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REACTIONS OF ORGANOZINC COORDINATION COMPOUNDS

IV*. REACTIONS WITH CARBON DIOXIDE IN RELATION TO THE ACTION OF CARBONIC ANHYDRASE**

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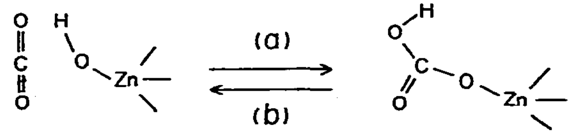
SUMMARY

The reaction of carbon dioxide with diethylzinc has been found to be specifically accelerated by *N*-methylimidazole. In view of the analogy between this reaction and the carbon dioxide/water reaction catalyzed by carbonic anhydrase, a zinc enzyme, it is suggested that coordination of the imidazole group(s) of histidine to the zinc atom in this enzyme makes an important contribution to the high catalytic activity of the enzyme.

INTRODUCTION

The reactivity of dialkylzinc towards active hydrogen compounds such as secondary amines[1,2] and carbon acids[3] is enhanced by the coordination with some complexing agents such as 2,2′-bipyridine and *N,N,N′,N′*-tetramethylethylenediamine. A physiologically important reaction of carbon dioxide and water is catalyzed by a zinc enzyme, carbonic anhydrase, it is thus of interest to examine the effect of complex formation in the reaction between organozinc compounds and carbon dioxide.

The high enzymatic activity of carbonic anhydrase in the reversible reaction of carbon dioxide and water to form carbonic acid has not yet been fully explained. Carbonic anhydrase is a metalloenzyme containing one zinc atom per molecule, and a mechanism for the enzymatic action was suggested by Riepe and Wang[4] as follows:



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[*] For Part III see ref. 3.

[**] Read at the Fifth International Conference on Organometallic Chemistry (1971, Moscow).

The reaction is considered to involve a nucleophilic attack of zinc-bound *O*H on carbon dioxide accompanied by proton transfer [eqn. (1a)] and a nucleophilic attack of zinc-bound *O*(CO) to hydrogen [eqn. (1b)]. On the other hand, coordination of a nitrogen atom to the zinc atom in the enzyme has been suggested. In particular, Dobry-Duclaux and May[5] suggested that there is coordination of the imidazole group(s) of the histidine residue to the zinc atom. The role of such coordination has not, however, been discussed in relation to the high enzymatic activity.

We studied the reaction of carbon dioxide with diethylzinc, which is analogous to reaction [eqn. (1a)]; *i.e.*, a nucleophilic attack of zinc-bound carbon on carbon dioxide, and observed a specific accelerating effect of an imidazole derivative. In the present paper, the accelerating effect of some complexing agents, including *N*-methylimidazole, is described, and discussed in relation to the action of carbonic anhydrase.

RESULTS

The reaction of diethylzinc with carbon dioxide is known to be very slow at ordinary temperatures and pressures. We have found that it is greatly accelerated by the presence of an equimolar amount of certain complexing agents, such as *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA), 2,2′-bipyridine (Bipy) and *N*-methylimidazole (Mim).

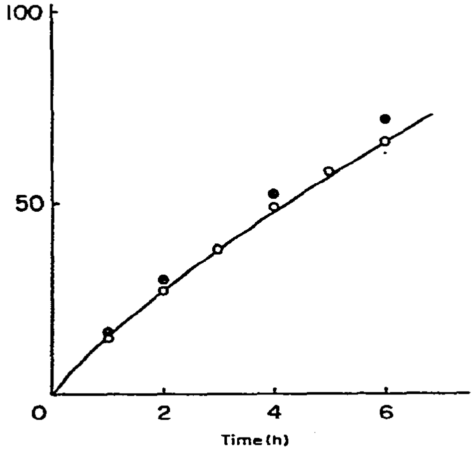


Fig. 1. Reaction of carbon dioxide with diethylzinc/Bipy system. In benzene at 20°; diethylzinc, 0.5 *M*. As followed by ○ CO_2 absorption (CO_2 760 mmHg) and ● propionic acid formation (CO_2 850 mmHg).

