coordinates, bond distances, bond angles, and observed and calculated structure factors (13 pages). Ordering information is given on any current masthead page.

References and Notes

(1) T. Isobe, T. Kamikawa, I. Kubo, and T. Kubota, *Bull. Chem. Soc. Jpn.*, **46**, 583 (1973).

(2) T. Kubota and I. Kubo, *Bull. Chem. Soc. Jpn.*, **42**, 1778 (1969).

(3) The cytotoxicity (KB) effect (LD_{50}) are as follows ($\mu g/mL$): shikodonin, 4.2; oridonin, 2.8; shikokianin, >10. See E. Fujita, Y. Nagao, M. Node, K. Kaneko, S. Nakazawa, and H. Kuroda, *Experientla*, **32**, 203 (1976).

(4) M. Yamaguchi, M. Taniguchi, I. Kubo, and T. Kubota, *Agr. Biol. Chem.*, in press.

(5) I. Kubo, M. Taniguchi, M. Yamaguchi, and T. Kubota, unpublished work.

(6) The results are based on a combination of proton–noise decoupling, off-resonance decoupling, and Fourier transform off-resonance decoupling techniques. See P. Zanno, I. Miura, K. Nakanishi, and D. Elder, *J. Am. Chem. Soc.*, **97**, 1975 (1975).

(7) M. M. Woolfson and G. Germain, *Acta Crystallogr., Sect. B*, **24**, 91 (1968).

(8) All calculations were performed on a FACOM 270/30 computer at the Computer Center of Osaka City University.

(9) T. Sakurai, Ed., "The Universal Crystallographic Computing System (I)", The Crystallographic Society of Japan, 1967.

(10) The work at Columbia University was partially supported by NIH Grant AI 10187.

Isao Kubo,* Michael J. Pettel

Department of Chemistry, Columbia University
New York, New York 10027

Ken Hirotsu,* Hideyuki Tsuji

Faculty of Science, Osaka City University
Sumiyoshi-ku, Osaka 558, Japan

Takashi Kubota

School of Medicine, Kinki University
Sayama-cho, Osaka 589, Japan

Received May 31, 1977

Copper Complex Acting as a Reversible Carbon Dioxide Carrier

Sir:

Use of CO_2 in synthetic organic reactions is important. Activation of CO_2 and its transfer to organic substrates by means of a transition metal complex acting as a reversible CO_2 carrier may be a promising approach. Among biological carboxylations, the biotin-dependent carboxylations are known to involve the intermediate formation of enzyme-bound carboxybiotin as a reversible carrier of activated CO_2.[1] Despite several recent reports on transition metal complexes having an ability for reversible CO_2 fixation,[2] examples effecting organic reactions of the CO_2 fixed in these complexes are few.[3] Here we report both a reversible decarboxylation of a copper(I) cyanoacetate-phosphine complex and a transcarboxylation by the complex to cyclohexanone.

All experiments described below were carried out under nitrogen atmosphere. Previously, we reported that copper(I) cyanoacetate underwent a quantitative and irreversible decarboxylation in dimethylformamide (DMF) at 50 °C to give isolable cyanomethylcopper(I).[4] Now it has been found that, in the presence of a $PBu_3{}^n$ ligand which dissolves copper(I) cyanoacetate in organic solvents, the decarboxylation of copper(I) cyanoacetate becomes reversible.

$$NCCH_2CO_2Cu \cdot (PBu_3{}^n)_x$$
$$\rightleftharpoons NCCH_2Cu \cdot (PBu_3)_x + CO_2 \quad (1)$$

