Tris(pyrazolyl)hydroboratozinc Hydroxide Complexes as Functional Models for Carbonic Anhydrase: On the Nature of the Bicarbonate Intermediate

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Received December 11, 1992

Abstract: The reactions of CO_2 with the zinc hydroxide complexes $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}$ZnOH ($3\text{-Bu}^t\text{-5-Mepz} = 3\text{-Bu}^t\text{-5-MeC}_3N_2H$) and $\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}$ZnOH ($3,5\text{-Pr}^i_2\text{pz} = 3,5\text{-Pr}^i_2C_3N_2H$), which are proposed to be structural models for the active site of the enzyme carbonic anhydrase, have been investigated. The hydroxide $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}$ZnOH reacts immediately with CO_2 to give a bicarbonate complex $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}$Zn(OCO$_2$H) that has been characterized by IR spectroscopy. ^{1}H NMR studies reveal that the reaction with CO_2 is rapid and reversible on the NMR time scale at room temperature. A subsequent slower transformation involves the formation of the carbonate complex $[\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-CO}_3)$, in which the carbonate ligand bridges the two zinc centers with a symmetric unidentate coordination mode. In contrast, the corresponding reaction of the less sterically encumbered hydroxide $\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}$ZnOH rapidly gives the carbonate complex $[\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^2\text{-CO}_3)$, in which the carbonate ligand bridges the two zinc centers in an asymmetric manner and is unidentate to one zinc center but bidentate to the other. $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}$ZnOH is also a functional model for carbonic anhydrase, since it effectively catalyzes the exchange of oxygen atoms between CO_2 and $H_2{}^{17}O$. Structural and reactivity studies on the unidentate and bidentate carbonate complexes, together with structural studies on a series of nitrate complexes, suggest that facile access to a unidentate bicarbonate species may be a critical factor for carbonic anhydrase activity.

Introduction

Carbonic anhydrase, the first zinc enzyme to be discovered, continues to be a subject of intense investigation.[1] The enzyme is ubiquitous in nature, occurring in animals, plants, and bacteria, with an essential physiological function to catalyze the reversible hydration of carbon dioxide (eq 1). As such, carbonic anhydrase

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+ \qquad (1)$$
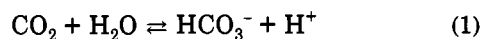
plays important roles with respect to aspects of (i) respiration, transporting CO_2 between metabolizing tissues and the lungs, and (ii) intracellular CO_2/HCO_3^- equilibration.[2] The structures of several forms of the enzyme have been determined by X-ray diffraction, confirming that the active site is composed of a pseudotetrahedral zinc center coordinated to three histidine imidazole groups and either a water molecule $[(His)_3ZnOH_2]^{2+}$ (His = histidine) or a hydroxide anion $[(His)_3ZnOH]^+$, depending upon pH.[3,4]

The catalytic mechanism for carbonic anhydrase activity is now generally considered to comprise several steps, as summarized

Scheme I. Simplified Scheme for the Catalytic Mechanism of Carbonic Anhydrase Activity

in Scheme I:[1,2,5] (i) deprotonation of the coordinated water (with a $pK_a \approx 7$) to give the active zinc hydroxide derivative $[(His)_3ZnOH]^+$,[6] (ii) nucleophilic attack of the zinc-bound hydroxide at the carbon dioxide substrate to give a bicarbonate intermediate $[(His)_3ZnOCO_2H]^+$, and (iii) displacement of the bicarbonate anion by H_2O to complete the catalytic cycle.

Although this sequence illustrates the salient features of the proposed carbonic anhydrase mechanism, many questions remain unanswered or debatable. One important question centers on the nature of the bicarbonate intermediate. For example, is the bicarbonate ligand bound to the zinc center via only one oxygen atom (unidentate) or via two oxygen atoms (bidentate)? Theoretical treatments have explored the possibilities of both unidentate[7] and bidentate[7d,7e] coordination modes of the bicarbonate ligand, while an X-ray absorption study on carbonic anhydrase in the presence of bicarbonate has suggested that the
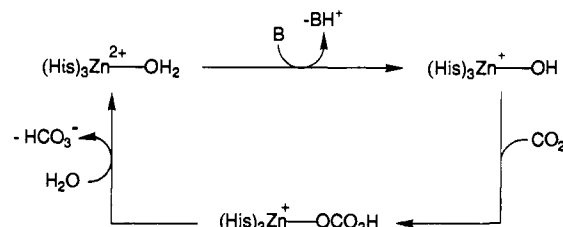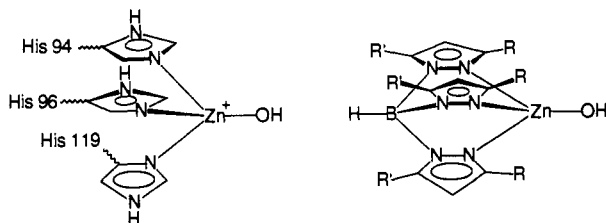
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(6) Since the pK_a of the coordinated water in CAII is ca. 7, the upper limit for proton transfer to bulk water would only be ca. 10^3 s^{-1} (assuming a diffusion-limited rate constant of 10^{10} M^{-1} s^{-1} for the reverse reaction of proton transfer to the coordinated hydroxide), a factor of 10^3 *slower* than the maximum turnover rate. In order to explain the very high turnover rate, it has been proposed that a buffer (B) is required to accept the release of the proton. (a) Eigen, M.; Hammes, G. G. *Adv. Enzymol.* **1963**, *25*, 1–38. (b) Silverman, D. N.; Tu, C. K. *J. Am. Chem. Soc.* **1975**, *97*, 2263–2269. (c) Jonsson, B.-H.; Steiner, H.; Lindskog, S. *FEBS Lett.* **1976**, *64*, 310–314.

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Figure 1. Comparison between the structure of the active site of carbonic anhydrase (left) and the structure of the model {η³-HB(3,5-RR'pz)₃}ZnOH (right).

zinc center remains four-coordinate.[8] In order to address questions of the type raised above, we are presently investigating systems that are designed to be both *structural* and *functional* models of the enzyme carbonic anhydrase. Specifically, the sterically demanding tris(pyrazolyl)hydroborato ligand system [HB-(3,5-RR'pz)₃]⁻ (RR'pz = substituted pyrazole) has provided an excellent role in stabilizing monomeric tetrahedral derivatives of the type {η³-HB(3,5-RR'pz)₃}ZnX.[9] In these complexes, the three nitrogen atom donors of the tris(pyrazolyl)hydroborato ligand bind to the zinc center in a manner closely analogous to that of the histidine imidazole groups in carbonic anhydrase. This analogy is exemplified by the zinc hydroxide complex {η³-HB(3-Buᵗ-5-Mepz)₃}ZnOH (3-Buᵗ-5-Mepz = 3-Buᵗ-5-MeC₃N₂H),[10] which represents an excellent structural model for the active site of carbonic anhydrase (Figure 1). In this paper we describe further studies of tris(pyrazolyl)hydroborato complexes of zinc, with a view to providing information concerning the nature of the bicarbonate intermediate of the carbonic anhydrase catalytic cycle.[11]

Results and Discussion

Over recent years the coordination environment of the zinc center in carbonic anhydrase has been structurally modeled by several research groups using a variety of different ligand systems.[12] The most recent examples are provided by Kimura's triazacyclododecane derivatives and, in particular, the zinc hydroxide complex {[12]aneN₃Zn(OH)}(ClO₄).[13] However, this complex was shown by X-ray diffraction to be a hydrogen-bonded trimer in the solid state associated with a molecule of perchloric acid, {[12]aneN₃Zn(OH)}₃(ClO₄)₃·(HClO₄).[13a] Therefore, the complex {η³-HB(3-Buᵗ-5-Mepz)₃}ZnOH,[10] which represents the

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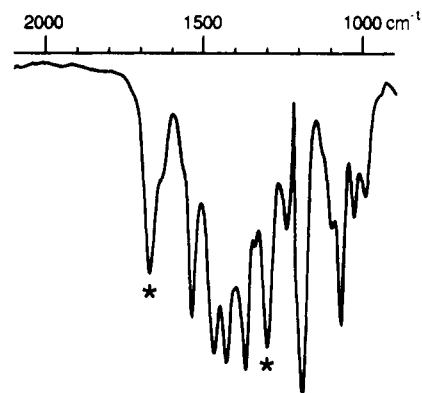
Figure 2. IR spectrum of the bicarbonate complex {η³-HB(3-Buᵗ-5-Mepz)₃}Zn(OCO₂H). * indicates absorptions (1675 and 1302 cm⁻¹) due to the bicarbonate ligand.

first structurally characterized monomeric terminal hydroxide complex of zinc, offers considerable potential with respect to modeling the reactivity and function of the enzyme. At the outset, however, it should be emphasized that the reactions reported in this paper have been performed in hydrocarbon (and not aqueous) solvents in order to establish the reactivity of the Zn–OH functionality in a well-defined environment. Thus, although these studies depict the reactivity of a Zn–OH functionality in a hydrocarbon medium, direct comparison with carbonic anhydrase requires a degree of caution.

