Biochim. Biophys. Acta* 1964, 85, 462. (c) Lindskog, S. *Struct. Bonding (Berlin)* 1970, 8, 153.

(4) Bertini, I.; Luchinat, C.; Scozzafava, A. *Inorg. Chim. Acta* 1980, 46, 85.

(5) (a) Jacob, G. S.; Brown, R. D.; Koenig, S. *Biochemistry* 1980, 19, 3755. (b) Koenig, S. H.; Brown, R. D.; Jacob, G. S. In "Biophysics and Physiology of CO₂"; Bauer, C., Gros, G., Bartels, H., Eds.; Springer-Verlag: Berlin, 1980; pp 238-253.

(6) (a) Pesando, J. M. *Biochemistry* 1975, 14, 681. (b) Appleton, D. W.; Sarkar, B. *Proc. Natl. Acad. Sci. U.S.A.* 1974, 71, 1686. (c) Gupta, R. K.; Pesando, J. M. *J. Biol. Chem.* 1975, 250, 2630.

(7) (a) For a discussion of the histidine and Zn²⁺-OH⁻ mechanisms, see: Dunn, M. F. *Struct. Bonding (Berlin)* 1975, 23, 61. (b) Pocker, Y.; Storm, D. R. *Biochemistry* 1968, 7, 1202.

(8) Kannan, K. K.; Petef, M.; Ad-Dresdner, H.; Lövgren, S. *FEBS Lett.* 1977, 73, 115.

(9) For criticisms of the Zn-imidazolate mechanisms, see: (a) Martin, R. B. *Proc. Natl. Acad. Sci. U.S.A.* 1974, 71, 1986. (b) Söavåg, I.; Kiss, T.; Gergely, A. *J. Chem. Soc., Dalton Trans.* 1978, 964. (c) For discussions of the proposed mechanisms, see ref 1.

(10) (a) Demoulin, D.; Pullman, A.; Sarkar, B. *J. Am. Chem. Soc.* 1977, 99, 8498. (b) Woolley, P. *Nature (London)* 1975, 258, 677. (c) Bertini et al. (Bertini, I.; Cantù, G.; Luchinat, C.; Mani, F. *Inorg. Chim. Acta* 1980, 46, L91) have reported a pK_a of 8.8 ± 0.2 attributable to H₂O ionization in cobalt(II) [(tris(3,5-dimethyl-1-pyrazolyl)methyl)amine] hydrate perchlorate. (d) Coates, J. H.; Gentle, G. J.; Lincoln, S. F. *Nature (London)* 1974, 249, 773; five-coordinate Zn²⁺-OH₂, pK_a = 9.0.

(11) Davis, R. P. *J. Am. Chem. Soc.* 1959, 81, 5674.

(12) H₂O coordinated to trivalent cations has a significantly lowered pK_a ~ 6.5-7 depending on the system: (a) Harrowfield, J. MacB.; Norris, V.; Sargeson, A. M. *J. Am. Chem. Soc.* 1976, 98, 7782. (b) Chaffee, E.; Dasgupta, T. P.; Harris, G. M. *Ibid.* 1973, 95, 4169. (c) Palmer, D. A.; Harris, G. M. *Inorg. Chem.* 1974, 13, 965.

(13) (a) Huguet, J.; Brown, R. S. *J. Am. Chem. Soc.* 1980, 102, 7571. (b) Curtis, N. J.; Huguet, J.; Brown, R. S. *Ibid.* 1981, 103, 6953.

(14) Read, R. J.; James, M. N. G. *J. Am. Chem. Soc.*, in press.

$M^{2+}\text{-OH}^-$, although on the basis of other evidence, we favor its involvement in catalysis.^{13b}

Herein, we report subsequent studies with a slightly more flexible tris(imidazole)phosphine oxide ligand (**2b**) which we believe not only provides reasonable evidence for a low pK_a for a metal-associated ionization of H_2O but as its Co(II) complex displays interesting parallels with Co(II) CA with respect to its pH-dependent absorption spectrum in the presence of various anions. Additionally, **2b**· M^{2+} shows modest catalysis of CO_2 hydration and the hydrolysis of *p*-nitrophenyl picolinate (**5**), the latter process resulting from what appears to be an enzyme-like pathway involving a preequilibrium formation of a ternary complex that subsequently breaks down to products.

Experimental Section

N-(Diethoxymethyl)-2,4,5-trimethylimidazole (3). A mixture of 31 g (0.28 mol) of 2,4,5-trimethylimidazole,^{15a} 0.1 g of *p*-toluenesulfonic acid, and 150 mL of triethyl orthoformate was heated at reflux until the requisite amount of ethanol was distilled (~5 h).^{15b} The remaining volatiles were then removed by rotary evaporation, and the oily residue was transferred to a distillation flask along with 1 g of solid Na_2CO_3 . Vacuum distillation afforded 42.4 g (71%) of **3**, bp 85 °C (0.01 torr); 1H NMR ($CDCl_3$) δ 1.21 (t, 6 H), 2.08 (s, 3 H), 2.18 (s, 3 H), 2.41 (s, 3 H), 3.08–4.0 (m, 4 H), 5.86 (s, 1 H); mass spectrum, m/e calcd for $C_{11}H_{20}N_2O_2$, 212.1526; obsd, 212.1521.

Tris[(4,5-dimethyl-2-imidazolyl)methyl]phosphine Oxide (2b). To 19.83 g (0.0935 mol) of **3** in 300 mL of dry ether held at –40 °C under N_2 was added 44.52 mL (0.094 mol) of 2.1 M *n*-BuLi in hexane via syringe at such a rate that the temperature did not exceed –40 °C. The resulting reddish-brown solution was then stirred an additional 30 min, at which time 4.27 g (0.031 mol) of freshly distilled PCl_3 was added to it. The mixture was stirred, warmed to room temperature overnight, and then quenched by the addition of 80 mL of NH_4OH . The ether layer was separated, dried over $MgSO_4$, and filtered, and volatiles were removed. To the residue was added 50 mL of H_2O and 100 mL of acetone, and the mixture was refluxed overnight to effect the deprotection.^{13b} Following this, the acetone and water were removed by rotary evaporation, and the final residual oil was taken up in $CHCl_3$, which was then dried over $MgSO_4$. Removal of $CHCl_3$ yields a light yellow solid (6.7 g), which was recrystallized from ethanol/ether to afford 5.5 g (46%) of colorless crystals of **2b**, mp 304 °C (dec). Spectral and analytical properties confirmed that P oxidation had occurred during the workup to give the phosphine oxide. 1H NMR ($CDCl_3$) δ 2.18 (s, 18 H), 3.40 (broad d, 6 H); IR ($CDCl_3$ cast) 1070, 1105, 2890 cm^{-1} ; mass spectrum, m/e calcd for $C_{18}H_{27}N_6PO$, 374.1984; obsd, 374.1983. Anal. C, H, N.