The reversibility was supported by the following experimental results. (i) Cyanomethylcopper(I) absorbed CO_2 gas in the presence of 3 equiv of $PBu_3{}^n$ at 0 °C and methyl cyanoacetate was produced in a yield of 53% on treating the reaction mixture with methyl iodide at 0 °C to room tempera-
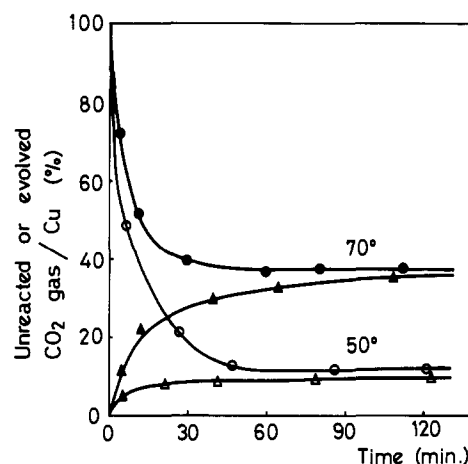

Figure 1. Reversibility of the carboxylation starting from 0.86 mmol of $NCCH_2Cu$ (O) and 0.77 mmol of $NCCH_2Cu$ (●) (ratio of $PBu_3{}^n/Cu$ = 3), and the decarboxylation starting from 0.95 mmol of $NCCH_2COOCu$ (▲) and 0.89 mmol of $NCCH_2COOCu$ (△) (ratio of $PBu_3{}^n/Cu$ = 3) in 5 mL of DMF: $NCCH_2COOCu \cdot (PBu_3{}^n)_x \rightleftharpoons NCCH_2Cu \cdot (PBu_3{}^n)_x + CO_2$.
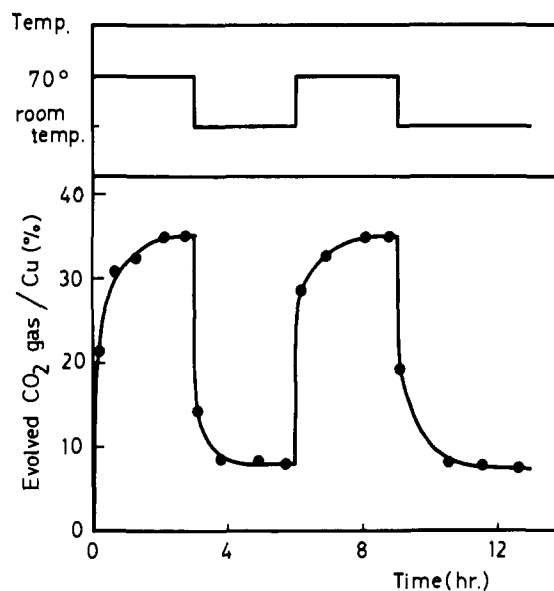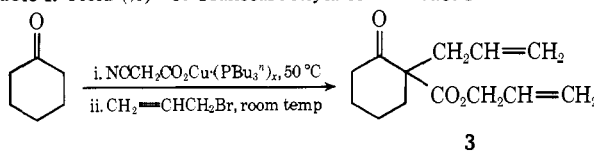
Figure 2. Cycle of CO_2 gas evolution–absorption by alternate heating and cooling of $NCCH_2CO_2Cu \cdot (PBu_3{}^n)_x$: ratio of $PBu_3{}^n/NCCH_2CO_2Cu$ (1.30 mmol) = 3; 5 mL of DMF.

ture. (ii) As shown in Figure 1, nearly the same state of equilibrium was attained from either direction of eq 1. (iii) A cycle of carboxylation-decarboxylation caused by changing the reaction temperature was repeatable (Figure 2).

As the molar ratios of $PBu_3{}^n/NCCH_2CO_2Cu$ and $PBu_3{}^n/NCCH_2Cu$ were increased from 1 to 2 and 3, the decarboxylation was depressed and the carboxylation was favored. However, an addition of 1 more equiv of $PBu_3{}^n$ beyond $PBu_3{}^n/Cu$ = 3 did not cause any effect on the decarboxylation of $PBu_3{}^n/NCCH_2CO_2Cu$ = 3 and the carboxylation of $PBu_3{}^n/NCCH_2Cu$ = 3. Based on these findings, it might be assumed that three $PBu_3{}^n$ ligands are coordinated to copper(I) cyanoacetate and cyanomethylcopper(I), respectively, forming coordinatively saturated complexes.[5] Determination of the number of the phosphine ligand coordinated to the copper complexes involved in the reversible decarboxylation by isolating the copper–phosphine complexes is impossible, because $NCCH_2CO_2Cu \cdot (PBu_3{}^n)_x$ (x = 1, 2, and 3) is too unstable toward decarboxylation at ambient temperature to permit its isolation. General expressions $NCCH_2CO_2Cu \cdot (PBu_3{}^n)_x$ and