Reactivity of {η³-HB(3-Buᵗ-5-Mepz)₃}ZnOH toward CO₂: Unidentate Intermediates. A critical sequence of the proposed carbonic anhydrase catalytic cycle (Scheme I) involves the reaction of a nucleophilic zinc hydroxide group with carbon dioxide to give a bicarbonate species. We have also observed that the zinc hydroxide complex {η³-HB(3-Buᵗ-5-Mepz)₃}ZnOH reacts rapidly (and reversibly) with CO₂ to give the bicarbonate derivative {η³-HB(3-Buᵗ-5-Mepz)₃}Zn(OCO₂H) (eq 2), which thereby provides

$$\{\eta^3\text{-HB(3-Bu}^t\text{-5-Mepz)}_3\}ZnOH + CO_2 \rightleftharpoons$$
$$\{\eta^3\text{-HB(3-Bu}^t\text{-5-Mepz)}_3\}Zn(OCO_2H) \quad (2)$$
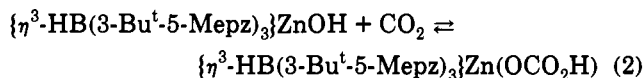
an excellent model step for the proposed carbonic anhydrase catalytic cycle. Spectroscopic evidence for the formation of the bicarbonate complex {η³-HB(3-Buᵗ-5-Mepz)₃}Zn(OCO₂H) has been provided by IR studies, as shown in Figure 2. Specifically, addition of CO₂ to a solution of {η³-HB(3-Buᵗ-5-Mepz)₃}ZnOH in CHCl₃ results in the immediate observation of two bands at 1675 and 1302 cm⁻¹ (marked *) in the IR spectrum, which may be assigned to the bicarbonate ligand of {η³-HB(3-Buᵗ-5-Mepz)₃}Zn(OCO₂H). Although IR data on structurally characterized bicarbonate complexes are rare, by the use of similar arguments that have been used for distinguishing between unidentate and bidentate coordination of carboxylate (O₂CR) ligands,[14] we believe that the positions of the bands (1675 & 1302 cm⁻¹) strongly suggest that the bicarbonate ligand is *unidentate*. For monomeric carboxylate derivatives, the transition from bidentate to unidentate coordination is accompanied by an increasing separation between the symmetric and asymmetric CO₂ stretching bands: $\nu_{asym}(CO_2)$ shifts to higher frequencies as the bonding becomes more localized in the unidentate coordination mode, and $\nu(CO)$ for the uncoordinated group may approach

(14) For monomeric carboxylate derivatives, the separation between the symmetric and asymmetric CO₂ stretching bands, $\Delta\nu = [\nu_{asym}(CO_2) - \nu_{sym}(CO_2)]$, provides a useful indication of the coordination mode: complexes which exhibit values of $\Delta\nu$ greater than 200 cm⁻¹ invariably possess unidentate coordination. Deacon, G. B.; Phillips, R. J. *Coord. Chem. Rev.* **1980**, *33*, 227–250.

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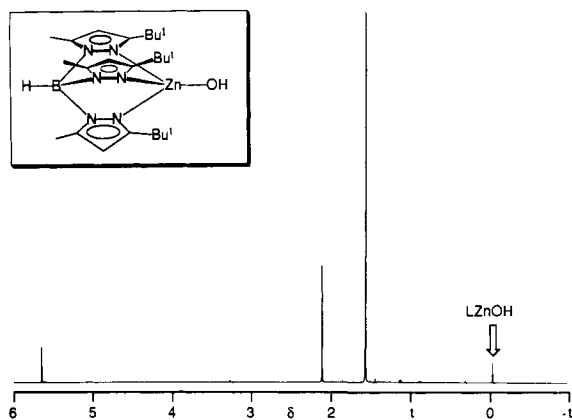
Figure 3. ¹H NMR spectrum of {η^3-HB(3-Bu^t-5-Mepz)$_3$}ZnOH in C$_6$D$_6$. The ZnO*H* group is observed as a sharp singlet at δ –0.06 ppm.

close to that observed for a number of organic carbonyl complexes.[14] Although bicarbonate complexes exhibit more than two ν(CO) vibrations, the transition from bidentate to unidentate coordination would also be expected to be accompanied by a shift of the highest energy CO stretch to higher frequencies as its bond order increases and in the extreme would be expected to approach those of organic carbonates that are typically in the range 1750–1780 cm^{-1}.[15,16] The stretching frequencies of bicarbonate complexes that have been structurally characterized by X-ray diffraction include (i) bidentate RhH$_2$(PPr$^i_3$)$_2$(η^2-O$_2$COH) (1587 and 1338 cm^{-1}; $\Delta\nu$ = 249 cm^{-1})[17] and (ii) unidentate *trans*-Pd(CH$_3$)(PEt$_3$)$_2$(η^1-OCO$_2$H) (1634 and 1353 cm^{-1}; $\Delta\nu$ = 281 cm^{-1})[18] and *cis*-Pd(dppe)(η^1-OCO$_2$H)$_2$ (1700, 1630, 1410, 1300, and 850 cm^{-1}).[19] These data, therefore, support the notion that the higher frequency absorption of the bicarbonate ligand increases as the coordination mode changes from bidentate to unidentate.[20] As such, the stretching frequencies (1675 and 1302 cm^{-1}; $\Delta\nu$ = 373 cm^{-1}) observed for {η^3-HB(3-Bu^t-5-Mepz)$_3$}Zn(OCO$_2$H) are in accord with a unidentate bicarbonate ligand. Additional support is also provided by the observation of similar stretching frequencies for the methyl carbonate derivative {η^3-HB(3-Bu^t-5-Mepz)$_3$}Zn(η^1-OCO$_2$CH$_3$) (1689 and 1297/1280 cm^{-1}; $\Delta\nu$ = 392–409 cm^{-1}), which Vahrenkamp has shown by X-ray diffraction to possess a unidentate methyl carbonate ligand.[11]

The formation of the bicarbonate complex {η^3-HB(3-Bu^t-5-Mepz)$_3$}Zn(OCO$_2$H) is reversible, and removal of the CO$_2$ atmosphere results in regeneration of {η^3-HB(3-Bu^t-5-Mepz)$_3$}ZnOH. Evidence for the reversibility of the reaction is readily provided by ¹H NMR spectroscopy. The ¹H NMR spectrum of {η^3-HB(3-Bu^t-5-Mepz)$_3$}ZnOH in C$_6$D$_6$ is shown in Figure 3 and is characterized by a sharp resonance at –0.06 ppm assigned to the ZnO*H* group. Addition of CO$_2$ to {η^3-HB(3-Bu^t-5-Mepz)$_3$}ZnOH results in significant broadening (into the base line) and shifting of the ZnO*H* resonance. Small shifts of the resonances associated with the tris(pyrazolyl)hydroborato ligand are also observed. The partial ¹H NMR spectra shown in Figure 4 illustrate the changes that are observed upon the incremental removal of the CO$_2$ atmosphere: the hydroxy resonance becomes sharper as the CO$_2$ atmosphere is removed and eventually returns

Figure 4. ¹H NMR evidence for rapid, reversible, reaction of {η^3-HB(3-Bu^t-5-Mepz)$_3$}ZnOH with CO$_2$. The upper spectrum illustrates the ZnO*H* resonance of {η^3-HB(3-Bu^t-5-Mepz)$_3$}ZnOH (* represents an internal standard). Upon exposure to CO$_2$ (1 atm), the bottom spectrum is obtained, in which the ZnO*H* resonance is broadened into the baseline. Upon incremental removal of the CO$_2$, the resonance emerges from the baseline, ultimately returning to that for {η^3-HB(3-Bu^t-5-Mepz)$_3$}ZnOH.

to that of {η^3-HB(3-Bu^t-5-Mepz)$_3$}ZnOH, with a sharp ZnO*H* resonance. The data clearly provide strong evidence that the reversible reaction of {η^3-HB(3-Bu^t-5-Mepz)$_3$}ZnOH with CO$_2$ is rapid on the NMR time scale at room temperature. A measure of the rate of the reaction may be estimated from the lifetime broadening of the ZnO*H* resonance. Under 1 atm of CO$_2$ (solubility ca. 0.1 M)[21] the broadening is at least 50 Hz, so that the rate constant is at least of the order 10^2–10^3 M^{-1} s^{-1}.