Titrimetric pK_a and $pK_{M^{2+}}$ values were determined as previously described¹⁶ and required, for reasons of solubility, media containing either 20% or 80% ethanol– H_2O (v/v). The values reported in Table I are averages of at least three determinations and are uncorrected for the highly organic content of the medium, although in principle this could be done.¹⁷

UV and visible absorption spectra were obtained with a Cary 210 UV–vis spectrophotometer at 25.0 °C in 80% ethanol– H_2O solutions. The Co(II) spectra as a function of pH were obtained by using unbuffered solutions, the pH of which was adjusted by the addition of aliquots of 1 N NaOH directly to the cuvettes. pH values are simply those read from a Radiometer Model GK 2322C combination electrode directly immersed in the solution. For the catalytic studies, metal solutions were made from their perchlorate salts and standardized by EDTA titration. *p*-Nitrophenyl picolinate was prepared by published procedure¹⁸ and used as a stock 5×10^{-3} M solution in 95% EtOH. Hydrolysis was monitored at 25.0 \pm 0.02 °C by observing the appearance of *p*-nitrophenoxide at 400 nm, as a function of variable quantities of **2b**:Co(II) at various pHs. Reaction products were confirmed by monitoring the absorption spectra at completion, which were identical with those obtained from adding appropriate concentrations of catalyst, metal, and *p*-nitrophenol to the buffered solutions of the same pH. Buffers employed were Hepes (for

CO_2 hydration) and Ches (for *p*NPP hydrolysis), and ionic strength was kept at 0.2 M and 0.048 M, respectively, with $NaClO_4$.

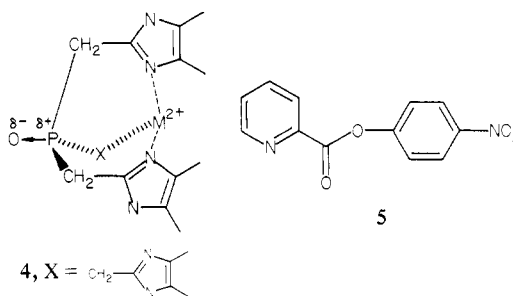
Pseudo-first-order rate constants (k_{obsd}) for the liberation of *p*-nitrophenoxide from *p*-nitrophenyl picolinate were evaluated by fitting the experimental absorbance vs. time values by a nonlinear least-squares program kindly provided by Professor R. E. D. McClung of this department. Values reported in Table III are averages of at least three sequential determinations.

Kinetic determinations of the reaction rates for establishing equilibrium between HCO_3^- and CO_2 were performed by stopped-flow techniques under carefully buffered conditions as previously described.^{13b} The reaction was monitored by observing the rate of change in concentration of an indicator anion (bromocresol purple anion, $\lambda = 600$ nm), whose response to small changes in $[H^+]$ that accompany bicarbonate dehydration (or CO_2 hydration) is rapid relative to the dehydration process itself. Under appropriate buffering conditions, $\Delta[H^+]$ can be held to less than 0.04 pH unit (8%) such that the process can be analyzed in terms of a pseudo-first-order equilibrium situation in which $k_{obsd} = k_{hydration} + k_{dehydration}$, the sum of all the forward and reverse rate constants, which includes those dependent upon the various forms of the buffer and other species present in the medium. Catalytic rate constants (k_{cat}) reported in Table II are taken to be the difference in k_{obsd} determined in the presence and absence of **2b**· Zn^{2+} divided by $[2b \cdot Zn^{2+}]$.^{13b} Control experiments established that the k_{obsd} values with either **2b** or Zn^{2+} alone were identical with those found with neither present in solution.

Results

pK_a and $pK_{M^{2+}}$ Values. Shown in Table I are the pK_a and $pK_{M^{2+}}$ values for ligand **2b** and for comparison purposes ligand **1**.¹³ For **2b**, a modest upward shift in each of the pK_a values occurs in passing from 80% to 20% ethanol, as is expected if the dielectric constant of the medium increases. On the other hand, the ability of **2b** to bind Zn^{2+} is higher in the more organic solvent, although the reverse seems true for Co^{2+} binding ability. Like our other ligands which catalyze CO_2 hydration,^{13a} **2b** binds Zn^{2+} stronger than it does Co^{2+} , paralleling that situation in the enzyme,¹⁹ although not nearly so strongly.

Originally, we designed ligand system **2** to bind metal more strongly than does **1**, since the latter appeared to have considerable strain when bound to metal (i.e., the N– Zn^{2+} –N bond angles are about 95°¹⁴). It was hoped that extension of the imidazoles outward from the central phosphorus anchor would allow an optimization of the chelation by an adjustment of the “pitch” of the propeller-shaped ligand which would lead to an enhanced metal-binding ability. In part, this appears true, since both the $pK_{Zn^{2+}}$ and $pK_{Co^{2+}}$ values are larger for **2b** than for **1**. However, the structural change falls far short of what we expected, which may be due to an unfavorable interaction between the O–P dipole and the bound M^{2+} ion as illustrated in **4**.



This might be minimized with phosphine **2**, which unfortunately undergoes spontaneous oxidation such that we were unable to prepare it.

Finally, in 20% ethanol– H_2O , precipitation was observed for the Co(II) complex at pH readings above 7.5, which was tied to the consumption of OH^- . Henceforth, discussion will be limited to results obtained in the 80% ethanol– H_2O medium in which pH values in excess of 9 could be obtained with no precipitation.

Titration of $2b \cdot 2H^+$. Quantitative titrations of 5×10^{-3} M **2b**· $2HNO_3$ in an 80% ethanol– H_2O medium containing 0.2 M

(15) (a) Cowgill, R. W.; Clark, W. M. *J. Biol. Chem.* **1952**, *198*, 33. (b) Curtis, N. J.; Brown, R. S. *J. Org. Chem.* **1980**, *45*, 4038.

(16) Brown, R. S.; Huguet, J. *Can. J. Chem.* **1980**, *58*, 889.

(17) Bates et al. (Bates, R. G.; Paabo, M.; Robinson, R. A. *J. Phys. Chem.* **1963**, *67*, 1833) have offered correction factors for pH values determined in alcohol–water solvents of various compositions in terms of $pH^* = pH - \delta$. pH^* is the corrected value for the particular solvent mixture while pH is simply the value indicated by an electrode immersed directly into the solution. For the present work, 80% v/v ethanol: H_2O media (74 wt % ethanol) requires a correction of $\delta \approx 0.2$ unit.

(18) Sigman, D. S.; Jorgensen, C. T. *J. Am. Chem. Soc.* **1972**, *94*, 1724.

