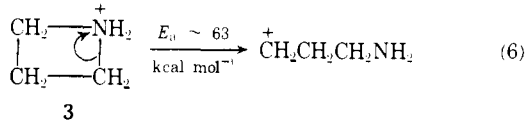
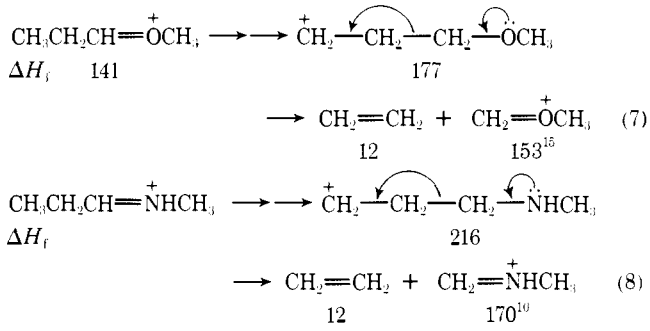
of **3** (eq 6). Thus, dissociation from **3** would be stepwise rather than concerted.



3

If the above descriptions of the reaction surfaces are appropriate, then it should be possible to predict (in a qualitative sense) the kinetic energy releases that will occur in similar reactions. Therefore we have examined reactions 7 and 8 in which a heteroatom X-H group of the earlier examined reactants is replaced by a X-$CH_3$ group.



In contrast to eq 1, the intermediate primary carbonium ion in eq 7 should now correspond to a transition state, since the methoxymethyl cation ($\Delta H_f$ = 153 kcal $mol^{-1}$) is considerably more stable than the hydroxymethyl cation ($\Delta H_f$ = 170 kcal $mol^{-1}$). Thus, the addition of $C_2H_4$ to $CH_2{=}OCH_3^+$ involves an activation energy of ~12 kcal $mol^{-1}$, and on the basis that approximately one-third of this should pass into kinetic energy (vide supra) in the reverse step (dissociation), the kinetic energy release should be ca. 4 kcal $mol^{-1}$. The kinetic energy release profile for reaction 7 is indeed much broader than that for reaction 1 and corresponds to an average kinetic energy release (computed from the width at half-height[11]) of 3 kcal $mol^{-1}$. Analogous arguments for eq 8 show that the reverse activation energy should be ca. 34 kcal $mol^{-1}$, and ca. 11 kcal $mol^{-1}$ should be released as kinetic energy. The energy release profile is similar to that given in Figure 2, but the computed kinetic energy release (11 kcal $mol^{-1}$) is indeed larger than for eq 2, and in good agreement with the predicted value.

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## Carbon-13 Nuclear Magnetic Relaxation Study on Cobalt Carbonic Anhydrase: Evidence on the Location of Enzyme Bound $CO_2$ and $HCO_3^-$

*Sir:*

Zinc metalloenzymes constitute a class of enzymes that can generally be substituted with Co(II) at the active site. This introduces a paramagnetic probe that allows NMR measurements that determine distances from individual atoms of bound ligands to the metal. In earlier studies, the relaxation times ($T_1$ and $T_2$) of $^{13}C$-enriched $HCO_3^-$ bound to bovine cobalt or zinc carbonic anhydrase (EC 4.2.1.1) were measured.[1] This communication reports work on the low activity human B form of the enzyme that gives information on the location of both bound substrate and product on the actively functioning enzyme.

It is well known that paramagnetic ions can decrease the longitudinal relaxation time, $T_1$, of nearby $^{13}C$ nuclei. With carbonic anhydrase, the paramagnetic contribution to the $^{13}C$ relaxation rate of the substrate gives the distance between the paramagnetic metal and the carbon nucleus of the bound substrate.[1] Much of the methodology required for studies of this kind has been developed over a decade ago in studies of inorganic complexes, and then extended to protein systems.[2-4]

The paramagnetic contribution to the longitudinal relaxation rate was calculated from

$$\frac{1}{T_{1_p}} = \frac{1}{T_1} - \frac{1}{T_1{}^0} \tag{1}$$

where $1/T_1$ is the measured [$^{13}C$]substrate relaxation rate in cobalt carbonic anhydrase solutions and $1/T_1{}^0$ is the corresponding rate in zinc carbonic anhydrase solutions.[5] Longitudinal relaxation was measured with a Bruker HFX-10 or a JEOL FX-60 using a conventional inversion recovery pulse sequence, with an equilibrium mixture of substrate and product (which is the substrate in the reverse direction), 90% carbon-13 enriched. The paramagnetic contribution was normalized by the factor $f$, $[\text{enzyme}]_0/[\text{substrate}]_0$, to yield $1/fT_{1_p}$.