Complete NMR characterization of {η^3-HB(3-Bu^t-5-Mepz)$_3$}-Zn(OCO$_2$H) has proved difficult as a result of the rapid equilibrium that exists between {η^3-HB(3-Bu^t-5-Mepz)$_3$}-Zn(OCO$_2$H) and {η^3-HB(3-Bu^t-5-Mepz)$_3$}ZnOH. Even low-temperature ¹H NMR studies at –90 °C in toluene-d_8 were unable to reveal both species. Furthermore, ¹⁷O NMR studies demonstrate that the ¹⁷O-labeled derivative {η^3-HB(3-Bu^t-5-Mepz)$_3$}Zn¹⁷OH (vide infra) undergoes rapid exchange of oxygen atoms with excess CO$_2$, resulting in transfer of the ¹⁷O label from the hydroxy group to CO$_2$ (eq 3). The rapidity of this exchange

$$\{\eta^3\text{-HB(3-Bu}^t\text{-5-Mepz)}_3\}\text{Zn}^{17}\text{OH} + \text{CO}_2 \rightleftharpoons$$
$$\{\eta^3\text{-HB(3-Bu}^t\text{-5-Mepz)}_3\}\text{Zn(OCO}^{17}\text{OH)} \rightleftharpoons$$
$$\{\eta^3\text{-HB(3-Bu}^t\text{-5-Mepz)}_3\}\text{ZnOH} + {}^{17}\text{OCO} \quad (3)$$

process presumably prevents the observation of ¹⁷O NMR

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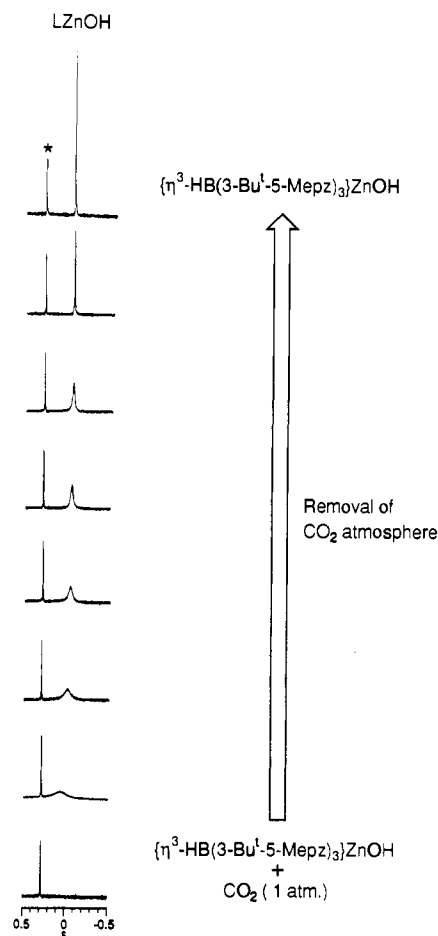
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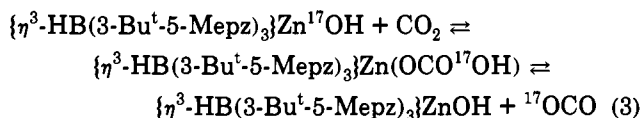
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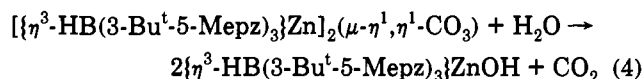
(21) The mole fraction solubility of CO$_2$ in C$_6$H$_6$ is x_g = 0.009 12 (ca. 0.1 M) at 298.2 K and a partial pressure of 1.013 bar. Fogg, P. G. T.; Gerrard, W. *Solubility of Gases in Liquids*; Wiley: New York, 1991.

Scheme II. Formation of a Symmetric Bridging Carbonate Complex

Figure 5. ^{17}O NMR spectrum of $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn^{17}OH$ in C_6D_6.
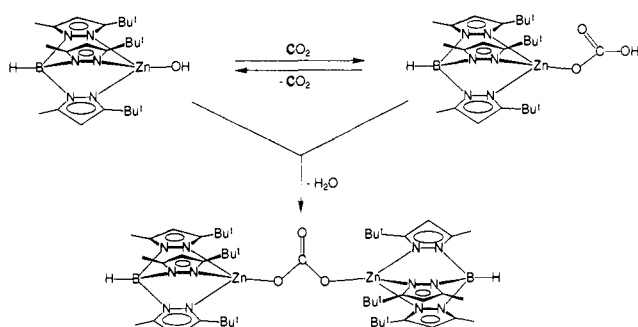
resonances for the bicarbonate complex. Moreover, we were also unable to observe a resonance in the ^{13}C NMR spectrum of a solution of $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$ in C_6D_6 in the presence of enriched $^{13}CO_2$ (1 atm) that could be assigned with confidence to a bicarbonate ligand, again presumably due to the rapid exchange.

In view of the labile equilibrium between $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-}$ Mepz)_3\}Zn(OCO_2H)$ and $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$, attempts to crystallize $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn(OCO_2H)$ were conducted under an atmosphere of CO_2. However, under these conditions, the bridging carbonate complex $[\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-}$ Mepz)_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-CO}_3)$ was the product that was isolated (Scheme II).[22] $[\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-CO}_3)$ may be regarded as a product of the condensation of $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-}$ 5-Mepz)_3\}Zn(OCO_2H)$ and $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$. Preferential crystallization of $[\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-}$ CO_3)$ from the reaction mixture is presumably a consequence of its lower solubility, since once isolated, it reacts instantaneously with water to give $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$ (eq 4). The

$$[\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-CO}_3) + H_2O \rightarrow$$
$$2\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH + CO_2 \quad (4)$$

molecular structure of $[\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-}$ CO_3)$ has been determined by X-ray diffraction, confirming that the bridging carbonate is coordinated to each zinc center in a *unidentate* fashion.[23] As such, the bis(unidentate) coordination mode of the bridging carbonate ligand provides additional support for the presence of a unidentate versus bidentate bicarbonate ligand in $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn(OCO_2H)$. Symmetric bis(unidentate) coordination of bridging carbonate ligands has been observed previously.[24] Additional characterization of the carbonate complex $[\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-CO}_3)$ is provided by IR and NMR spectroscopy. IR studies on $[\{\eta^3\text{-}$ HB(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-CO}_3)$ reveal that the bridging carbonate ligand shows $\nu(CO)$ absorptions at 1587 and 1311 cm^{-1}.[25] The ^{13}C NMR spectrum of isotopically enriched samples shows a resonance at δ 164 ppm (in C_6D_6) that is assigned to the bridging CO_3 group. Thus, whereas we were unable to observe a ^{13}C NMR resonance for the bicarbonate ligand of $\{\eta^3\text{-HB}(3\text{-}$
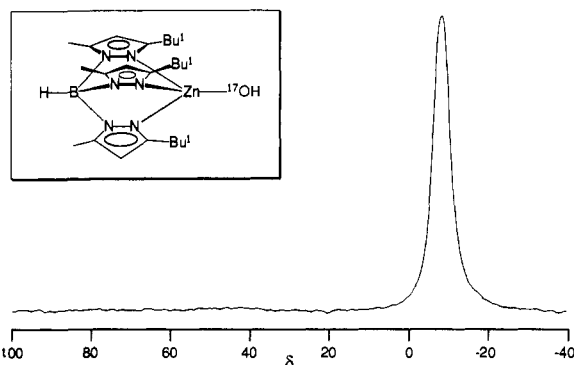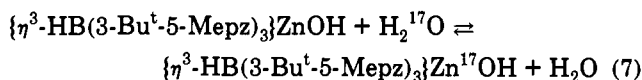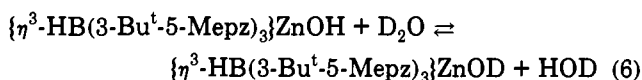
$Bu^t\text{-5-Mepz})_3\}Zn(OCO_2H)$ due to chemical exchange, there is no difficulty in locating the ^{13}C NMR resonance for the carbonate ligand of $[\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-CO}_3)$, which is not in equilibrium with the hydroxide in the absence of water.

