erizations of both **1b** and **1c** are faster reactions than the conversion of **1a** to **2a**. These results demonstrate that substitution of a methyl group at C-4 in **1a** by a phenyl group (**1b**) and substitution of a methyl group at C-2 in **1b** by a proton (**1c**) both increase the rate of isomerization of the 1-azacyclobutene 1-oxides. These observations are consistent with the known effects of substitution at the various positions of cyclobutenes on the rate of isomerization.[15] Taking into account these known substituent effects on the rate of isomerization of cyclobutenes and assuming that the *N,N*-diethylcarbamoyl group at C-4 will decrease the activation energy by 1-2 kcal mol^{-1},[16] we have calculated a value of 28-29 kcal mol^{-1} for the activation energy of the isomerization of the unknown cyclobutene that corresponds with the 2,3-dihydroazete 1-oxide (**1a**). Therefore we conclude that substitution of an sp^2-hybridized carbon atom of a cyclobutene ring by an N-O group has only a small effect on the activation energy of the isomerization reaction. More important, this substitution does not change the stereochemical pathway of the reaction, which means that the Woodward-Hoffmann rules can be extended to electrocyclic reactions of 1-azacyclobutene 1-oxides.

Acknowledgment. This investigation was supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for Advancement of Pure Research (ZWO).

Supplementary Material Available: Tables of atomic positional and thermal parameters, interatomic distances and angles, and a list of observed and calculated structure factors (9 pages). Ordering information is given on any current masthead page.

---

(15) Willcott, M. R.; Cargill, R. L.; Sears A. B. *Prog. Phys. Org. Chem.* **1972**, *9*, 25-98.

(16) The decrease in activation energy caused by $COOCH_3$ substitution at C-4 is 2-3 kcal mol^{-1}. See: Dalrymple, D. L.; Russo, W. B. *J. Org. Chem.* **1975**, *40*, 492-495.

Marcel L. M. Pennings, David N. Reinhoudt*
Laboratory of Organic Chemistry
Twente University of Technology, Enschede
The Netherlands

Sybolt Harkema, Gerrit J. van Hummel
Laboratory of Chemical Physics
Twente University of Technology, Enschede
The Netherlands

Received July 14, 1980

Catalytically Active Models for the Active Site in Carbonic Anhydrase

Sir:

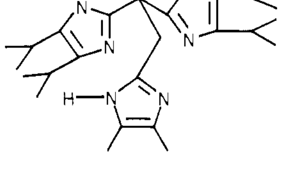
Carbonic anhydrase (CA) is a ubiquitous enzyme which catalyzes the interconversion of CO_2 and HCO_3^-. Its active site consists of a Zn^{2+} ion bound pseudotetrahedrally to three histidine imidazoles and either a water molecule or OH^- ion.[2] The activity of CA is governed by the ionization of at least one group with a pK_a around 7.[1] Recently some model systems exhibiting CO_2 hydration catalysis have been reported,[3] but as yet no catalytically active model which attempts to approximate the known Zn^{2+} binding site for CA has appeared. Herein we report preliminary results concerning two models for the active site of CA which show catalytic activity toward CO_2 hydration.

Table I. Rates of CO_2 Hydration Catalyzed by Complexes **1**-Zn^{2+} and **2**-Zn^{2+} [a]

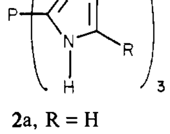
catalyst	pH	k_{cat},[b] M^{-1} s^{-1}	k_{cat}/K_M, M^{-1} s^{-1}
$(imidazole)_2Zn^{2+}$ [c]	7.5	2.0	
1-Zn^{2+}	6.5	$7.6 \pm 0.5 \times 10^2$	
1-Zn^{2+}	7.5	$2.4 \pm 0.2 \times 10^2$	
2a-Zn^{2+}	7.5	*d*	
2b-Zn^{2+}	7.5	*d*	
2c-Zn^{2+}	6.5	*d*	
2c-Zn^{2+}	7.0	$2.0 \pm 0.4 \times 10^2$	
human CAB[e]			1×10^7
human CAC[e]			8×10^7

[a] Determined under pseudo-first-order conditions (with respect to CO_2) at 25 °C in 76% ethanol-H_2O according to procedures outlined in ref 6. Experiments with **2a**-Zn^{2+} and **2b**-Zn^{2+} for reasons of solubility were performed in H_2O. pH values are those directly read from electrode immersed in solution. [b] $k_{cat} = (k_{obsd} - k_{uncat})/[cat]$, $[cat] = 5 \times 10^{-4}$ M. [c] Reference 3a. [d] k_{obsd} did not differ from that observed in the absence of catalyst. [e] Khalifah, R. G. *J. Biol. Chem.* 1971, *246*, 251, ref 6.