At binding saturation, with fast exchange

$$\frac{1}{fT_{1_p}} = \frac{1}{T_{1_m}} \tag{2}$$

The distance $r$ was calculated from the dipolar term of the Solomon-Bloembergen equation[4]

$$r = C[T_{1_m} f(\tau_c)]^{1/6} \tag{3}$$

where $C$ is a product of physical constants, equal to 460 Å $s^{-1/3}$ in the present work,[6] and $f(\tau_c)$ is the well-known function of the correlation time for dipolar interaction, $\tau_c$.[4] We have used a $\tau_c$ value of $1.2 \times 10^{-11}$ s.[1,7]

Carbon-13 longitudinal relaxation times of $CO_2$ and $HCO_3^-$ in solutions of cobalt(II) carbonic anhydrase at several pH values are given in Table I. In the experiments, the equilibrium between substrate and product varies from about 97% $HCO_3^-$ at pH 7.8 to about 87% $CO_2$ at pH 5.5. In all cases, the $^{13}C$ longitudinal relaxation times are substantially decreased by

**Table I.** Paramagnetic Enhancement of Longitudinal Relaxation of $[^{13}C]CO_2$ and $[^{13}C]HCO_3^-$ in the Presence of Cobalt(II) Carbonic Anhydrase B[a]

| pH | Enzyme | Substrate | $T_1$ (s) | $fT_{1_p}$ (ms) |
|---|---|---|---|---|
| 7.8 | 0.79 mM CoCA | 110 mM $HCO_3^-$ | 0.68 | 7.7 |
| | 0.45 mM CoCA | 55 mM $HCO_3^-$ | 0.48 | 5.1 |
| | 0.50 mM CoCA | 37 mM $HCO_3^-$ | 0.33 | 5.4 |
| | 0.26 mM CoCA | 27 mM $HCO_3^-$ | 0.48 | 6.0 |
| 6.3 | 0.40 mM CoCA | 20 mM $HCO_3^-$ | 0.47 | 5.3 |
| | | 20 mM $CO_2$ | 0.44 | 4.9 |
| 5.5 | 0.20 mM CoCA | 3 mM $HCO_3^-$ | —[b] | —[b] |
| | | 20 mM $CO_2$ | 0.40 | 4.1 |
| 7.8 | 0.90 mM ZnCA | 95 mM $HCO_3^-$ | 2.2 | — |
| 6.5 | None | 25 mM $HCO_3^-$ | 2.1 | — |
| 5.8 | None | 20 mM $CO_2$ | 35 | — |

[a] Abbreviations: ZnCA, human zinc carbonic anhydrase B; CoCA, human cobalt(II) carbonic anhydrase B. $T_1$ values at pH 6.3 are the average from seven separate samples; $T_1$ value at pH 5.5 is the average from two separate samples. [b] Resonance of $HCO_3^-$ was not detectable at this concentration.

the presence of relatively small amounts of cobalt carbonic anhydrase. Such an effect is not seen in the presence of zinc carbonic anhydrase.

At pH 7.8 a single resonance due to $^{13}C$-enriched $HCO_3^-$ is observed. Variation of the concentration of $HCO_3^-$ does not significantly affect $fT_{1_p}$ indicating that the enzyme binding site is saturated with this substrate. The $^{13}C$-Co(II) distance calculated from $fT_{1_p}$ is 3.6 Å (±0.2 Å standard deviation).

At pH 6.3, $CO_2$ and $HCO_3^-$ are present in nearly equal amounts, and the enzyme catalyzed exchange is slow enough that separate $^{13}C$ resonances for $CO_2$ ($\delta$ 125 ppm) and $HCO_3^-$ ($\delta$ 162 ppm) are observed. But, the $T_1$ values of $CO_2$ and $HCO_3^-$ are the same within experimental uncertainty. This is because $T_1$ is long compared to the $CO_2$–$HCO_3^-$ exchange time so that both resonances give the same average $T_1$.[8] Individual distances, therefore, cannot be calculated from the two relaxation times. The weighted average $^{13}C$-Co(II) distance for enzyme-bound $CO_2$ and $HCO_3^-$ is 3.6 Å (±0.2 Å standard deviation). At pH 5.5 the concentration of $HCO_3^-$ is very low, and only a single resonance due to $CO_2$ is observed. The average $^{13}C$-Co(II) distance calculated from $fT_{1_p}$ for this resonance is 3.4 Å.