(19) (a) Lindskog, S. *J. Biol. Chem.* **1963**, *238*, 945; $pK_{Zn^{2+}} = 10.5$, $pK_{Co^{2+}} = 7.2$. (b) Lindskog, S.; Nyman, P. O. *Biochim. Biophys. Acta* **1964**, *85*, 462.

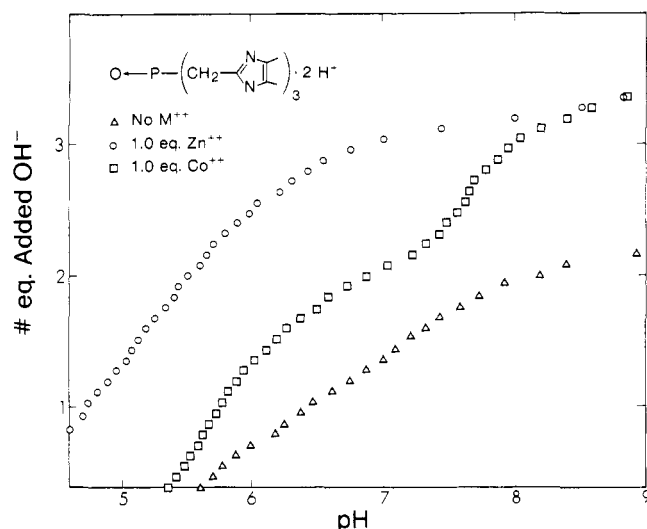


Figure 1. Titration curves for 5×10^{-3} M **2b**- 2H^+ in the presence of no added M^{2+} (Δ), 1.0 equiv of added Zn^{2+} (\circ), and 1.0 equiv of added Co^{2+} (\square); 80% ethanol/ H_2O ; $\text{M} = 0.2$ M (NaClO_4).

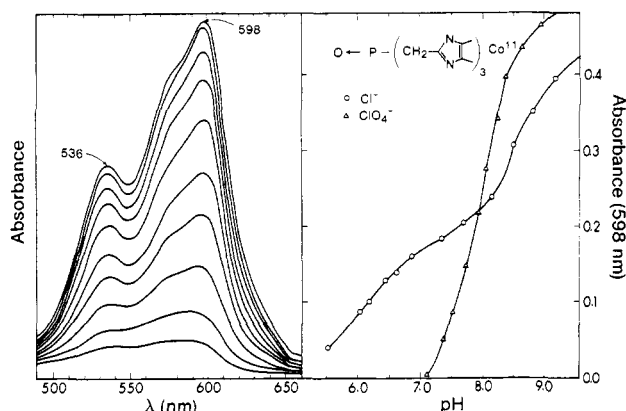


Figure 2. Visible absorbance curve and pH profile for **2b**- Co(II) in the presence of added ClO_4^- (Δ) and Cl^- (\circ); 80% ethanol/ H_2O (8.3×10^{-4} M **2b**- Co(II)); 0.2 M NaClO_4 or 0.2 M NaCl).

NaClO_4 were determined in the absence, and presence, of 1.0 equiv of $\text{Zn}(\text{ClO}_4)_2$ or $\text{Co}(\text{ClO}_4)_2$, the curves being shown in Figure 1. In the absence of metal, 2 equiv of OH^- are consumed after pK_a^3 for the ligand has been passed, corresponding to the titration of the two added H^+ ions. In the presence of Zn^{2+} , the analogous titration shows the consumption of 3 equiv of OH^- before pH 7, indicating a formal titration of one additional acidic group associated with the 1:1 **2b**: Zn^{2+} complex having a $\text{pK}_a \sim 6$. Additionally, with 1 equiv of added Co^{2+} , two OH^- are consumed before pH 7 (indicating complete complex formation by that point), and consumption of a third equivalent of OH^- is clearly indicated by a titration curve having an apparent pK_a of ~ 7.8 . In the latter two cases, no sign of precipitation occurs up to a measured pH of at least 9, and importantly all titrations are completely reversible.

The visible absorption spectrum of an 80% ethanol- H_2O solution containing 8.3×10^{-4} M each of **2b** and $\text{Co}(\text{ClO}_4)_2$ and 0.2 M NaClO_4 as a function of pH shows that the titrimetric pK_a , measured as above, is also tied to the formation of an intense blue solution, indicative of a four- and/or five-coordinate²⁰ Co(II) species ($\epsilon_{598} \approx 550 \text{ M}^{-1}/\text{cm}^{-1}$ at "pH" 8.8, assuming unique binding) as is shown in Figure 2. These observations indicate that while a Co(II) complex is formed by pH 7 (the 2 equiv of added H^+ are titrated), formal titration of some additional group associated with the Co(II) complex (either H_2O or imidazole)

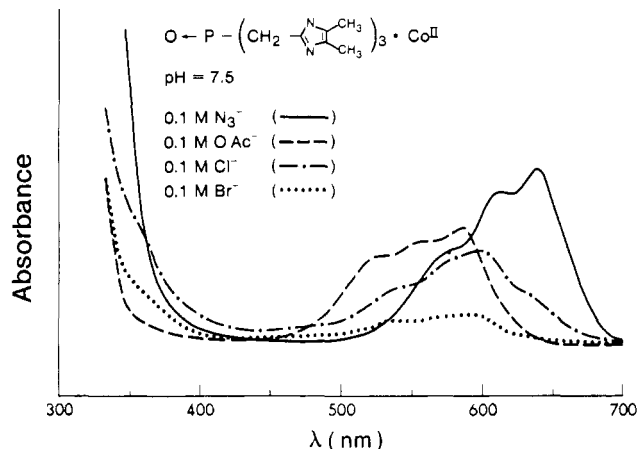


Figure 3. Visible absorption spectra of **2b**- Co(II) in the presence of added anions; pH 7.5.

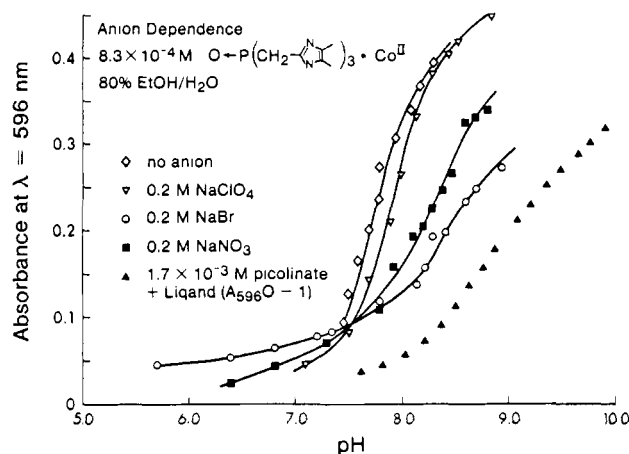


Figure 4. Spectrophotometric pH-dependent titration curves of **2b**- Co(II) in the presence of added anions.

reduces the coordination number around Co(II)^{21} from six to five or four.