Table I. Yield (%)[a] of Transcarboxylation Product 3[b]

Ratio of	Solvent	
$PBu_3{}^n/NCCH_2CO_2Cu$	DMF	C_6H_6
0	5	
0.5	38	36
1.0	87[c]	88[c]
1.5	83	70
3.0	29	38

[a] Yields are based on $NCCH_2CO_2Cu$. [b] $NCCH_2CO_2Cu$, 1.0 mM; ratio of c-$C_6H_{10}O/NCCH_2CO_2Cu$ = 5; solvent, 5 mL; ratio of CH_2=$CHCH_2Br/NCCH_2CO_2Cu$ = 10. [c] CH_3CN was detected quantitatively.

$NCCH_2Cu\cdot(PBu_3{}^n)_x$ have been used throughout this study. A ligand with a higher σ-donating ability favored the carboxylation probably owing to an increased carbanionic character of the $NCCH_2$ group and/or a stabilization of the carboxylate group;[2g] the equilibrium values of the evolved CO_2 gas (L/$NCCH_2CO_2Cu$ = 3, DMF, 50 °C) were 62% (P(OPh)$_3$), 55% (P(OMe)$_3$), 25% (PPh$_3$), 19% (PEt$_3$), and 9.8% (PBu$_3{}^n$).

Several analogous reversible CO_2 insertions involving a transition metal-oxygen[2a,b,g,h] bond have been reported. However, there has been only one precedent for the reversible CO_2 insertion into a transition metal-carbon bond involved commonly in a variety of transition metal-catalyzed reactions: the reversible CO_2 insertion into $PhC≡CCu\cdot(PBu_3{}^n)_x$ which has been reported very recently by us.[2f] As it is known that an electron-withdrawing functional group on the α carbon of a carboxylic acid promotes its decarboxylation by facilitating the release of CO_2,[6] these reversible CO_2 insertions into the copper-carbon bonds seem to require the presence of the electron-withdrawing groups such as -CN and -C≡CPh on α carbons of the organocoppers which control the carbanionic character of the α-carbon atoms to effect both the CO_2 insertion and the decarboxylation simultaneously.

Now the $NCCH_2CO_2Cu\cdot(PBu_3{}^n)_x$ complex has been found to function as a reversible CO_2 carrier, transcarboxylating its CO_2 moiety to an active methylene compound. The $NCCH_2CO_2Cu\cdot(PBu_3{}^n)_x$ complex reacted with cyclohexanone to produce effectively allyl 1-allyl-2-oxocyclohexanecarboxylate after the treatment with allyl bromide (Table I). The reaction path of the transcarboxylation is depicted in Scheme I which is supported by the following results. Acetonitrile was detected quantitatively after the treatment with allyl bromide. The structure of the allylated transcarboxylation product suggests an intermediacy of 1 in the transcarboxyla-