The actual amounts of enzyme-bound $CO_2$ and $HCO_3^-$ are unknown, although the enzyme is likely saturated with these substrates. Thus, the essential invariance of the calculated distances with pH indicates either that the relative amounts of bound $CO_2$ and $HCO_3^-$ are pH independent, so that the same weighted average distance is obtained at all pH values, or that the $^{13}C$-Co(II) distances of bound $CO_2$ and $HCO_3^-$ are comparable, so that the average distance does not vary with any variation in the relative amounts of enzyme-bound $CO_2$ and $HCO_3^-$. In any case, these distances rule out all proposals that suggest one or more water molecules bridge a space between bound substrate and metal hydroxide.[9] They are in accord with mechanisms that suggest a metal-liganded hydroxyl adds directly to the carbon of enzyme-bound $CO_2$,[10] so that dehydration proceeds via $HCO_3^-$ coordinated directly to the metal.[1] In addition, the distances indicate that $CO_2$ could be bound close to the metal, suggesting that the metal could effectively polarize the carbonyl, thereby favoring formation of metal-bound bicarbonate.[11,12]

A metal-bound $CO_2$ mechanism fits the recent x-ray structural studies of Kannan and co-workers,[13] who determine that the competitive inhibitor imidazole (Khalifah[12]) is distantly coordinated as a fifth ligand, without replacing the metal-bound $H_2O$ or $OH^-$. On the other hand, Riepe and Wang[14] conclude from infrared studies that $CO_2$ is not bound to the zinc of bovine carbonic anhydrase, but this conclusion is disputed by Khalifah.[12] To help resolve these and other questions, we plan additional NMR studies on the cobalt(II) substituted enzyme, beginning with a look at the effects of various inhibitors on $[^{13}C]CO_2$ and $[^{13}C]HCO_3^-$ nuclear relaxation.

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other bicarbonate to the human B enzyme, and it was not taken into account. If it is present, this will not materially alter any of the results and conclusions given in this communication.
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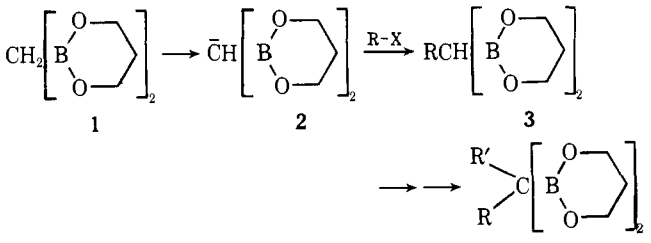
## Carbanions from Deprotonation of *gem*-Diboronic Esters

*Sir:*

Deprotonation of bis(trimethylenedioxyboryl)methane (**1**) with lithium 2,2,6,6-tetramethylpiperidide yields the diborylcarbanion (**2**), which with alkyl halides gives high yields of *gem*-diboronic esters (**3**), which can in turn be deprotonated and alkylated.

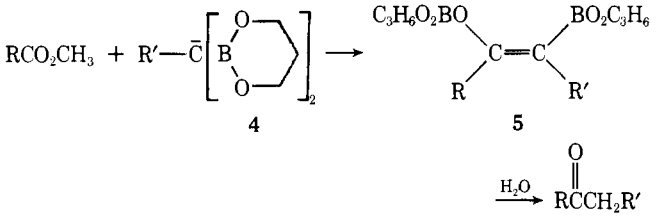


The carbanion from **3** (R = *n*-pentyl) converts esters, $R'CO_2CH_3$, to ketones, $R'COCH_2R$.

A close analogue of **2** has been shown previously to homologate aldehydes efficiently via alkeneboronic esters,[1] and several related boron substituted carbanions have been shown to have potential for synthetic applications,[2] but their use has been discouraged by the need to prepare them by deboronation of exotic tris- and tetrakis(dialkoxyboryl)methanes.[3] *gem*-Diboronic esters are accessible from the direct condensation of dichloromethane, lithium, and trimethyl borate[3] and also from dilithiomethane,[4] bis(iodomercuri)methane,[5] or, for higher members of the series, hydroboration.[6]

Bases normally attack boron in preference to carbon-bound protons,[1-3] but the success of Rathke and Kow in deprotonating *B*-methyl-9-borabicyclononane and alkenyldisiamylboranes with lithium 2,2,6,6-tetramethylpiperidide[7] prompted us to try this highly hindered base. Boronic esters offer more ways for the reaction to be diverted. The homologation of heptanal to octanal[1] was chosen as the test reaction. Low yields were obtained using the ethylene glycol ester, $CH_2(BO_2C_2H_4)_2$, and another ethylene glycol ester in a parallel study[8] gave no carbanion. The 1,3-propanediol ester **1** was then tested, and with inclusion of tetramethylethylenediamine in the reaction mixture the yield of octanal reached 62%. This is not as good as by the former procedure,[1] perhaps because the reaction medium is more basic. The problem is not in the conversion of **1** to the anion **2**, since reaction of **2** with 1-iodoheptane followed by oxidation[1] yielded 82% of octanal. The results of a series of alkylations of **2** are summarized in Table I.