Anion Dependence of the Co(II) Absorption Spectrum. The **2b**- Co(II) visible absorption spectrum is markedly dependent upon the presence of added anions as is shown in Figure 3, indicating an association of monovalent anions such as Cl^- , I^- , Br^- , AcO^- , and NO_3^- with the metal. No unique spectrum indicative of a new 4 (5-) coordinate species is observed in the presence of ClO_4^- , which we believe shows that it is only weakly associated with the metal center. However, spectral determinations of **2b**- Co(II) in the presence of 0.2 M NaCl as a function of pH clearly show the buildup of a unique 4- and (5-) coordinate species (λ_{max} 607 nm (ϵ 312); 632 nm (ϵ 280) at pH 8.10), which gives way on further addition of OH^- to the same absorption spectrum as was observed in the analogous ClO_4^- titration experiment (Figure 2, pH profile). Similar observations are made in the presence of other anions.

Finally, we consider the absorption profile as a function of pH for **2b**- Co(II) in the presence of added anions, as is shown in Figure 4. In the absence of any added anion (other than that which is used to make up the 8.3×10^{-4} M $\text{Co}(\text{ClO}_4)_2$ solution) the absorbance increase at 596 nm relates to a process having a pK_a of ~ 7.6 . However, in the presence of 0.2 M ClO_4^- , Br^- , Cl^- , or NO_3^- , this spectrophotometric pK_a is shifted upward to progressively higher values, with the final spectrum observed in all cases being the same as the one observed at high pH in the absence of anion. Picolinate at 0.2 M binds to Co(II) so strongly that no involvement

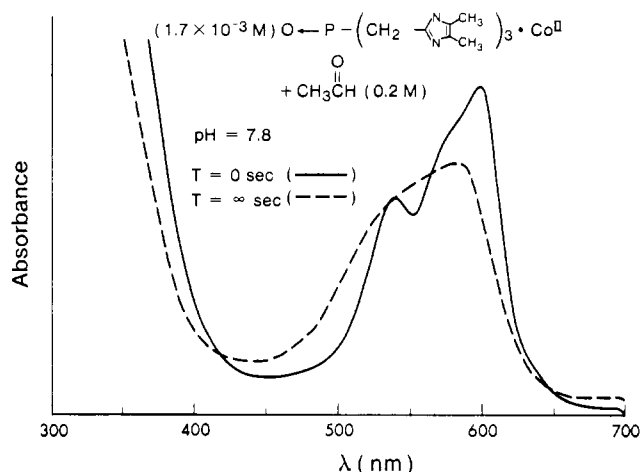
(20) Bertini, I.; Canti, G.; Luchinat, C.; Scozzafava, A., *J. Am. Chem. Soc.* **1978**, *100*, 4873 and references therein.

(21) (a) This observation has been observed before^{10c,21bc} although not with ligand systems containing only three imidazoles. (b) Tang, C. C.; Davalian, D.; Huang, P.; Breslow, R. *J. Am. Chem. Soc.* **1978**, *100*, 3918. (c) Bertini, I.; Canti, G.; Luchinat, C.; Mani, F. *Inorg. Chem.* **1981**, *20*, 1670.

Table II. Pseudo-First-Order Rate Constants for Attainment of $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$ Equilibrium from the Direction of HCO_3^- Dehydration^a

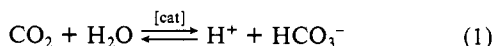
initial pH	$k_{\text{obsd}}, \text{s}^{-1}$, no cat.	$k_{\text{obsd}}, \text{s}^{-1}$, $2\text{b} \cdot \text{Zn}^{2+}$ ^b	$k_{\text{cat}}, \text{M}^{-1} \text{s}^{-1}$ ^c	$k_{\text{obsd}}, \text{s}^{-1}$, 2b^d	$k_{\text{obsd}}, \text{s}^{-1}$, Zn^{2+} or Co^{2+} ^e
6.40	0.822 ± 0.007	0.900 ± 0.004	156 ± 22		0.831 ± 0.004
6.70	0.455 ± 0.006	0.536 ± 0.004	162 ± 20	0.451 ± 0.004	0.450 ± 0.005
7.00	0.295 ± 0.006	0.341 ± 0.006	92 ± 20		

^a 5×10^{-4} M NaHCO_3 , 5×10^{-5} M bromocresol purple, 2.5×10^{-2} M HEPES buffer, 0.2 M NaClO_4 , 80% ethanol- H_2O , $T = 25.0^\circ\text{C}$. ^b 5×10^{-4} M 2b ; 5×10^{-4} M $\text{Zn}(\text{ClO}_4)_2$. ^c $k_{\text{cat}} = (k_{\text{obsd}}(\text{cat}) - k_{\text{obsd}}(\text{no cat})) / [2\text{b} \cdot \text{Zn}^{2+}] \text{M}^{-1} \text{s}^{-1}$. ^d 5×10^{-4} M 2b . ^e 5×10^{-4} M M^{2+} (ClO_4)₂; ref 13b.

**Figure 5.** The visible absorption spectrum of $2\text{b} \cdot \text{Co}(\text{II})$ at the beginning and end of an acetaldehyde hydration reaction.

of 2b at 8.3×10^{-1} M is seen; spectra indicative of octahedral $\text{Co}(\text{II})$ are observed. However, if the picolinate concentration is reduced to 1.7×10^{-3} M (the $[2\text{b} \cdot \text{Co}(\text{II})]$ being the same value), the profile shown in Figure 4 is observed, again showing an upward displacement from that with no added anion.

Kinetic Studies of $2\text{b} \cdot \text{M}^{2+}$. Studies of catalysis of attainment of equilibrium for the process in eq 1 were determined under



pseudo-first-order conditions as previously described.^{13b} Equilibrium was approached from the direction of HCO_3^- dehydration in buffered solutions using NaClO_4 to maintain the ionic strength at 0.2 M. Pseudo-first-order rate constants (k_{obsd}) presented in Table II, refer to the sum of the pseudo-first-order rate constants ($k_{\text{hyd}} + k_{\text{dehyd}}$) for the hydration and dehydration process, which was monitored by an indicator technique in which the rate of change of [bromocresol purple anion] (which rapidly responds to $\Delta[\text{H}^+]$) was monitored at 600 nm on an Aminco-Morrow stopped-flow apparatus at 25.0°C .^{13b} It is clear that some modest catalysis by $2\text{b} \cdot \text{Zn}^{2+}$ is evident at pH 6.40 and 6.70 that is diminished at pH 7.20 and disappears completely at higher values where the kinetic traces become clearly biphasic and precipitation occurs. No catalysis is seen for 2b or Zn^{2+} alone, indicating that a cooperative interaction of the two is required. The complex of $2\text{b} \cdot \text{Co}^{2+}$ does not catalyze the reaction.