tion. The 2 complex without PBu$_3{}^n$ was isolated by metalation of 2-oxocyclohexanecarboxylic acid with 2 equiv of *t*-BuOCu in THF at −10 °C and identified as follows: Cu content by
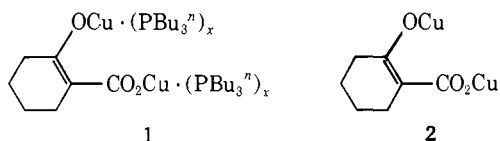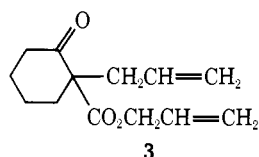
Scheme I
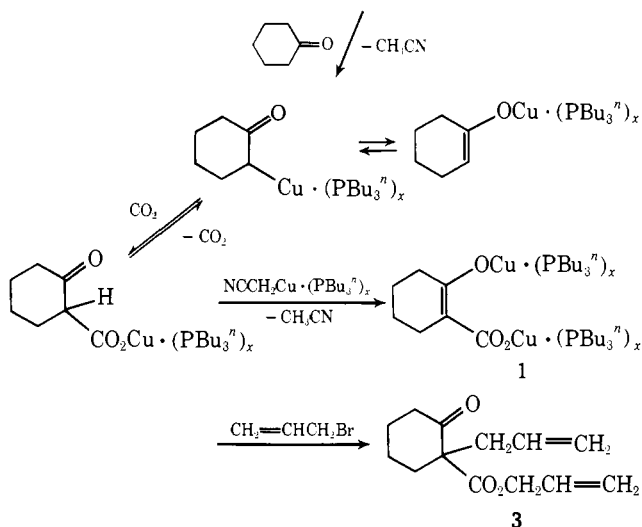
iodometry 46.8% (calcd 47.6%); IR (Nujol) 1545 and 1484 cm^{-1} (νC=CCO_2); formation of 3 by treatment with CH_2=$CHCH_2Br$, 68% (without PBu$_3{}^n$) and 70% (with PBu$_3{}^n$). In a separate study,[7] the 2 complex also has been

$$\xrightarrow{4} \quad (2)$$
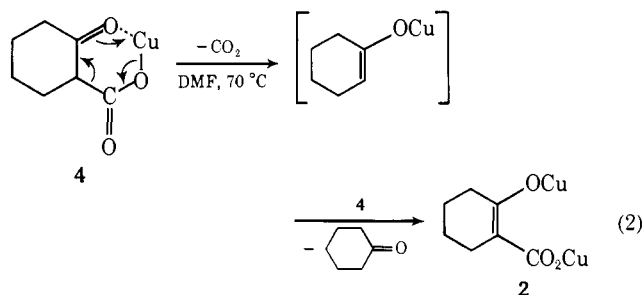
isolated by the decarboxylation of 4 which is promoted by the presence of an electron-withdrawing β-keto group[6] (eq 2). This process also is closely related to Scheme I. 2 is stable to the decarboxylation owing to the absence of such β-keto group. Heating 2 at 70 °C in DMF with or without PBu$_3{}^n$ evolved only a negligible amount of CO_2. The decarboxylation of 2 required a higher reaction temperature and proceeded quantitatively at 120 °C after 4 h in DMF. Based on these results, it may be concluded that the intermediate formation of 1 which is reluctant to decarboxylate is responsible for the efficient transcarboxylation from $NCCH_2CO_2Cu\cdot(PBu_3{}^n)_x$ to cyclohexanone. The similar reaction using another reversible CO_2 carrier, $PhC≡CCO_2Cu\cdot(PBu_3{}^n)_x$,[2f] instead of $NCCH_2CO_2Cu\cdot(PBu_3{}^n)_x$, gave 3 in only 20% yield in DMF. In the above-mentioned reactions, methyl iodide can replace allyl bromide with similar effectiveness.