Ligand **1**[4] in the presence of 1 equiv of Zn^{2+} shows reversible



1



2a, R = H
b, R = CH_3
c, R = $CH(CH_3)_2$

consumption of 1 equiv of OH^- with an apparent pK_a of 6.5 in 76% ethanolic H_2O.[5] CO_2 hydration[6] in the presence of **1**-Zn^{2+} shows catalysis at pH 6.5 (Table I) which diminishes at pH >7 to a final k_{cat} of 240 ± 20 M^{-1} s^{-1} at pH 7.5. No catalysis by **1** is observed in the absence of Zn^{2+}. Although we do not have good evidence for the nature of the catalytically active species, UV spectra of **1**-Co^{2+} show the presence of what might be interpreted as tetrahedrally coordinated Co^{2+}.[7] The fact that the apparent catalysis is reduced at higher pH's indicates to us that the reversible titration is best explained by complex hydrolysis[4] leading to a less active species.

In order to circumvent this hydrolysis which we feel is probably due to relatively poor binding of Zn^{2+} by **1**,[4] we turned to phosphines **2a-c**[8] which appear from models to be reasonable tridentate ligands for the CA metal binding site. ^1H NMR spectra of **2c** in methanol-d_4-D_2O as a function of increasing $[Zn^{2+}]$ show the appearance of a well-defined 1:1 complex when $[\mathbf{2c}]/[Zn^{2+}] = 1$; no 2:1 complex is observed.[8b] UV spectra of **2a** and **2b** in the presence of $CoCl_2$ show little if any evidence for 4-coordinate ligation.[9] On the other hand, the isopropyl phopsphine **2c** in the presence of $CoCl_2$ shows reversible formation of a tetrahedral species at increasing pH with bands appearing at 588 (285), 622 (450), 646 (516), 662 (501) nm (ε).[9c] The **2c**-Co^{2+} spectra are highly anion dependent (Figure 1), reminiscent of the situation for the Co^{2+}-enzyme.[10] In the presence of ClO_4^- and NO_3^- the

---

(1) For recent reviews, see: Lindskog et al. *Enzymes, 3rd Ed.* **1971**, *5*, 587. (b) Pocker, Y.; Sarkanen, S. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1978**, *47*, 149. (c) Golding, B. T.; Leigh, G. J. *Inorg. Biochem.* (*London*), **1979**, *1*, 50. (d) Kannan, K. K.; Petef, M; Cid-Dresdner, H.; Lövgren, S. *FEBS Lett.* **1977**, *73*, 115.

(2) Notstrand, B.; Vara, I; Kannan, K. K. *Isozymes, Curr. Top. Biol. Med. Res.* **1975**, *1*, 575.

(3) (a) Tabushi, I.; Kuroda, Y.; Mochizuki, A. *J. Am. Chem. Soc.* **1980**, *102*, 1152. (b) Woolley, P. *Nature* (*London*) **1975**, *258*, 677. (c) Harrowfield, J. McB.; Norris, V.; Sargeson, A. M. *J. Am. Chem. Soc.* **1976**, *98*, 7282.

(4) Brown, R. S.; Huguet, J. *Can. J. Chem.* **1980**, *58*, 899.

(5) In a less polar medium the pK_a is reduced to 5.8 (60% aqueous dioxane) pH's were determined directly from electrode readings in these media.

(6) Gibbons, B. H.; Edsall, J. T. *J. Biol. Chem.* **1963**, *238*, 3502.

(7) This observation is tempered by the fact that the UV spectra of **1**-Co^{2+} are not completely reversible (reproducible) as a function of pH.

(8) (a) Curtis, N. J.; Brown, R. S. *J. Org. Chem.* **1980**, *45*, 4038-4040. (b) The ^1H NMR spectra of **2c** and **2c**-Zn^{2+} in CH_3OH-d_4-D_2O show resonances at δ 1.22 (36 H, d, *J* = 7 Hz), 3.00 (6 H, sept *J* = 7 Hz) and 1.22 (18 H, d, *J* = 7 Hz), 1.34 (18 H, d, *J* = 7 Hz), 3.17 (3 H, m), 3.53 (3 H, m), respectively.