Since carbonic anhydrase will also catalyze the hydration of certain aldehydes²² albeit far less efficiently than CO_2 hydration, we looked at the ability of $2\text{b} \cdot \text{M}^{2+}$ to catalyze the hydration of acetaldehyde. No such catalysis could be demonstrated for either the Zn^{2+} or Co^{2+} complex at 1.7×10^{-3} M, although the visible absorption spectrum of the latter does change as the reaction progresses, clearly indicating an association of the hydrate with the complex as shown in Figure 5.

Table III. Observed Pseudo-First-Order Rate Constants for the Hydrolysis of *p*-Nitrophenyl Picolinate (**5**) under Various Conditions^a

pH	ratio of added [complex]/[pNPP]	[added complex], ^b $\text{M} \times 10^5$	[basic complex], ^c $\text{M} \times 10^5$	$k_{\text{obsd}}, \text{s}^{-1} \times 10^4$
7.5	40	200	39.7	139
	30	150	25.0	120
	20	100	12.5	96.7
	10	50	4.52	59.1
	0	0	0	2.18 ^d
7.9	30	150	54.8	162
	10	50	9.71	97.6
	0	0	0	3.32 ^d
8.15	30	150	100	193
	10	50	21.4	139
	0	0	0	6.34 ^d
8.40	30	150	118	201
	10	50	25.7	141
	0	0	0	5.65 ^d
8.70	40	200	175	198
	30	150	124	169
	20	100	73.7	149
	10	50	24.0	101
	0	0	0	6.28 ^d

^a 80% ethanol- H_2O ; 0.048 M Ches; 0.048 M NaClO_4 , $T = 26.0^\circ\text{C}$ [pNPP] = 5×10^{-5} M. k_{obsd} values $\pm 5\%$. ^b Comprised of equivalent concentrations of $\text{Co}(\text{ClO}_4)_2 + 2\text{b}$. ^c Based on ϵ_{max} of 550 $\text{M}^{-1} \text{cm}^{-1}$ for $2\text{b} \cdot \text{Co}(\text{II})$ at 598 nm. ^d k_{obsd} in the absence of added catalyst represents k_{uncat} .

The ability of $2\text{b} \cdot \text{Co}(\text{II})$ to catalyze the hydrolysis of *p*-nitrophenyl picolinate (**5**) was monitored at several pH and $[2\text{b} \cdot \text{Co}^{2+}]$ concentrations, the k_{obsd} values being summarized in Table III. Control experiments established that the reaction is not catalysed by 2b alone at the concentrations employed. Catalysis is seen at pH < 7.9 with $\text{Co}(\text{ClO}_4)_2$ alone; however, above pH 7.9 at the concentrations employed the solutions are heterogeneous, with visible precipitation of what appears to be $\text{Co}(\text{OH})_x$ being observed. Since no evidence of such precipitation occurs in the presence of equimolar 2b and $\text{Co}(\text{II})$, we believe that the species responsible for catalysis is a $2\text{b} \cdot \text{Co}(\text{II})$ complex and not free $\text{Co}(\text{II})$.

It can be seen from the rate constant data in Table III that the uncatalyzed k_{obsd} values are always substantially less than 6% of those observed in the presence of added catalyst. Since we have spectroscopic evidence that picolinate, the product of hydrolysis, interacts with the complex apparently by chelation of the Co^{2+} (vide supra), at least 10-fold excesses of complex to pNPP (**5**) were employed to maintain the pseudo-first-order conditions for hydrolysis of **5**. Nevertheless, as illustrated in Figure 6 at pH 7.5 and 8.7, plots of the total concentration of added complex (Δ or \blacktriangle) or the basic form of the complex (\square or \blacksquare), that giving rise to the characteristic 4- (5-) coordinate $\text{Co}(\text{II})$ visible absorption spectrum) against k_{obsd} are not linear but show saturation effects indicative of a pre-equilibrium binding of pNPP and complex prior to hydrolysis.

Discussion

Titration of $2\text{b} \cdot 2\text{HNO}_3$. From the quantitative titration data for $2\text{b} \cdot 2\text{H}^+$ in the absence and presence of equimolar Zn^{2+} or Co^{2+} , it is apparent that both metals bind reasonably well to the ligand and labilize one additional group associated with the complex.

(22) (a) Pocker, Y.; Meany, J. E. *J. Am. Chem. Soc.* **1965**, *87*, 1809. (b) Pocker, Y.; Meany, J. E. *Ibid.* **1967**, *89*, 631. (c) Pocker, Y.; Meany, J. E. *Biochemistry* **1965**, *4*, 2535. (d) Pocker, Y.; Meany, J. E. *Ibid.* **1967**, *6*, 237. (e) Pocker, Y.; Dickerson, D. G. *Ibid.* **1968**, *7*, 1995.

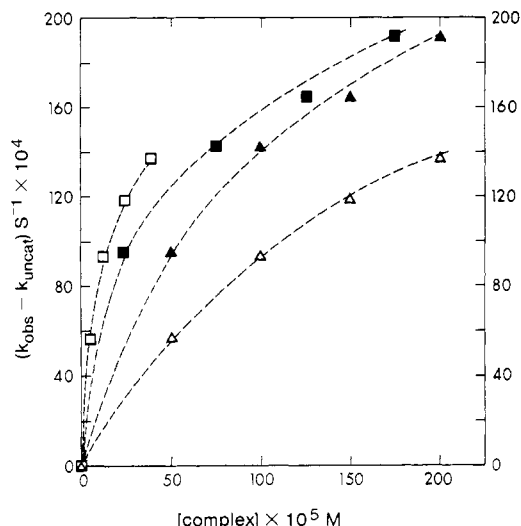
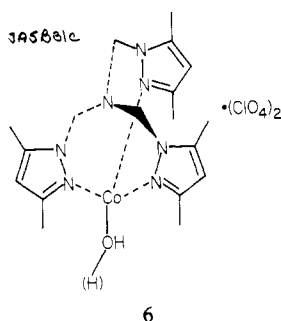


Figure 6. Plots of k_{obs} for the hydrolysis of pNPP at pH 7.5 and 8.7 against the concentrations of total added complex (Δ) and spectrophotometrically determined basic form of the complex (\square). (Open symbols refer to pH 7.5, closed symbols refer to pH 8.7.)