References and Notes

(1) J. Moss and M. D. Lane, *Adv. Enzymol.*, **35**, 321 (1971).
(2) (a) T. Tsuda and T. Saegusa, *Inorg. Chem.*, **11**, 2561 (1972); (b) M. Hidai, T. Hikita, and Y. Uchida, *Chem. Lett.*, 521 (1972); (c) S. Komiya and A. Yamamoto, *J. Organomet. Chem.*, **46**, C58 (1972); (d) C. Floriani and G. Fachinette, *J. Chem. Soc., Chem. Commun.*, 615 (1974); (e) B. R. Flynn and L. Vaska, *ibid.*, 703 (1974); (f) T. Tsuda, Y. Chujo, and T. Saegusa, *ibid.*, 963 (1975) (see also A. D. English and T. Herskovitz, *J. Am. Chem. Soc.*, **99**, 1648 (1977); (g) T. Tsuda, S. Sanada, K. Ueda, and T. Saegusa, *Inorg. Chem.*, **15**, 2329 (1976); (h) M. H. Chisholm, W. W. Reichert, F. A. Cotton, and C. A. Murillo, *J. Am. Chem. Soc.*, **99**, 1652 (1977).
(3) T. Tsuda, S. Sanada, and T. Saegusa, *J. Organomet. Chem.*, **116**, C10 (1976); see also T. Tsuda, Y. Chujo, and T. Saegusa, *J. Chem. Soc., Chem. Commun.*, 415 (1976).

(4) T. Tsuda, T. Nakatsuka, T. Hirayama, and T. Saegusa, *J. Chem. Soc., Chem. Commun.*, 557 (1974).
(5) For the isolations of CH₃Cu·(PPh₂Me)₃ and CH₃CO₂Cu·(PPh₂Me)₃, see (a) A. Miyashita and A. Yamamoto, *Bull. Chem. Soc. Jpn.*, **50**, 1102 (1977), and (b) A. Miyashita and A. Yamatoto, *J. Organomet. Chem.*, **113**, 187 (1976), respectively.
(6) For a decarboxylation of β-keto acids, see, for example, H. H. Wasserman in "Steric Effects in Organic Chemistry", M. S. Newman, Ed., Wiley, New York, N.Y., 1956, p 351.
(7) T. Tsuda, Y. Chujo, S. Takahashi, and T. Saegusa, unpublished result.

Tetsuo Tsuda, Yoshiki Chujo, Takeo Saegusa*

Department of Synthetic Chemistry,
Faculty of Engineering, Kyoto University, Kyoto, Japan
Received May 6, 1977

## Specificity in Enzymatic Decarboxylation

Sir:

Specificity is a key feature of enzymatic reactions. At the first level of sophistication, specificity may be expressed either in binding or in catalysis. Discrimination between these two possibilities may often be accomplished by determining Michaelis constants and turnover rates for different substrates. If the specificity arises in catalysis, steady-state kinetics often does not permit assignment of the specificity to a single step or group of steps. For example, studies of chymotrypsin conducted by Niemann,[1] Neurath,[2] and their collaborators revealed examples of both types of specificity. To identify reaction steps responsible for specificity in catalysis, a number of people have measured acylation and deacylation rates for numerous tightly binding substrates.[3] Both acylation and deacylation rates parallel changes in catalytic rate. Thus, in the case of chymotrypsin specificity is not manifested in a single reaction step.

However, it is possible that in many cases specificity is manifested principally in a single reaction step. Few methods are available for dissecting enzymatic reaction mechanisms at the necessary level of sophistication. We show here that heavy-atom isotope effects are useful in probing the details of enzyme specificity. We are able by this method to identify the step responsible for specificity of the inducible form of the pyridoxal 5'-phosphate dependent arginine decarboxylase (E.C. 4.1.1.19) from *E. coli*.

Most amino acid decarboxylases require pyridoxal 5'-phosphate for activity. These enzymes have stringent specificity requirements.[4] Binding involves the α carboxyl and amino groups and usually a distal group (often charged) as well.[5] The chain which connects the α carbon to this distal group probably lies on the enzyme in its most extended conformation. For example, glutamate decarboxylase acts very slowly on aspartic acid or α-aminoadipic acid and inhibition of the enzyme by dicarboxylic acids is optimum for inhibitors whose carboxyl-carboxyl distance corresponds to that of the extended conformation of glutamic acid.[6]

Kinetic data summarized in Table I reveal that a similar pattern of specificity is observed with arginine decarboxylase. Arginine and canavanine are the proper size and are decar-