Exchange Reactions Catalyzed by $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}$-ZnOH. The chemistry described above indicates that, within the tris(pyrazolyl)hydroboratozinc hydroxide system, we have observed key transformations that are required for catalytic activity. Furthermore, we have also obtained additional evidence which indicates that the zinc hydroxide complex $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-}$ Mepz)_3\}ZnOH$ is indeed a *functional* model for carbonic anhydrase. Specifically, ^{17}O NMR spectroscopy demonstrates that the zinc hydroxide complex $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$ is an effective catalyst for the exchange of oxygen between CO_2 and $H_2^{17}O$ in benzene solution (eq 5). Although the physiological

$$CO_2 + H_2^{17}O \rightleftharpoons CO^{17}O + H_2O \quad (5)$$
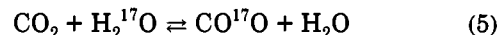
importance of carbonic anhydrase rests with the catalytic formation of bicarbonate (HCO_3^-), the above exchange process is also naturally catalyzed by the enzyme. Indeed, this method of measuring oxygen atom exchange between CO_2 and $H_2^{17}O$ at equilibrium is analogous to the original ^{18}O method for measuring the uncatalyzed rate of hydration of CO_2 (and dehydration of HCO_3^-).[26,27]

The experiments that reveal the ability of $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-}$ Mepz)_3\}ZnOH$ to catalyze oxygen exchange between CO_2 and $H_2^{17}O$ are summarized in Figures 5 and 6. The initial experiment was to demonstrate that, analogous to carbonic anhydrase,[6b,28] the model hydroxide complex $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$ also undergoes rapid exchange with labeled water (eqs 6 and 7). The

$$\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH + D_2O \rightleftharpoons$$
$$\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOD + HOD \quad (6)$$

$$\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH + H_2^{17}O \rightleftharpoons$$
$$\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn^{17}OH + H_2O \quad (7)$$

(22) For a general review and some recent papers on metal carbonates see: (a) Palmer, D. A.; Van Eldik, R. *Chem. Rev.* **1983**, *83*, 651–731. (b) Alvarez, R.; Atwood, J. L.; Carmona, E.; Pérez, P. J.; Poveda, M. L.; Rogers, R. D. *Inorg. Chem.* **1991**, *30*, 1493–1499. (c) Einstein, F. W. B.; Willis, A. C. *Inorg. Chem.* **1981**, *20*, 609–614. (d) Kolks, G.; Lippard, S. J.; Waszczak, J. V. *J. Am. Chem. Soc.* **1980**, *102*, 4832–4833.

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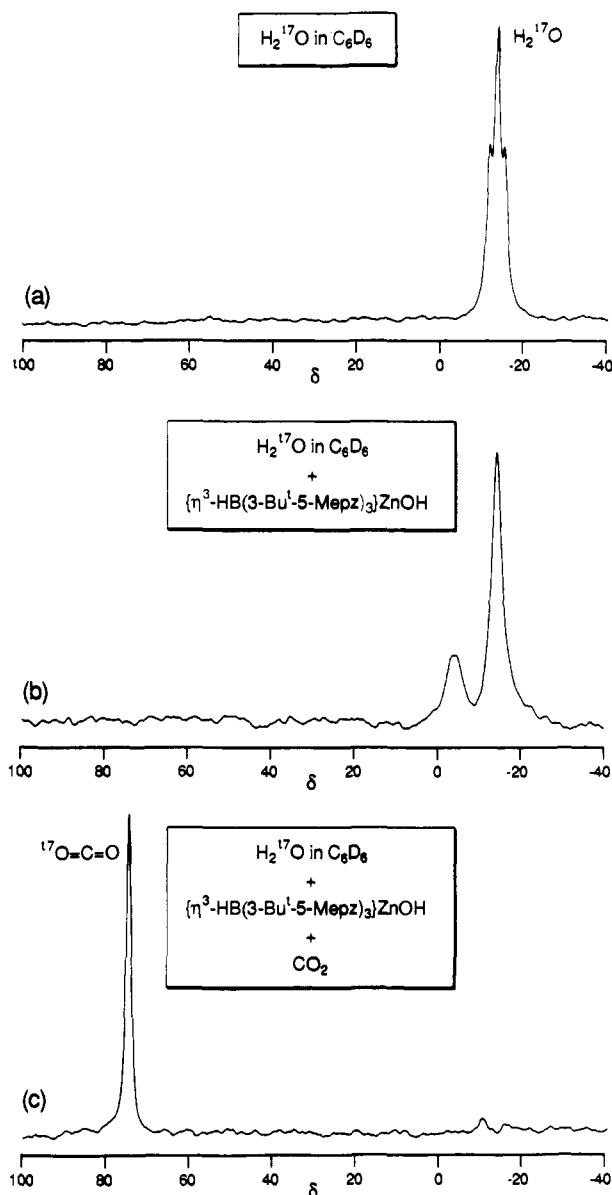
Figure 6. ^{17}O NMR evidence for the catalytic exchange of oxygen atoms between $H_2^{17}O$ and CO_2: (a) ^{17}O NMR spectrum of a saturated solution of $H_2^{17}O$ in C_6D_6; (b) ^{17}O NMR spectrum obtained upon addition of {η^3-HB(3-Bu^t-5-Mepz)$_3$}ZnOH to sample (a); (c) ^{17}O NMR spectrum obtained upon addition of CO_2 to sample (b), illustrating complete transfer of ^{17}O label from $H_2^{17}O$ to CO_2.

existence of these exchange processes was readily confirmed using ^2H and ^{17}O NMR spectroscopy. The ^{17}O NMR spectrum of the enriched complex {η^3-HB(3-Bu^t-5-Mepz)$_3$}Zn^{17}OH obtained by this method is shown in Figure 5, revealing that the Zn^{17}OH group is characterized by a resonance at −8 ppm.
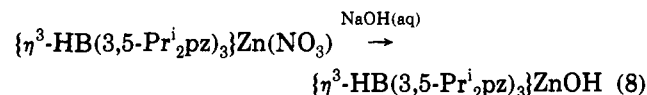
Experiments to demonstrate the catalytic oxygen exchange between CO_2 and $H_2^{17}O$ also centered on ^{17}O NMR spectroscopy. The ^{17}O NMR spectrum of ^{17}O-enriched water in benzene is shown in Figure 6(a). Addition of {η^3-HB(3-Bu^t-5-Mepz)$_3$}ZnOH results in the observation of two resonance, one due to $H_2^{17}O$ and one due to {η^3-HB(3-Bu^t-5-Mepz)$_3$}Zn^{17}OH produced by an exchange process [Figure 6(b)]. Significantly, upon addition of excess CO_2, the ^{17}O NMR spectrum reveals that the ^{17}O label has been completely transferred from $H_2^{17}O$ to carbon dioxide within a period of minutes at room temperature [Figure 6(c)]. In contrast, the exchange process for a sample prepared under similar conditions but in the absence of the {η^3-HB(3-Bu^t-5-Mepz)$_3$}ZnOH catalyst takes several days to proceed to com-

Scheme III. Potential Mechanism for the Catalytic Exchange of Oxygen Atoms between H_2O^* and CO_2 (H_2O^* = $H_2^{17}O$)
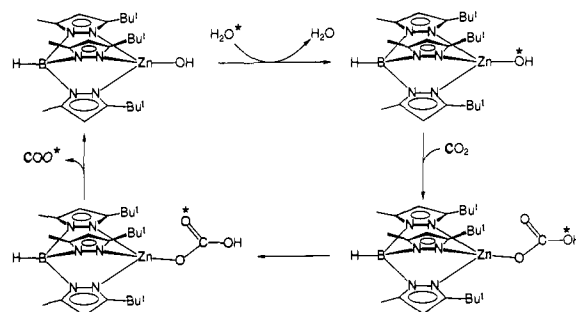
pletion,[29] thereby clearly demonstrating the catalytic efficiency of the zinc hydroxide complex. A simplified cycle for oxygen atom exchange catalyzed by {η^3-HB(3-Bu^t-5-Mepz)$_3$}ZnOH is shown in Scheme III, but alternative mechanisms are also possible.