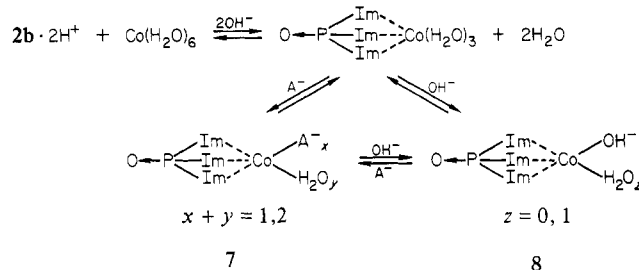
This may be either a metal-associated H_2O or imidazole, although for the following reasons we favor the former.

Martin¹⁸ has reported that tris complexes of 2-(2'-pyridyl)-imidazole with Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , and Zn^{2+} show ligand absorption bands near 298 and 265 nm, which on pyrrole N-H ionization appear near 295 nm with an additional peak or shoulder near 330 nm. Although one must be cautious in extrapolating observations made on that more aromatic system to the present case,^{23c} we note that UV measurements of ligand **2b** (λ_{max} 233 nm (ϵ_{max} $2.3 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$), pH ~ 7) in the presence of 1 equiv of $\text{Zn}(\text{ClO}_4)_2$ show only a minor change (λ_{max} 238 nm (ϵ_{max} $2.3 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$), pH 7.7) in peak position but certainly no evidence of a pH-dependent additional peak at higher wavelength that would be expected should pyrrole N-H ionization be occurring. In the presence of 1.0 equiv of $\text{Co}(\text{ClO}_4)_2$, the situation is quite different in the UV-visible region, with an increasingly large tailing of the UV band being evident up to $\sim 400 \text{ nm}$ occurring coincidentally with the pH-dependent spectral profile seen in Figure 2. This tailing is likely ascribable to $\text{HO}^- \rightarrow \text{Co}(\text{II})$ charge transfer similar to that which can be seen in systems where alkoxide or RS^- are $\text{Co}(\text{II})$ ligands.²⁴ A recent report of the absorption spectrum of cobalt(II) [(tris(3,5-dimethyl-1-pyrazol)methyl)amine hydrate perchlorate (**6**), which has no



possibility of pyrrole N-H ionization, has been similarly analyzed in terms of ionization of a $\text{Co}(\text{II})\text{-H}_2\text{O}$ which then gives rise to a 4- (5-) coordinate $\text{Co}(\text{II})$.^{10c,21c}

Scheme I



We believe the titration data and pH-dependent spectral profile shown in Figures 1 and 2 can therefore best be analyzed by the process shown in Scheme I.

As introduced into solution, **2b** exists as its diprotonated form. Addition of 2 equiv of OH^- deprotonates the salt and allows full coordination of **2b** to $\text{Co}(\text{II})$ by pH 7 since all added proton is titrated by that point. This complex must be predominantly 6-coordinate, as evidenced by the lack of characteristic absorptions which are attributable to 4- and 5-coordinate $\text{Co}(\text{II})$. Further addition of 1 equiv of OH^- deprotonates a metal-associated H_2O ($\text{pK}_a \sim 7.6$ in the absence of added anions) and reduces the coordination number from 6 to 4 or 5, as is evidenced by the appearance of the characteristic blue species attributable to the basic form of the complex (**8**). This situation is strikingly similar to that with $\text{Co}(\text{II})$ CA in that it also is characteristically blue, as is expected for a 4- (5-) coordination of the active-site $\text{Co}(\text{II})$.^{7a,5,19,25,26}

Anion Dependence of the $\text{Co}(\text{II})\text{-2b}$ Spectra. Originally, Lindskog noted that monovalent anions perturbed the absorption spectrum of $\text{Co}(\text{II})$ CA, which indicated an association of the anion with the active-site metal. In addition it was observed¹⁹ that the spectral effects of added anions could be reversed by an increase in pH such that the inhibitory anion is displaced from the metal to ultimately yield the absorption spectrum characteristic of the high-pH, or basic, form of the enzyme. Monovalent anions also inhibit the CO_2 hydration activity of both $\text{Co}(\text{II})$ and $\text{Zn}(\text{II})$ CA, as well as its esterase activity.^{28,29} One should point out that the inhibition by all anions may not adhere to a single common mechanism, since some complexes (i.e., CN^-) appear to be tetrahedrally coordinated, while others show distinct distortion from this geometry and may reflect addition of the anion as a fifth metal ligand.²⁸ The overall effect has been recently discussed by Bertini⁴ and Koenig,⁵ the latter's views that the pH dependence of anion inhibition results from a competition of $\text{OH}^-(\text{H}_2\text{O})$ and anion for a common site on the metal being strongly supported by the following observations.

Interestingly, **2b**- $\text{Co}(\text{II})$ displays a quite similar behavior to the enzyme in that it too exhibits anion-dependent $\text{Co}(\text{II})$ absorption spectra (Figure 3). More interestingly, if one considers the pH dependency of the **2b**- $\text{Co}(\text{II})$ absorption spectra in the presence of added anions (Figure 4), it is evident that the "titration" curves for formation of the basic form of the complex are shifted upward in the presence of anions. By the interpretation in Scheme I, it would therefore appear that anions associate with the $\text{Co}(\text{II})$ complex to form new 4- (5-) coordinate species (**7**), which at higher concentrations of OH^- give rise to the basic complex **8**. Since the only anion common to *all* titrations is OH^- , the basic form of the complex must be one having an associated OH^- . This is again supported by the quantitative titration data depicted in Figures 1 and 2, since 1 equiv of OH^- per equiv of **2b**- $\text{Co}(\text{II})$ is consumed, leading to the basic form. The situation

(23) (a) Boggess, R. K.; Martin, R. B. *Inorg. Chem.* **1974**, *13*, 1525. (b) Sundberg and Martin conclude that pyrrole-H ionizations induced by divalent metal ions of the first or second transition series complexed across the imidazole ring take place with pK_a 's > 10 . Sundberg, R. J.; Martin, R. B. *Chem. Rev.* **1974**, *74*, 471. (c) R. B. Martin, personal communication.

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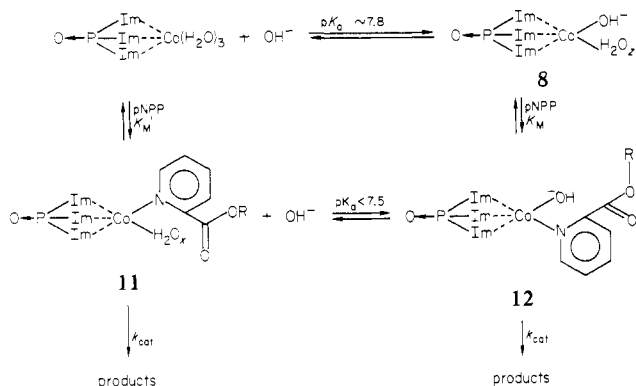
(25) Bertini, I.; Canti, G.; Luchinat, C.; Scozzafava, A. *J. Am. Chem. Soc.* **1978**, *100*, 4873.