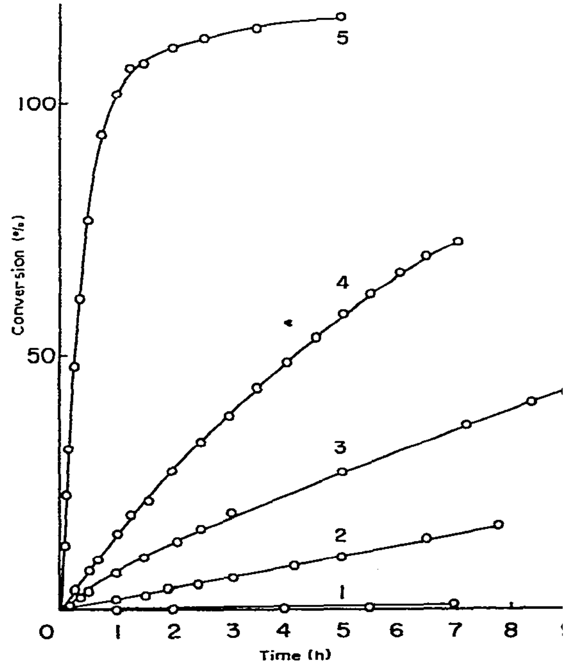
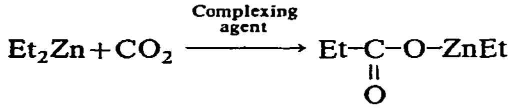


Fig. 2. Reaction of diethylzinc with carbon dioxide. In benzene at 20°; diethylzinc, 0.5 *M*; carbon dioxide 1 atm. Conversion for one Et–Zn bond of diethylzinc. Complexing agent (mole ratio to diethylzinc): 1. None, 2. TMEDA(1), 3. Mim(1), 4. Bipy(1), 5. Mim(2).

The occurrence of the reaction was confirmed by the formation of propionic acid (after acid hydrolysis of the product) and by the absorption of carbon dioxide, as shown in Fig. 1 for the diethylzinc–Bipy system. The reaction was usually carried out in benzene at 20° under 1 atmosphere pressure of carbon dioxide, and followed by the absorption of carbon dioxide.



$$Et_2Zn + CO_2 \xrightarrow{\text{Complexing agent}} Et\text{–}\underset{\underset{O}{\|}}{C}\text{–}O\text{–}ZnEt \qquad (2)$$

Neither diethyl ketone nor triethylcarbinol was detected in the reaction products.

As shown in Fig. 2, the reaction of diethylzinc with carbon dioxide was very slow, but was accelerated by TMEDA, Bipy and Mim. The effect of Mim was very remarkable, and when a 2/1 Mim/Zn molar ratio was used, the second ethyl group of the diethylzinc also reacted. In agreement with this, ethylzinc propionate was found to react slowly with carbon dioxide in the presence of two equivalents of Mim.

At low conversions the reaction showed pseudo first-order kinetics with respect to diethylzinc at a constant concentration of carbon dioxide. Pseudo first-order rate constants thus evaluated are listed in Table 1. As shown in the Table, the presence of two equivalents of Mim brought about more than a 3700-fold acceleration.

Pyridine and its derivatives, when used in two molar ratio to zinc, were moderately effective, and the catalytic activities were in the order: Bipy > 4-methylpyridine > TMEDA ≈ pyridine > ethyl isonicotinate ≈ pyrazine > 2,4,6-trimethylpyridine. The order for the substituted pyridines parallels their basicity, except for 2,4,6-trimethylpyridine for which there would be much steric hindrance in the complex formation. Thiophene was ineffective.