(9) (a) Carlin, R. L. In "Transition Metal Chemistry"; Marcel Dekker: New York, 1965; Vol 1, p 1. (b) Ciampolini, M. *Struct. Bonding* (*Berlin*) **1969**, *6*, 52. (c) 10^{-1} M, $CoCl_2$, 10^{-3} M, **2c**, 4.1, ethanol-H_2O.

(10) (a) Lindskog, S. *J. Biol. Chem.* **1963**, *238*, 945. (b) Bertini, I.; Canti, G.; Luchinat, C.; Scozzafava, A. *J. Am. Chem. Soc.* **1978**, *100*, 4873. (c) Lindskog, S., *Struct. Bonding* (*Berlin*) **1970**, *8*, 153.

0002-7863/80/1502-7571$01.00/0

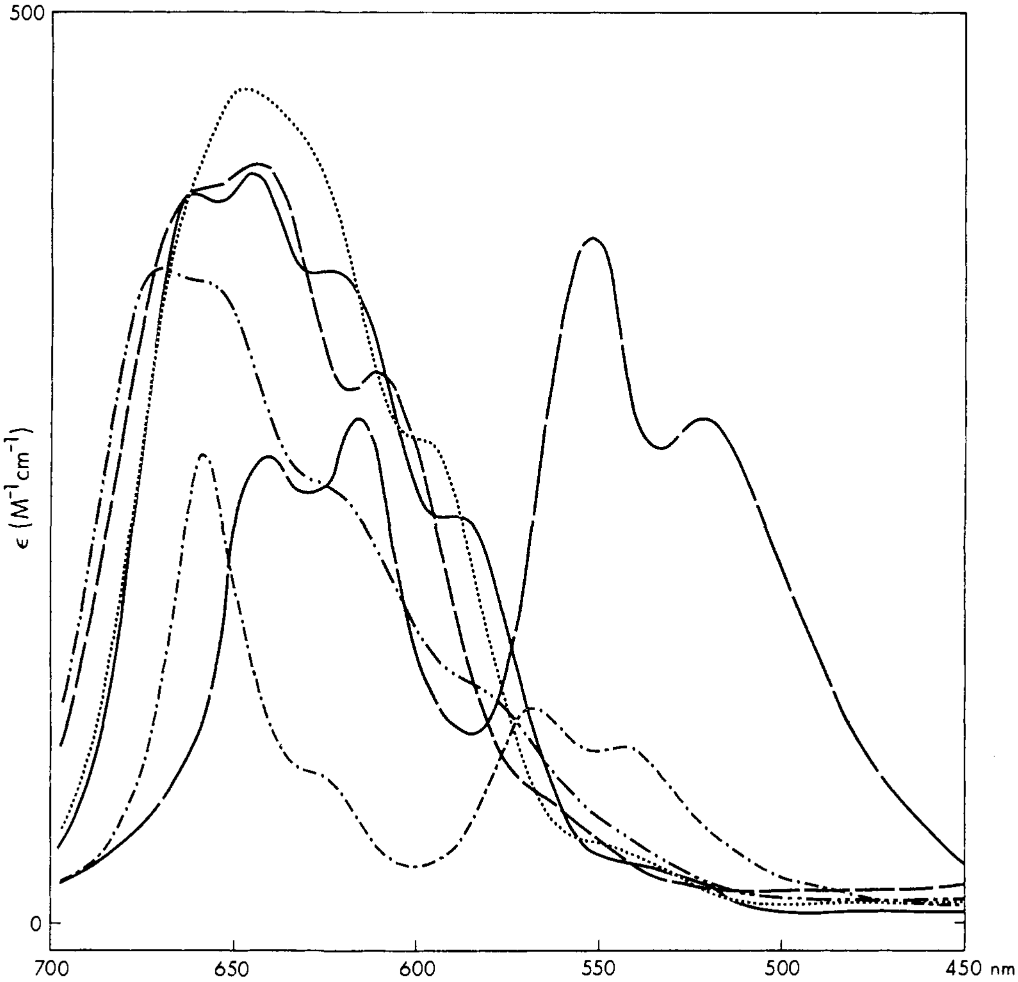


Figure 1. UV–visible spectra of 1:1 **2c**–Co(NO$_3$)$_2$ solutions saturated with different anions in 4:1 ethanol–H$_2$O; Cl$^-$ (—); Br$^-$ (- - -); I$^-$ (– – –); F$^-$ (— - — -); N$_3$$^-$ (— – —). The spectrum of Co^{2+} bovine carbonic anhydrase in unbuffered solution at pH 8.8 (— —) redrawn from: Bertini, I.; Luchinat, C.; Scozzafava, A. *Inorg. Chim. Acta* **1980**, *46*, 85.