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(27) Kernohan, J. C. *Biochim. Biophys. Acta* **1965**, *96*, 304.

(28) For an excellent review of the inhibitory effects of monovalent anions, see ref 1a and 1c.

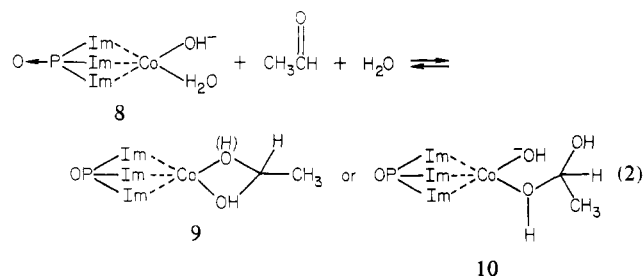
(29) (a) Lindskog, S. *Biochemistry* **1966**, *5*, 2641. (b) Pocker, Y.; Stone, J. T. *Ibid.* **1968**, *7*, 2936; 3021.

Scheme II. Hydrolysis of *p*NPP by **2b**·Co(II)

for the Zn(II) complex is likely to be similar, although of course it displays no visible spectrum.

Cautiously, we might extrapolate these observations one step further to the inhibition of CA catalysis by monovalent anions. It is well to reiterate that many authors have observed that monovalent anions inhibit the enzyme by apparently shifting the pK_a responsible for activity to alkaline pH.^{28,30} Although we have yet to determine the effects of added anions on catalysis afforded by **2b**·M²⁺, one might speculate on the basis of the above findings that the enzymic anionic perturbation of pK_a does not result from a mass action effect in which increasingly larger [OH⁻] is required to displace the inhibitory anions from the metal center. In this interpretation, equivalent to that put forward recently by Koenig,⁵ the inhibitory effect of anions is such that when bound, they prevent access of reactants (CO₂, OH⁻(H₂O)) to the metal. Should this indeed be the case, an obvious corollary would require that there be a linear relationship between [OH⁻] and inhibitory effect of the anions, with those anions giving the largest inhibition shifting the " pK_a " to highest values.

Kinetic Studies. As is illustrated by the data given in Table II, **2b**·Zn(II) catalyzes the interconversion of CO₂ and HCO₃⁻, albeit weakly. Unfortunately, at pH values much in excess of 7, precipitation accompanies the reaction; this is likely due to the formation of insoluble ZnCO₃. Acetaldehyde hydration is apparently not enhanced by the presence of **2b**·Co(II) at the concentrations or pHs employed, but it is interesting to note that the integrity of the basic spectrum of the Co(II) complex changes as the hydration proceeds (Figure 5). Thus the acetaldehyde hydrate interacts with the complex as in eq 2, perhaps to give **9** or **10**, either



of which must be 4- (5-) coordinate as judged by the Co(II) absorption spectrum. Bertini has recently reported that trichloroacetaldehyde hydrate is bound to the metal of Co(II) CA and similarly perturbs the absorption spectrum by displacing the metal-associated H₂O of the active site.³¹

Since CA also catalyzes the hydrolysis of certain esters (notably *p*-nitrophenyl acetate), the catalysis of *p*NPA by **2b**·Zn(II) was investigated at pH 7.75, *T* = 25 °C. Uncatalyzed, the reaction

proceeds with a pseudo-first-order rate constant of $7.89 \times 10^{-6} \text{ s}^{-1}$. The rate of reaction increases linearly with added **2b**·Zn(II), indicative of a process involving the catalyst and *p*NPA, with the second-order-rate constant being about $5.5 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$. In order to increase the affinity of the ester substrate, we chose to study *p*-nitrophenyl picolinate hydrolysis mediated by **2b**·M(II), since the picolinate nitrogen is expected to target this substrate on the metal,¹⁸ perhaps allowing a facile subsequent hydrolysis.

The catalysis of hydrolysis of *p*NPP (**4**) by **2b**·Co(II) was extensively investigated between pH 7.5 and 8.7 as a function of [**2b**·Co(II)]. Since the picolinate product interacts with the complex, the [**2b**·Co(II)] was kept in large excess of **5** to ensure good pseudo-first-order kinetics, ratios of (10–40):1 being employed. In the absence of Co(II), **2b** alone does not catalyze the reaction at these concentrations. However, up to pH 7.9, Co(II) alone is more effective a catalyst (pH 7.9, 30 equiv of Co(II)·(ClO₄)₂, $k_{\text{obsd}} = 3.2 \times 10^{-2} \text{ s}^{-1}$) than the complex. Above this pH however, visible precipitation of Co(OH)_x is observed, although the remaining Co(II) in solution does catalyze hydrolysis ca. one-fifth as well as **2b**·Co(II) at pH 8.4. Although it is not surprising that Co(II) will catalyze the hydrolysis of **5** by binding to the pyridine N, we believe that free Co(II) is not responsible for the catalysis seen in the presence of **2b**·Co(II). Firstly, the titration data of equimolar **2b**·2H⁺ and Co(II) indicate that virtually all ligands, and therefore metal, are bound as a complex by ~pH 7; hence free [Co(II)] must be very low at the larger pHs employed here. Secondly, above pH 7.9, with no added **2b**, Co(OH)_x precipitates from the medium, although no precipitation is evident in the presence of equimolar **2b** up to at least pH 9. Thirdly, monitoring the **2b**·Co(II) absorption spectrum gives a quantitative determination of the amount basic form of the complex (**8**) (ϵ 598 nm 550 M⁻¹ cm⁻¹), these values under various conditions being given in Table III along with the rate constant data. At pH 8.7, we can account for ~75–95% of the added Co(II) as the basic complex (**8**) (at 20–40 equiv of **2b**·Co(II) to *p*NPP), the remaining amount being the octahedral complex shown in Scheme I and the pK_a relating the two species being ~7.7–7.8 under these conditions. Of course, as the concentration of added **2b**·Co(II) is diminished, to 10 equiv relative to *p*NPP, the amount actually present as complex must diminish as is required by the relatively low Co(II) binding constant of **2b**, and the amount of basic form observed (50%) also diminishes.