Reactivity of {η^3-HB(3,5-Pr^i$_2$pz)$_3$}ZnOH toward CO_2: Bidentate Intermediates. The *tert*-butyl groups of the substituted tris(pyra-zolyl)hydroborato ligand [η^3-HB(3-Bu^t-5-Mepz)$_3$]$^-$ create a very sterically demanding coordination environment about the zinc center in the above complexes. Indeed, the steric demands of this ligand may be one of the main factors responsible for the formation of the four-coordinate unidentate bicarbonate and carbonate complexes, rather than the alternative five-coordinate bidentate derivatives. In order to examine this suggestion, we have investigated ligands that would create more spacious pockets around the metal center than is provided by the *tert*-butyl derivatives described above and thereby promote increased coordination at the zinc center. Although substitution at the 3-position by methyl would create a very spacious pocket around zinc, our previous studies would suggest that the six-coordinate sandwich complex [η^3-HB(3,5-Me$_2$pz)$_3$]$_2$Zn is likely to provide a thermodynamic sink of the system.[30] Therefore, for the purpose of this study we have utilized the tris(3,5-diisopropylpyrazolyl)-hydroborato ligand system developed by Kitajima and Moro-oka.[31] The iodide and nitrate derivatives {η^3-HB(3,5-Pr^i$_2$pz)$_3$}ZnX (3,5-Pr^i$_2$pz = 3,5-Pr^i$_2$C$_3$N$_2$H; X = I, NO$_3$) are readily prepared by the direct reaction of K{HB(3,5-Pr^i$_2$pz)$_3$} with ZnX$_2$. The iodide derivative has been structurally characterized by X-ray diffraction, confirming the monomeric nature of the complex.[23,32] The zinc hydroxide {η^3-HB(3,5-Pr^i$_2$pz)$_3$}ZnOH complex is synthesized by reaction of the nitrate derivative with NaOH (eq 8)[33]

$$\{\eta^3\text{-HB}(3,5\text{-Pr}^i{}_2\text{pz})_3\}\text{Zn(NO}_3) \xrightarrow{\text{NaOH(aq)}} $$
$$\{\eta^3\text{-HB}(3,5\text{-Pr}^i{}_2\text{pz})_3\}\text{ZnOH} \quad (8)$$

and has also been obtained independently by Kitajima using an alternative route.[34] The hydroxide functionality in {η^3-HB(3,5-Pr^i$_2$pz)$_3$}ZnOH is characterized by a ν(OH) absorption at 3668 cm^{-1} in the IR spectrum and resonances at δ −0.29 and δ −36 in the ^1H and ^{17}O NMR spectra, respectively, similar to those for

(29) Note that the exchange process in benzene solution is significantly slower than that in water, which greatly facilitates rate measurements by ^{17}O NMR spectroscopy.

(30) (a) Han, R.; Parkin, G. *J. Organomet. Chem.* **1990**, *393*, C43–C46. (b) Looney, A.; Parkin, G. Unpublished results.

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(32) X-ray diffraction data on the nitrate derivative {η^3-HB(3,5-Pr^i$_2$pz)$_3$}Zn(NO$_3$) also confirm that the complex is monomeric, but details of the nitrate ligand are obscured due to disorder.

(33) {η^3-HB(3,5-Pr^i$_2$pz)$_3$}ZnOH may also be obtained by the reaction of {η^3-HB(3,5-Pr^i$_2$pz)$_3$}ZnI with either NaOH or Bu^n$_4$OH, but in lower yield. Han, R.; Parkin, G. Unpublished results.

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Tris(pyrazolyl)hydroboratozinc Hydroxide Complexes

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Scheme IV. Reaction of $\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}$ZnOH with CO_2

Figure 7. Variation of coordination mode of the nitrate ligand in $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{pz})_3\}M(NO_3)$ as a function of M (M = Zn, Co, Ni, Cu).

Scheme V. Different Stabilities of Bridging Carbonate Complexes toward H_2O
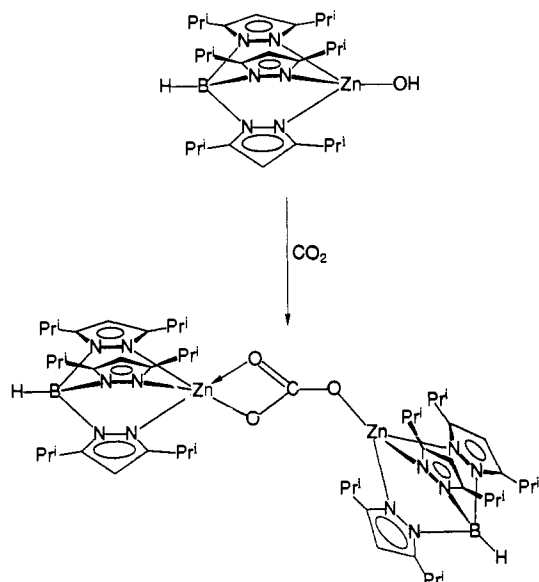
$\{\eta^3\text{-HB}(3\text{-Me-5-Bu}^t\text{pz})_3\}$ZnOH. In this regard, we note that Kitajima was unable to observe resonances attributable to the zinc hydroxide group of $\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}$ZnOH.[34] However, we have confirmed our assignment for the hydroxy resonance in the ^{1}H NMR spectrum by ^{2}H NMR spectroscopy on the deuterated complex $\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}$ZnOD, obtained by the exchange reaction with D_2O.

Similar to $\{\eta^3\text{-HB}(3\text{-Me-5-Bu}^t\text{pz})_3\}$ZnOH, the less sterically encumbered hydroxide $\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}$ZnOH also reacts instantaneously with CO_2. However, as shown in Scheme IV, in marked contrast to $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}$ZnOH, the product that is observed immediately upon addition of CO_2 is not the bicarbonate derivative but rather the bridging carbonate complex $[\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$, an observation also noted by Kitajima.[34] Furthermore, the molecular structure of $[\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$, as determined by X-ray diffraction,[23,34] reveals that the carbonate ligand is coordinated in an asymmetric manner, with unidentate coordination to one zinc center and bidentate to the other zinc center, i.e. $\mu\text{-}\eta^1,\eta^2\text{-}CO_3$.[35] As such, the coordination mode of the bridging carbonate ligand in $[\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$ is noticeably distinct from that of $[\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$, in which the carbonate ligand bridges in a symmetric unidentate mode. In this regard it is also of interest to contrast the unidentate/bidentate coordination mode of the bridging carbonate ligand with that observed by Kitajima and Moro-oka in the corresponding complexes $[\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}M]_2(\mu\text{-}\eta^2,\eta^2\text{-}CO_3)$ (M = Fe, Co, Ni, Cu), in which the carbonate ligand bridges in a *bidentate* fashion to each metal center.[34,36]

Consequences of Unidentate versus Bidentate Coordination. Significantly, the difference in coordination modes of the bridging carbonate ligands of $[\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$ and $[\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$ is manifested in their reactivities. Thus, whereas the complex $[\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$, with symmetric unidentate coordi-

nation, reacts instantaneously with H_2O to give $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}$ZnOH, the carbonate complex $[\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$ is stable to H_2O at room temperature (Scheme V). It is likely that the increased thermodynamic stability of $[\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$ toward H_2O may be due to additional ground-state stabilization imparted by bidentate coordination of the carbonate ligand at one of the zinc centers.