Noltes and Boersma[6] reported that diethylzinc forms very stable 1/1 complexes with TMEDA and Bipy in benzene. Thus when TMEDA or Bipy is used as the complexing agent, the reactivity represented by the rate constant in the Table should actually be ascribed to the diethylzinc–TMEDA or diethylzinc–Bipy complex.

The infrared spectra of diethylzinc–Mim systems were examined in a study of the complex formation (Fig. 3). In the low wave number region of the infrared spectrum of diethylzinc in benzene, absorptions due to $\gamma_r(CH_2\text{–}Zn)$ and $\nu_{as}(C\text{–}Zn\text{–}C)$ occur at 620 and 561 cm^{-1}, respectively. Upon addition of an equimolar amount of

TABLE 1

PSEUDO FIRST-ORDER RATE CONSTANTS FOR THE DIETHYLZINC/CARBON DIOXIDE REACTION[a]

Complexing agent[b] (mole ratio to zinc)	*pKa*	*Rate constant (min^{-1})*
None		$< 10^{-5}$
TMEDA (1)	9.0	3.7×10^{-4}
Bipy (1)	4.1	2.7×10^{-3}
Mim (1)	6.9	1.0×10^{-3}
Mim (2)	6.9	3.7×10^{-2}

[a] In benzene at 20°; diethylzinc, 0.5 *M*; carbon dioxide, 1 atm. [b] TMEDA: *N,N,N′,N′*-tetramethylethylenediamine, Bipy: 2,2′-bipyridine, Mim: *N*-methylimidazole.

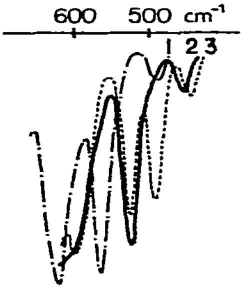


Fig. 3. Infrared spectra of diethylzinc/*N*-methylimidazole systems. 0.5 *M* in benzene at 20°. 1. Et_2 2. Et_2Zn/Mim system; 3. Et_2Zn/2Mim system.

Mim the band at 561 cm^{-1} disappears and a new band appears at 525 cm^{-1}, indicati the formation of a 1/1 complex. On further addition of Mim, a new absorption appe at 491 cm^{-1} and the band at 525 cm^{-1} decreases, and so the 491 cm^{-1} band is co sidered to be due to a 1/2 diethylzinc/Mim complex. From these observations, can conclude that the diethylzinc/Mim (1/1) system consists almost exclusively $Et_2Zn \cdot$ Mim complex, while the diethylzinc/Mim (1/2) system contains comparal amounts of the $Et_2Zn \cdot 2$Mim and $Et_2Zn \cdot$ Mim complexes (48 and 52%, respectivel It follows that the $Et_2Zn \cdot 2$Mim complex has a much higher reactivity than t $Et_2Zn \cdot$ Mim complex.

Not only compounds containing carbon–zinc bonds, but also those containi oxygen–zinc bonds are activated by coordination with Mim. Although ethylzi ethoxide EtZnOEt itself does not react with carbon dioxide, a reaction at the zin ethoxy bond took place in the presence of two equivalents of Mim; carbon dioxi was absorbed very rapidly to give a reaction mixture with strong absorption due COO–Zn in its IR spectrum, but no propionic acid was detected after the a hydrolysis of the mixture. The apparent pseudo first-order rate constant for t reaction was $k_1 = 2.5 \times 10^{-1}$ min^{-1}. The ethoxyzinc propionate/2 Mim system react with carbon dioxide at a similar rate.

DISCUSSION

The enhancement of the reactivity of diethylzinc is considered to be due, least in part, to the increase in the nucleophilicity of the ethyl–zinc bond by compl formation with the base. However, the extent of the acceleration cannot be explain in terms of the basicity of the complexing agent (p*K*a: TMEDA 9.0, Mim 6.9, Bi 4.1), the stability of the diethylzinc complex (TMEDA[6] > Bipy[6] ≫ Mim), or t strength of the coordination as indicated by the shift of ν_{as}(C–Zn–C) absorption[1] the infrared spectrum (None 561, Bipy 501, TMEDA 498, Mim 491 cm^{-1}).