It is evident from the data presented in Table III that a saturation phenomenon is occurring, since increases in either [**8**] or total added [**2b**·Co(II)] do not produce commensurate rate increases in hydrolysis. In the absence of added **2b**·Co(II), the spontaneous rate of hydrolysis is always less than 5% of that observed in the presence of 30 equiv of catalyst. Curiously the rate data obtained with 30 equiv of added catalyst do not reflect a strong dependence on [OH⁻], since less than a 2-fold increase in rate is seen in passing from pH 7.5 to 8.7. (In fact a slight diminution of rate is seen from pH 8.40 to 8.70.) This could be accounted for by contending that either the overall catalyzed process is insensitive to [OH⁻] or the kinetic pK_a responsible for catalysis by a ternary complex of **2b**·Co(II) and *p*NPP is somewhat less than 7.5. Overall the process might be depicted as in Scheme II.

Here, **2b**·Co(II) exists as its octahedral form or basic form (**8**), the two being tied by a thermodynamic pK_a of ~7.7–7.8 under these conditions. Added *p*NPP might associate with either form to give ternary complexes which probably resemble **11** or **12**, their equilibrium constants for formation being k_M and k_M' , respectively. Either **11** or **12** could break down to products. However, if such breakdown of **11** requires OH⁻, then one would expect a pH dependence for the process, which is not observed. This may be accounted for if the equilibrium position for $\text{OH}^- + \text{11} \rightleftharpoons \text{12}$ lies far to the right at the pHs employed here such that further addition of OH⁻ produces little increase in the [**12**]. Alternatively, **11** could break down to products with no involvement of OH⁻. An Eadie plot of $(k_{\text{obsd}} - k_{\text{uncat}})$ vs. $(k_{\text{obsd}} - k_{\text{uncat}})/[\text{total added } \text{2b} \cdot \text{Co(II)}]$ at pH 8.7, where k_{obsd} and k_{uncat} represent the pseudo-first-order rate constants for *p*NPP hydrolysis in the presence and absence,

(30) For pertinent references, see: Coleman, J. E. In "Biophysics and Physiology of CO₂"; Bauer, C., Gros, G., Bartels, H., Eds.; Springer-Verlag: Berlin, 1980; pp 133–150.

(31) Bertini, I.; Borghi, E.; Canti, G.; Luchinat, C. *J. Inorg. Biochem.* **1979**, *11*, 49.

respectively, of added complex, is linear, the intercept ($k_{\text{cat,max}} = k_{\text{cat}} + k_{\text{cat}}$) being $3.0 \times 10^{-2} \text{ s}^{-1}$ and slope ($-k_{\text{M,TOT}} = -(k_{\text{M}} + k_{\text{M}})$) being $1.0 \times 10^{-3} \text{ M}$, consistent with the process in Scheme II. Such plots from use of only the basic form of the complex (i.e., [8]) are decidedly nonlinear and indicate that it alone is not responsible for *p*NPP binding and hydrolysis.

Conclusion

Although one can never be certain that conclusions drawn from model studies can be extrapolated to the enzymic systems, we believe this tris(imidazolyl)phosphine oxide provides several important small-molecule precedents for observations made with carbonic anhydrase.

(1) Ligand **2b**, when bound to equimolar Zn(II) or Co(II), promotes the dissociation of a metal-bound H_2O having pK_a s of ~ 6 and 7.8 , respectively. Spectrophotometric evidence for the Co(II) complex indicates that titration of the $\text{Co}-\text{OH}_2$ reduces the coordination around the metal from 6 to 5 or 4 as evidenced by the characteristic absorption spectrum of the basic form of the complex.

(2) Monovalent anions such as halides, NO_3^- , and CH_3COO^- interact with the Co(II) complex to produce unique 4- (5-) coordinate complexes, similar to the situation in Co(II)CA. ClO_4^- appears not to be able to associate strongly enough to the complex to produce a characteristic species.

(3) As a function of pH, the absorption spectra of the Co(II)-**2b** complexes in the presence of various anions invariably change at high pH to that of the basic form of the complex. One observes that the spectrophotometric pK_a of the Co(II) complex is shifted to higher values in the presence of anions. The observation clearly stems from a mass action effect whereby the anion is displaced from the metal by increasing concentrations of OH^- . Certain anions such as picolinate associate with the complex so strongly

that very high $[\text{OH}^-]$ is required to displace them. These observations based on the model system explain in a straightforward way the analogous situation in Co(II)CA; the inhibition of catalysis by the enzyme by monovalent anions may be likewise explained.

(4) Zn(II)-**2b** will catalyze the interconversion of CO_2 and HCO_3^- , although very inefficiently. It is clear that the simple expectation that catalysts employing low M^{2+} coordination numbers and a metal-bound hydroxide alone are sufficient to lead to enormous rate accelerations for this process is an oversimplified one. Since our previous catalyst, [tris[4,5-diisopropyl-2-imidazolyl]phosphine]zinc(2+) was more active for this process than Zn(II)-**2b** and considerably more strained, it may be that severe distortion in the complex is required for optimal rates. Of course one cannot overlook the possibility that the enzyme provides a requisite H-bonding network that cannot be duplicated in these models.

(5) **2b**-Co(II) will catalyze the hydrolysis of *p*-nitrophenyl picolinate. Nonlinearity of plots of k_{obsd} vs. [**2b**-Co(II)] in either its total or basic form indicates preequilibrium binding of complex and *p*NPP.

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Electron Delocalization in Mixed-Valence Molybdenum Polyanions

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Abstract: Electron transfer in mixed-valence molybdenum polyanions has been studied by ESR and optical spectroscopy. Two series of compounds belonging to the Lindqvist (Mo_6O_{19}) and the Keggin ($\text{XMo}_{12}\text{O}_{40}$) structural types have been considered. The degree of valence trapping and ground-state delocalization have been measured by ESR at very low temperature (4 K). Optical activation energies have been deduced from the position of intervalence transfer absorption bands. Thermal activation energies have been measured from a detailed analysis of the ESR line width and line shape within the 4–200-K temperature range. Values of the transfer integral J between adjacent molybdenum sites have been estimated from a comparison between optical and thermal activation energies. Experiments show that all of the studied polyanions belong to class II of mixed-valence compounds. Thermally activated electron hopping appears to be much easier in the Keggin structure than in the Lindqvist structure.

A number of hetero- and isopolyanions of molybdenum are reducible to a series of mixed-valence compounds commonly named "heteropoly blues" containing Mo^{VI} and Mo^{V} . These species exhibit intense intervalence transfer bands and occasionally ESR signals that have been interpreted by assuming electron trapping at low temperature.^{1a,2} This description of the electronic

structure corresponds to class II systems in Robin and Day's classification³ of mixed-valence compounds. For such systems, two closely related electron transfer processes can occur: (i) a thermal electron transfer, analogous to the so-called "hopping" process in solids, and (ii) an optical electron transfer. If the electronic interaction between metal centers is vanishing, it has been shown by Hush⁴ that the thermal activation energy (E_{th}) should be one-fourth of the optical energy (E_{op}). For nonzero

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