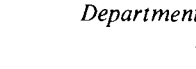
The alkylation product from **2** and 1-bromopentane (**3**, R = $n$-$C_5H_{11}$) was chosen to test anion formation with a higher homologue. Results are summarized in Table II. The resulting anion **4** was alkylated in the expected manner, and the efficient homologation of benzaldehyde to 1-phenyl-2-heptanone is analogous to aldehyde homologation. The remarkable conversions of methyl benzoate to 1-phenyl-1-heptanone and methyl butyrate to 4-decanone presumably involve enol borate intermediates (**5**), which would be immune to further attack by the carbanion (**4**) and would hydrolyze very rapidly.



R = $C_6H_5$, $C_3H_7$; R′ = $C_5H_{11}$

The procedure for preparing **1** from bis(dimethoxyboryl)methane[3] and 1,3-propanediol was similar to previously reported transesterifications,[1] recrystallized from ether/pentane, 78%, mp 42-43 °C, satisfactory C, H, and B analyses. To form the carbanion **2**, 3.5 mmol of butyllithium converted 0.49 g of 2,2,6,6-tetramethylpiperidine to the lithio derivative in the presence of 0.41 g of tetramethylethylenediamine in 10 mL of rigorously dried THF, and this was stirred at −75 °C during the addition of 0.64 g (3.5 mmol) of **1** in 15 mL of THF. After 1 h at −75 °C the mixture was allowed to warm to 0 °C, resulting in precipitation of **2**, and stirred 1 h. The mixture was again cooled to −75 °C, 3.33 mmol of the alkyl halide was added, and reaction was continued 3 h at 25 °C. The solution was concentrated under vacuum. For boronic ester isolation, the residue was chromatographed on a short silica gel column with dichloromethane/pentane and the product **3** was further purified by short path distillation at ~100 °C (0.1 mm). For gas chromatographic analysis, the residue was dissolved in 20 mL of dichloromethane and oxidized with sodium perborate in water, using authentic comparison samples of carbonyl compounds and internal standards as previously described.[1a]

Table I. Reactions of Lithium Bis(trimethylenedioxyboryl)methide (**2**)

| Reactant | Product | % yield | Method |
|---|---|---|---|
| $CH_3(CH_2)_3I$ | $CH_3(CH_2)_3CHO$ | 86 | GLC |
| $CH_3(CH_2)_3Br$ | | 79 | GLC |
| $CH_3(CH_2)_4Br$ | $CH_3(CH_2)_4CHO$ | 80 | GLC |
| | | 62 | Dist |
| | $CH_3(CH_2)_4CH(BO_2C_3H_6)_2$ | 71 | Dist[a] |
| $CH_3(CH_2)_6I$ | $CH_3(CH_2)_6CHO$ | 82 | GLC |
| $CH_3(CH_2)_6OTs$ | | 76 | GLC |
| $C_6H_5CH_2Br$ | $C_6H_5CH_2CHO$ | 71 | GLC |
| $Cl(CH_2)_4I$ | $Cl(CH_2)_4CHO$ | 83 | GLC |
| $Br(CH_2)_4CN$ | $(C_3H_6O_2B)_2CH(CH_2)_4CN$ | 49 | Dist[a] |
|  |  | 57 | Dist[a] |

[a] Satisfactory analyses for C, H, B (and N).

Table II. Reactions of 1-Lithio-1,1-bis(trimethylenedioxyboryl)hexane (**4**)

| Reactant | Product | % yield |
|---|---|---|
| $CH_3I$ | $CH_3(CH_2)_4COCH_3$ | 78 |
| $C_6H_5CH_2CH_2I$ | $C_6H_5CH_2CH_2$, $CH_3(CH_2)_4$ $C(BO_2C_3H_6)_2$ | 70[a] |
| $C_6H_5CHO$ | $C_6H_5CH_2CO(CH_2)_4CH_3$ | 84 |
| $C_6H_5CO_2CH_3$ | $C_6H_5CO(CH_2)_5CH_3$ | 99[b] |
| $CH_3(CH_2)_2CO_2CH_3$ | $CH_3(CH_2)_2CO(CH_2)_5CH_3$ | 66 |

[a] By short-path distillation. Hydrolyzed to boronic acid, mp 66-147 °C dec, satisfactory C, H, and B anal. [b] Isolated 71% 2,4-DNP.