The carbonic anhydrase catalytic cycle requires rapid displacement of bicarbonate by water. If the above observation that the reactivity of a carbonate ligand is dramatically influenced by its coordination mode may be applied to the corresponding bicarbonate derivatives, these results would suggest that the formation of a bidentate bicarbonate intermediate could inhibit a catalytic cycle. We have recently provided support for this suggestion by examination of metal-substituted systems, for which we proposed that the coordination mode of a bicarbonate ligand in a complex may be gleaned by comparison with the structure of the related isoelectronic nitrate derivative.[23,37] The coordination mode of the nitrate ligands in the complexes $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{pz})_3\}M(NO_3)$ (M = Co, Ni, Cu, Zn)[38] was shown to vary from unidentate for Zn to symmetric bidentate for Ni and Cu (Figure 7). In contrast, the cobalt derivative exhibits a noticeably asymmetric coordination mode of the nitrate ligand, which is intermediate between unidentate and symmetric bidentate (Figure

(35) The coordination mode of the bridging carbonate ligand in this complex resembles that observed in $[(C_6H_5)_4Sb]_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$,[35a] $Rh_2(PPh_3)_5(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$,[35b] $[Cu(NH_3)_2(CO_3)]$,[35c] and $[Mo(CO)(PMe_2Ph)_3(CO_3)]_2$.[35d] (a) Ferguson, G.; Hawley, D. M. *Acta Crystallogr.* **1974**, *B30*, 103–111. (b) Krogsrud, S.; Komiya, S.; Ito, T.; Ibers, J. A.; Yamamoto, A. *Inorg. Chem.* **1976**, *15*, 2798–2805. (c) Meyer, M. H.; Singh, P.; Hatfield, W. E.; Hodgson, D. J. *Acta Crystallogr.* **1972**, *B28*, 1607–1613. (d) Chatt, J.; Kubota, M.; Leigh, G. J.; March, T. C.; Mason, R.; Yarrow, D. J. *J. Chem. Soc., Chem. Commun.* **1974**, 1033–1034.

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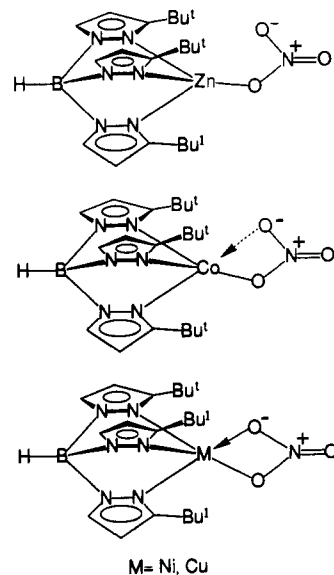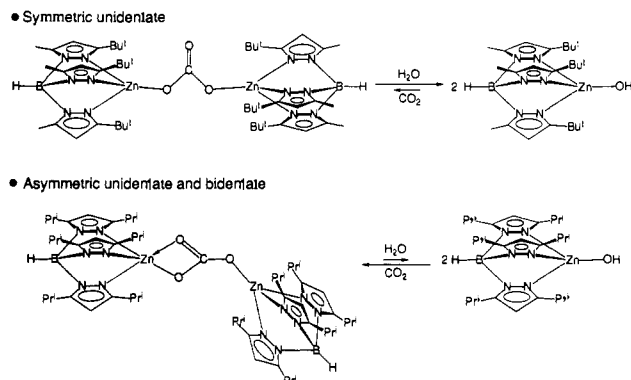
(37) Han, R.; Parkin, G. *J. Am. Chem. Soc.* **1991**, *113*, 9707–9708.

(38) The ligands $[\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3]^-$ and $[\eta^3\text{-HB}(3\text{-Bu}^t\text{pz})_3]^-$ differ only by methyl substitution at the 5-position of the pyrazolyl group.

7).[39] Studies on metal-substituted carbonic anhydrases[1,40,41] have revealed that the order of activity decreases across the series Zn > Co ≫ Ni and Cu, and it is striking how this sequence correlates with the observed coordination mode of the nitrate ligands in the model complexes $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{pz})_3\}M(NO_3)$ (M = Co, Ni, Cu, Zn). Furthermore, the cadmium derivative $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Cd(NO_3)$ also exhibits bidentate coordination of the nitrate ligand,[42] and cadmium-substituted carbonic anhydrase is also less active than the zinc enzyme.[43]

The ability of cobalt(II), nickel(II), copper(II), and cadmium(II) to exhibit a greater tendency than Zn(II) toward bidentate coordinate is also illustrated by structural comparisons within a series of 3-isopropyl-substituted tris(pyrazolyl)hydroboratometal carbonate complexes. Thus, of the bridging carbonate complexes $[\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}M]_2(\mu\text{-}CO_3)$ (M = Mn, Fe, Co, Ni, Cu, Zn), only the zinc derivative does not exhibit bidentate coordination at both metal centers.[34] Furthermore, Kitajima has also noted varying degrees of asymmetry of the bridging carbonate ligand in the complexes $[\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}M]_2(\mu\text{-}CO_3)$ (M = Mn, Fe, Co, Ni, Cu) that closely parallel the series of nitrate complexes described above. Thus, whereas the Ni and Cu complexes exhibit symmetric bridging coordination, the Fe and Co complexes exhibit asymmetric bridging coordination.[34]

Although many factors (e.g. the pK_a of the coordinated water) may be responsible for affecting the activity of metal-substituted carbonic anhydrases,[44] one interpretation of our results, if they can be applied to the isoelectronic bicarbonate derivatives, is that a progressive ground-state stabilization of the bicarbonate ligand would be observed for the sequence Zn < Co < Cu, Ni, and Cd. Stronger binding of the bicarbonate ligand across the series Zn < Co < Cu, Ni, and Cd may result in slower displacement of the bicarbonate ligand by H_2O and thereby prevent a catalytic cycle from operating for Cu and Ni derivatives, as a result of product inhibition. Therefore, a critical requirement for carbonic anhydrase activity may be the accessibility of a unidentate, rather than a bidentate, bicarbonate intermediate.[45] This suggestion supports the results of X-ray absorption studies on zinc- and cobalt-substituted carbonic anhydrases in the presence of bicarbonate, in which the zinc derivative is proposed to be four-coordinate whereas the cobalt derivative is proposed to be five-coordinate.[8]

Conclusion

The zinc hydroxide complex $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$ has been shown to be not only a structural model for the active site of carbonic anhydrase but also a functional model by providing an efficient catalytic cycle for the exchange of oxygen atoms between CO_2 and H_2O. Structural and reactivity studies on unidentate and bidentate carbonate complexes, together with

structural studies on a series of nitrate complexes $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{pz})_3\}M(NO_3)$ (M = Co, Ni, Cu, Zn), suggest that the order of activity of metal-substituted carbonic anhydrases (Zn > Co ≫ Ni and Cu) may in part reflect a change in coordination mode for the proposed bicarbonate intermediate. The increased tendency toward bidentate coordination across the series (Zn < Co < Ni and Cu) would be expected to result in stronger binding of the bicarbonate ligand and hence slower displacement by H_2O. Facile access to a unidentate bicarbonate species may therefore be a critical factor for carbonic anhydrase activity.

Experimental Section

General Considerations. All manipulations were performed using a combination of glovebox, high-vacuum, or Schlenk techniques.[46] Solvents were purified and degassed by standard procedures. ^1H, ^2H, ^{13}C, and ^{17}O NMR spectra were measured on Varian VXR 200, 300, and 400 spectrometers. ^2H NMR spectra are referenced relative to natural abundance deuterium present in the protio solvent (C_6H_5D, 7.15 ppm; $CDCl_3$, 7.24 ppm). ^{17}O NMR spectra are referenced relative to external H_2O (0.0 ppm). IR spectra of isolated complexes were recorded as Nujol mulls or KBr pellets on a Perkin-Elmer 1420 spectrophotometer and are reported in cm⁻¹. IR spectra of the bicarbonate complex $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn(OCO_2H)$ were obtained in $CHCl_3$ solution in a cell using NaCl windows. Elemental analyses were measured using a Perkin-Elmer 2400 CHN Elemental Analyzer. Mass spectra were obtained on a Nermag R10-10 mass spectrometer using chemical ionization (NH_3 or CH_4) techniques. $K\{HB(3\text{-Bu}^t\text{-5-Mepz})_3\}$,[47] $K\{HB(3,5\text{-Pr}^i_2\text{pz})_3\}$,[31] $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnI$,[23] and $\{\eta^3\text{-HB}(3,5\text{-Pr}^i_2\text{pz})_3\}ZnI$[23] were prepared as reported elsewhere.