2c–Co^{2+} spectra are not indicative of tetrahedral coordination but are more consistent with predominant 5- and/or 6-coordinate Co^{2+}.[9,10] Thus the metal binding site of **2c** is flexible enough to allow access of one or more additional ligands other than the three from the phosphine. This feature may be important if the transition state for CO$_2$ hydration by **2c**–Zn^{2+} involves 5-coordinate Zn^{2+}.[1d]

Catalytically, the **2a**–**c**–Zn^{2+} complexes are interesting. While **2a**,**b**–**Zn**$^{2+}$ show negligible catalysis toward CO$_2$ hydration, **2c**–Zn^{2+} appears to be active (Table I). No catalysis by **2a**, **2b**, or **2c** is observed in the absence of Zn^{2+}. The observation that catalysis by **2c**–Zn^{2+} is reduced at pH 6.5 relative to what is observed at 7.0 may indicate that some basic form of this complex is catalytically viable.[11]

Ligand **2c** shows other features in common with CA. Small ligands such as bipyridyl and terpyridine characteristically bind to Zn^{2+} very rapidly (10^6 M^{-1} s^{-1})[12] with slightly positive E_a's and slightly negative $\Delta S^\ddagger$ values (8.4 kcal/mol and −5 eu, respectively).[12] Apo-CA binds Zn^{2+} somewhat slower (2 × 10^4 M^{-1} s^{-1}, pH 7.5),[13] with a high E_a and large positive $\Delta S^\ddagger$ (20.8 kcal/mol and +30 eu).[13] Between pH 5 and 7, **2c** also binds Zn^{2+} slowly (3 × 10^4 M^{-1} s^{-1}), in agreement with its being a reasonable model for the CA metal site; however, its activation parameters (9.9 kcal/mol and −4 eu, respectively) are more indicative of those for a smaller ligand.

Although the catalysis afforded by **2c**–Zn^{2+} is encouraging, it is modest when compared to that exhibited by the enzyme. Further study of this and related ligands is clearly warranted to delineate the minimum features responsible for activity.

Acknowledgment. We thank the University of Alberta and NSERCC for financial support of this work, Professor R. B. Jordan for assistance in determining CO$_2$ hydration rates by stopped flow kinetics, and Dr. A. Khalaj for preliminary synthetic efforts directed toward the phosphines.

J. Huguet, R. S. Brown*
Department of Chemistry, University of Alberta
Edmonton, Alberta, Canada T6G 2G2

Received June 26, 1980

---

[11] Titration experiments on **2c**–Zn^{2+} show an ionization of some associated group which we tentatively assign as **2c**–Zn^{2+}–OH$_2$. However, the titration curve is not easily analyzed as having arisen from a single well-defined event. **Note Added in Proof.** More recent experiments with **2c**–Zn^{2+} indicate that its catalysis toward CO$_2$ hydration drops at pH 7.5 to ~60 M^{-1} s^{-1}. This may indicate that **2c**–Zn^{2+} suffers some complex hydrolysis at increased pH as does **1**–Zn^{2+}. Experiments performed under slightly different conditions indicate that the k_{cat} terms for both CO$_2$ hydration and HCO$_3$$^-$ dehydration may be substantially larger than reported in Table I.

[12] Hoyler, R. H.; Hubbard, C. D.; Kettle, S. F. A.; Wilkins, R. G. *Inorg. Chem.* **1965**, *4*, 929; **1966**, *5*, 622.

[13] Henkens, R. W.; Sturtevant, J. M. *J. Am. Chem. Soc.* **1968**, *90*, 2669.

Ozonolysis of Trifluoroethylene, 1,1-Difluoroethylene, and Perfluoroethylene. Epoxide and Ozonide Formation

Sir:

Ozone and trifluoroethylene were mixed in 1,1,2-trichloro-1,2,2-trifluoroethane at 0 °C. The major volatile products have been identified as formyl fluoride, carbonyl fluoride, trifluoroethylene ozonide, and trifluoroethylene oxide. Obtained in lower

0002-7863/80/1502-7572$01.00/0