In view of the higher reactivity of the $Et_2Zn \cdot$ Bipy complex than of the Et_2Z TMEDA complex in the reaction with secondary amines[1,2], we suggested that bac donation from zinc to Bipy[6] plays an important role in this reaction; such an eff would facilitate the coordination of the reacting amine to the zinc atom. In t reaction of carbon dioxide with organozinc coordination compounds, coordinati of the carbon dioxide to zinc may be similarly important. Thus *N*-methylimidazole considered to be an especially effective complexing agent, since its relatively hi basicity is combined with the possibility of back-donation.

The higher reactivity of the $Et_2Zn \cdot 2Mim$ complex than of the $Et_2Zn \cdot Bipy$ complex is significant in view of the fact that Bipy is a better ligand than imidazole in the hydrolysis of diisopropyl fluorophosphate catalyzed by cupric ion[7]. The imidazole derivative may thus be regarded as an especially appropriate complexing agent for zinc in the present case.

In the light of the above discussion, the hydration of carbon dioxide catalyzed by carbonic anhydrase [eqn. (1a)] is thought to be much affected by coordination of the imidazole group(s) of histidine to the zinc atom in the enzyme. Although the enzymatic reaction takes place in water, the conclusions reached for the carbon dioxide/diethylzinc reaction in benzene can be applied since the active site of the enzyme is thought to be in a highly non-polar region[4]. Thus, the imidazole group(s) coordinated to the zinc atom would activate the oxygen–zinc bond and, furthermore, there is the possibility that the coordination of carbon dioxide to the zinc atom be facilitated by complex formation between the latter and imidazole. Although coordination of carbon dioxide to the zinc atom in carbonic anhydrase could not be detected by the infrared spectrum[4], the possibility of coordination in the transition state cannot be excluded.

As for the dehydration reaction [eqn. (1b)], a similar activation of the Zn–O(CO) bond can be postulated for the reaction with hydrogen. Even though coordination of the reacting H to zinc is unlikely, the activation of the Zn–O bond towards acid (CO-O–H) is considered much more pronounced than that towards carbon dioxide. Thus, the $Et_2Zn \cdot Bipy$ complex is about 270 times more reactive than Et_2Zn itself towards carbon dioxide (Table 1), but about 5000 times more reactive than Et_2Zn towards pyrrole (N–H)[2].

We conclude that *N*-methylimidazole is a remarkably effective complexing agent in catalysing the reaction of an organozinc compound with carbon dioxide, and in view of this coordination of the imidazole group(s) of histidine to the zinc atom in carbonic anhydrase is thought to make an important contribution towards the high catalytic activity of the enzyme.

EXPERIMENTAL

Materials

Diethylzinc was purified by distillation in nitrogen, b.p. 40°/40 mmHg.

Commercial high purity carbon dioxide was further purified by passage through a series of columns packed with cupric sulfate, potassium bicarbonate, reduced copper, phosphor pentoxide and activated cupric oxide before use.

N-Methylimidazole was prepared from imidazole, methyl iodide and sodium in liquid ammonia[8]. TMEDA was purified by distillation, and Bipy by recrystallization from n-hexane.

Reaction

The reaction was carried out as described for the reaction between diethylzinc and secondary amines[2]. A gas burette containing carbon dioxide at one atmosphere pressure was connected with the reaction flask containing the solution of complexing agent previously saturated with carbon dioxide. After the addition of diethylzinc to the solution, the absorption of carbon dioxide was followed by observing the gas burette, the pressure being maintained at 1 atm.

When the reaction was followed by the formation of the product, a measured amount of the reaction mixture was taken out at a suitable time interval and added to an excess of hydrochloric acid. GLC was used to determine the amount of propionic acid.

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