Preparation of $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$. $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$ was synthesized by an alternative procedure to that originally reported.[10] A suspension of $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnI$ (0.5 g, 0.8 mmol) in benzene (20 mL) was treated with a solution of Bu^n_4NOH in methanol (0.9 mL of 1.0 M, 0.9 mmol), giving a white precipitate. The mixture was stirred for 10 min at room temperature and filtered. The solvent was removed from the filtrate under reduced pressure, and the white solid was recrystallized from Et_2O, giving pure $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$ as white crystals (250 mg, 62%). ^1H NMR (C_6D_6) δ 1.55 (18 H, s, $\eta^3\text{-HB}\{C_3N_2H(CH_3)CMe_3\}_3$), 2.10 (9 H, s, $\eta^3\text{-HB}\{C_3N_2H\text{-}(CH_3)CMe_3\}_3$), 5.64 (3 H, s, $\eta^3\text{-HB}\{C_3N_2H(CH_3)CMe_3\}_3$), −0.06 (1 H, s, ZnO*H*). ^{13}C NMR (C_6D_6) δ 12.5 ($\eta^3\text{-HB}\{C_2N_2CH(CH_3)CMe_3\}_3$), 30.7 ($\eta^3\text{-HB}\{C_2N_2CH(CH_3)CMe_3\}_3$), 31.9 ($\eta^3\text{-HB}\{C_2N_2CH\text{-}(CH_3)CMe_3\}_3$), 102.8 ($\eta^3\text{-HB}\{C_2N_2CH(CH_3)CMe_3\}_3$), 143.7 and 163.2 ($\eta^3\text{-HB}\{C_2N_2CH(CH_3)CMe_3\}_3$).

Reaction of $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$ with CO_2. (a) A solution of $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$ (ca. 15 mg, 0.03 mmol) in C_6D_6 was saturated with CO_2 (1 atm) and monitored using ^1H, ^{13}C, and ^{17}O NMR spectroscopy. The CO_2 atmosphere was removed incrementally and the sample monitored by ^1H NMR spectroscopy. (b) A solution of $\{\eta^3\text{-}HB(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$ (ca. 50 mg, 0.1 mmol) in $CHCl_3$ was saturated with CO_2 (1 atm) and monitored using IR spectroscopy. (c) CO_2 was bubbled through a solution of $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$ (40 mg, 0.08 mmol) in Et_2O (10 mL). A white solid was precipitated and the purging was stopped when the volume of the solvent was reduced to ca. 4 mL. The white solid was filtered and dried in vacuo. ^1H NMR showed that the solid was composed of a mixture of $[\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$ and $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$ suitable for X-ray diffraction were obtained by slow evaporation of a benzene solution of $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$ under a CO_2 atmosphere. ^1H NMR (C_6D_6) δ 1.73 (18 H, s, $\eta^3\text{-HB}\{C_3N_2H(CH_3)CMe_3\}_3$), 2.12 (9 H, s, $\eta^3\text{-}HB\{C_3N_2H(CH_3)CMe_3\}_3$), 5.73 (3 H, s, $\eta^3\text{-HB}\{C_3N_2H(CH_3)CMe_3\}_3$). ^{13}C NMR (C_6D_6) δ 12.7 ($\eta^3\text{-HB}\{C_2N_2CH(CH_3)CMe_3\}_3$), 31.3 ($\eta^3\text{-}HB\{C_2N_2CH(CH_3)CMe_3\}_3$), 32.2 ($\eta^3\text{-HB}\{C_2N_2CH(CH_3)CMe_3\}_3$), 103.2 ($\eta^3\text{-HB}\{C_2N_2CH(CH_3)CMe_3\}_3$), 143.9 and 164.4 ($\eta^3\text{-HB}\{C_2N_2CH\text{-}(CH_3)CMe_3\}_3$), 164.0 ($\mu\text{-}CO_3$). IR data 1588 and 1311 ($\nu(CO)$), 2540 ($\nu(BH)$).

Reaction of $[\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$ with H_2O. A sample of $[\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}Zn]_2(\mu\text{-}\eta^1,\eta^1\text{-}CO_3)$ (ca. 10 mg), contaminated with $\{\eta^3\text{-HB}(3\text{-Bu}^t\text{-5-Mepz})_3\}ZnOH$ due to its sensitivity

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Tris(pyrazolyl)hydroboratozinc Hydroxide Complexes

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toward traces of water, in C_6D_6 (ca. 1 mL) was treated with H_2O (0.5 μL, 0.03 mmol). The reaction was monitored by ^1H NMR spectroscopy, which demonstrated the immediate and complete conversion to $\{\eta^3\text{-HB}(3\text{-}Bu^t\text{-}5\text{-Mepz})_3\}ZnOH$.

$\{\eta^3\text{-HB}(3\text{-}Bu^t\text{-}5\text{-Mepz})_3\}ZnOH$ Exchange with D_2O and $H_2^{17}O$. A solution of $\{\eta^3\text{-HB}(3\text{-}Bu^t\text{-}5\text{-Mepz})_3\}ZnOH$ (ca. 10 mg) in benzene (ca. 0.7 mL) was treated with D_2O (1.5 μL, 0.08 mmol) and left at room temperature for 1 h. The volatile components were removed under reduced pressure, and the product was examined by ^1H NMR, ^2H NMR, and IR spectroscopy. A similar experiment was performed with $H_2^{17}O$ (ca. 45% enriched), in which case the product was examined by ^{17}O NMR spectroscopy. ^1H NMR (C_6D_6) δ 1.55 (18 H, s, $\eta^3\text{-HB}\{C_3N_2H\text{-}(CH_3)CMe_3\}_3$), 2.10 (9 H, s, $\eta^3\text{-HB}\{C_3N_2H(CH_3)CMe_3\}_3$), 5.64 (3 H, $\eta^3\text{-HB}\{C_3N_2H(CH_3)CMe_3\}_3$), (-0.06 resonance for ZnOH absent). ^2H NMR (C_6H_6) δ -0.07 (ZnOD). IR (KBr disk, cm^{-1}) 2714 ($\nu(OD)$) ($\nu(OH)/\nu(OD) = 1.36$). ^{17}O NMR (C_6D_6) δ -8 ppm.

Oxygen Exchange between CO_2 and H_2O Catalyzed by $\{\eta^3\text{-HB}(3\text{-}Bu^t\text{-}5\text{-Mepz})_3\}ZnOH$. A saturated solution of $H_2^{17}O$ (ca. 45% enriched) in C_6D_6 (0.8 mL)[48] in an NMR tube was treated with $\{\eta^3\text{-HB}(3\text{-}Bu^t\text{-}5\text{-Mepz})_3\}ZnOH$ (ca. 2 mg) and then exposed to excess CO_2 (1 atm). The ^{17}O NMR spectrum was obtained within 10 min of mixing and revealed that complete transfer of the ^{17}O label from water to carbon dioxide had occurred. A similar experiment was performed with the exception that $\{\eta^3\text{-HB}(3\text{-}Bu^t\text{-}5\text{-Mepz})_3\}ZnOH$ was not added to the NMR tube. In this case complete transfer of the ^{17}O label took several days to go to completion.

Preparation of $\{\eta^3\text{-HB}(3,5\text{-}Pr^i_2pz)_3\}Zn(NO_3)$. A solution of $\{HB(3,5\text{-}Pr^i_2pz)_3\}K$ (2.0 g, 4.0 mmol) in THF (40 mL) was added dropwise to a suspension of $Zn(NO_3)_2\cdot6H_2O$ (1.4 g, 4.7 mmol) in THF (25 mL), resulting in the immediate formation of a white precipitate. The mixture was stirred for 10 min at room temperature and filtered. The filtrate was concentrated to ca. 10 mL and placed at 0 °C, giving a crop of colorless crystals. The crystals of $\{\eta^3\text{-HB}(3,5\text{-}Pr^i_2pz)_3\}Zn(NO_3)$ were isolated by filtration and dried in vacuo (1.72 g, 73%). Anal. Calcd for $\{\eta^3\text{-HB}(3,5\text{-}Pr^i_2pz)_3\}Zn(NO_3)$: C, 54.7; H, 7.8; N, 16.5. Found: C, 53.9; H, 7.4; N, 16.2. ^1H NMR (C_6D_6) δ 1.09 (18 H, d, $^3J_{H-H} = 6.9$, $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 1.14 (18 H, d, $^3J_{H-H} = 6.9$, $\eta^3\text{-HB-}\{C_3N_2H(CHMe_2)_2\}_3$), 3.36 (3 H, spt, $^3J_{H-H} = 6.9$, $\eta^3\text{-HB-}\{C_3N_2H(CHMe_2)_2\}_3$), 3.46 (3 H, spt, $^3J_{H-H} = 6.9$, $\eta^3\text{-HB-}\{C_3N_2H(CHMe_2)_2\}_3$), 5.78 (3 H, s, $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$). ^{13}C NMR (C_6D_6) δ 23.1 (6 C, q, $^1J_{C-H} = 127$; dq, $^2J_{C-H}$, $^3J_{C-H} = 5$; $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 23.3 (6 C, q, $^1J_{C-H} = 127$; dq, $^2J_{C-H}$, $^3J_{C-H} = 5$; $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 26.5 (3 C, d, $^1J_{C-H} = 128$; spt (partial resonance), $^2J_{C-H} = 4$; $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 27.8 (3 C, d, $^2J_{C-H} = 129$; spt (partial resonance), $^2J_{C-H} = 4$; $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 98.3 (3 C, d, $^1J_{C-H} = 173$, $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 156.8 (3 C, s, $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 161.0 (3 C, s, $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$). IR data 2580 ($\nu(BH)$).

Preparation of $\{\eta^3\text{-HB}(3,5\text{-}Pr^i_2pz)_3\}ZnOH$. A solution of $\{\eta^3\text{-HB}(3,5\text{-}Pr^i_2pz)_3\}Zn(NO_3)$ (0.53 g, 0.9 mmol) in Et_2O (40 mL) was treated with an aqueous solution of NaOH (0.9 mL, 1.0 M). The mixture was stirred for 5 min at room temperature, and the solvents were removed under reduced pressure. The residue was extracted into pentane and filtered, and the pentane was removed under reduced pressure. The product was crystallized from Et_2O at 0 °C, giving a crop of colorless crystals which were isolated by filtration and dried in vacuo (0.1 g, 20%). Anal. Calcd for $\{\eta^3\text{-HB}(3,5\text{-}Pr^i_2pz)_3\}ZnOH$: C, 59.2; H, 8.7; N, 15.3. Found: C, 58.2; H, 8.5; N, 15.0. ^1H NMR (C_6D_6) δ 1.13 (18 H, d, $^3J_{H-H} = 6.9$, $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 1.27 (18 H, d, $^3J_{H-H} = 6.9$, $\eta^3\text{-HB-}\{C_3N_2H(CHMe_2)_2\}_3$), 3.49 (3 H, spt, $^3J_{H-H} = 6.9$, $\eta^3\text{-HB-}\{C_3N_2H(CHMe_2)_2\}_3$), 3.51 (3 H, spt, $^3J_{H-H} = 6.9$, $\eta^3\text{-HB-}$

$\{C_3N_2H(CHMe_2)_2\}_3$), 5.81 (3 H, s, $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), -0.29 (1 H, s, ZnO*H*). ^{13}C NMR (C_6D_6) δ 23.3 (6 C, q, $^1J_{C-H} = 126$; dq, $^2J_{C-H}$, $^3J_{C-H} = 5$; $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 23.5 (6 C, q, $^1J_{C-H} = 127$; dq, $^2J_{C-H}$, $^3J_{C-H} = 5$; $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 26.5 (3 C, d, $^1J_{C-H} = 128$; spt (partial resonance), $^2J_{C-H} = 4$; $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 27.9 (3 C, d, $^1J_{C-H} = 128$, $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 97.9 (3 C, d, $^1J_{C-H} = 172$, $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 156.0 (3 C, s, $\eta^3\text{-HB-}\{C_3N_2H(CHMe_2)_2\}_3$), 160.3 (3 C, s, $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$). IR data 3668 ($\nu(OH)$), 2547 ($\nu(BH)$). MS m/e 546 (M$^+$).

$\{\eta^3\text{-HB}(3,5\text{-}Pr^i_2pz)_3\}ZnOH$ Exchange with D_2O and $H_2^{17}O$. Exchange reactions were performed in an analogous fashion to those for $\{\eta^3\text{-HB}(3\text{-}Bu^t\text{-}5\text{-Mepz})_3\}ZnOH$ described above. ^2H NMR (C_6H_6) δ -0.24 (ZnOD). ^{17}O NMR (C_6D_6) δ -36 ppm.

Preparation of $[\{\eta^3\text{-HB}(3,5\text{-}Pr^i_2pz)_3\}Zn]_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$. A solution of $\{\eta^3\text{-HB}(3,5\text{-}Pr^i_2pz)_3\}ZnOH$ (0.15 g, 0.27 mmol) in benzene (20 mL) was treated with CO_2 (1 atm). The solvent was removed immediately, giving $[\{\eta^3\text{-HB}(3,5\text{-}Pr^i_2pz)_3\}Zn]_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$ as a white powder in almost quantitative yield. Anal. Calcd for $[\{\eta^3\text{-HB}(3,5\text{-}Pr^i_2pz)_3\}\text{-}Zn]_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$: C, 58.9; H, 8.3; N, 15.0. Found: C, 59.8; H, 8.0; N, 13.7. ^1H NMR (C_6D_6) δ 1.13 (18 H, d, $^3J_{H-H} = 6.9$, $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 1.27 (18 H, d, $^3J_{H-H} = 6.9$, $\eta^3\text{-HB-}\{C_3N_2H(CHMe_2)_2\}_3$), 3.53 (3 H, spt, $^3J_{H-H} = 6.9$, $\eta^3\text{-HB-}\{C_3N_2H(CHMe_2)_2\}_3$), 3.82 (3 H, spt, $^3J_{H-H} = 6.9$, $\eta^3\text{-HB-}\{C_3N_2H(CHMe_2)_2\}_3$), 5.88 (3 H, s, $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$). ^{13}C NMR (C_6D_6) δ 23.5 (12 C, q, $^1J_{C-H} = 127$; dq, $^2J_{C-H}$, $^3J_{C-H} = 5$; $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 23.8 (12 C, q, $^1J_{C-H} = 126$; dq, $^2J_{C-H}$, $^3J_{C-H} = 5$; $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 26.5 (6 C, d, $^1J_{C-H} = 128$; spt (partial resonance), $^2J_{C-H} = 4$; $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 27.5 (6 C, d, $^1J_{C-H} = 130$; spt (partial resonance), $^2J_{C-H} = 4$; $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 97.6 (6 C, d, $^1J_{C-H} = 172$, $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 155.9 (6 C, s, $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 161.0 (6 C, s, $\eta^3\text{-HB}\{C_3N_2H(CHMe_2)_2\}_3$), 171.5 (1 C, s, $\mu\text{-}CO_3$). IR data 1537, broad ($\nu(CO)$), 2545 ($\nu(BH)$).

Reaction of $[\{\eta^3\text{-HB}(3,5\text{-}Pr^i_2pz)_3\}Zn]_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$ toward H_2O. A solution of $[\{\eta^3\text{-HB}(3,5\text{-}Pr^i_2pz)_3\}Zn]_2(\mu\text{-}\eta^1,\eta^2\text{-}CO_3)$ (ca. 10 mg) in C_6D_6 (0.7 mL) was treated with excess H_2O. The sample was monitored by ^1H NMR spectroscopy, which confirmed that no reaction had occurred over a period of 2 weeks at room temperature.

Note Added in Proof. After this manuscript was submitted, the X-ray structure of a bicarbonate complex of a mutant of carbonic anhydrase (Thr-200 $\rightarrow$ His human CA II) was reported.[49] The structure indicates that the bicarbonate ligand is coordinated to the zinc in an asymmetric fashion with inequivalent Zn–O bond lengths (2.2 and 2.5 Å). Furthermore, the structure of a bicarbonate complex of cobalt carbonic anhydrase II has also been recently reported.[50] However, a molecule of water is also coordinated at a distance of 2.33 Å to the cobalt center, such that the coordination geometry is described as distorted octahedral. The Co–O bond lengths to the bicarbonate ligand in this derivative are similar (2.39 and 2.44 Å).

(48) The solubility of water in benzene at 298 K is 70 mg per 100 g of solution (ca. 34 mM). *Solubility Data Series*; Kertes, A. S., Editor-in-Chief; Pergamon Press: New York, 1989; Vol. 37, Part I, p 72.

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