Scheme I
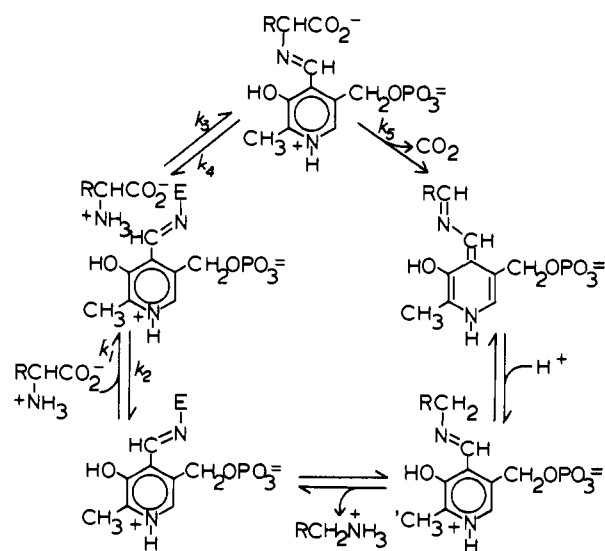
boxylated efficiently by the enzyme. Homoarginine and norarginine are proper except that the distal group is at the wrong distance from the α carbon. Michaelis constants for these substrates are similar to that of arginine, but the maximum velocities are down by about a hundredfold compared with arginine.

To establish that the Michaelis constants actually represent equilibrium constants for substrate binding, we measured inhibition constants for the inhibition of decarboxylation of arginine by canavanine, homoarginine, and norarginine. In each case (Table I) the observed inhibition constant is within experimental error of the corresponding Michaelis constant. Thus, binding of these substrates to the enzyme is an equilibrium process.[7]

Carboxyl carbon isotope effects for the decarboxylation of arginine, canavanine, homoarginine, and norarginine by arginine decarboxylase at pH 5.25 were measured by our usual procedure.[8] The isotope effects are summarized in Table I. The rapidly reacting substrates arginine and canavanine give isotope effects which are small but significantly different from unity, whereas the slowly reacting substrates homoarginine and norarginine give larger isotope effects.

The mechanism of the decarboxylation is shown in Scheme I. There are four steps following substrate binding, and one or more of these steps could control substrate specificity. However, the steps following decarboxylation are probably relatively rapid and have no role in specificity.[9] Decarboxylation is not reversible under our reaction conditions; so only reaction steps up through decarboxylation will be reflected in the carbon isotope effects.[10] Assuming that only the decarboxylation step shows a significant carbon isotope effect and that substrate binding is at equilibrium, the relation between the observed isotope effect and the mechanism shown in Scheme I is given by

Table I. Kinetic and Isotope Effect Data for Arginine Decarboxylase

Substrate	Formula	V_{max},[a] s⁻¹	K_m, mM	K_i, mM	k^{12}/k^{13} [b]
Arginine	(NH₂)₂⁺CNH(CH₂)₃CH(NH₂)CO₂H	560	1.0 ± 0.5		1.0144 ± 0.0004
Homoarginine	(NH₂)₂⁺CNH(CH₂)₄CH(NH₂)CO₂H	6	1.0 ± 0.3	6.0 ± 5.0	1.0535 ± 0.0002
Norarginine	(NH₂)₂⁺CNH(CH₂)₂CH(NH₂)CO₂H	9	3.7 ± 0.3	4.0 ± 3.0	1.0438 ± 0.0004
Canavanine	(NH₂)₂⁺CNHO(CH₂)₂CH(NH₂)CO₂H	140	2.0 ± 1.0	0.8 ± 0.2	1.0048 ± 0.0002

[a] Steady-state kinetic measurements were performed manometrically at pH 5.25, 37 °C, in 0.2 M sodium acetate buffer. The data were corrected using eq C of K. F. Gregory and H. C. Winter, *Anal. Biochem.*, **11**, 519 (1965). Velocities are given per enzyme active site, assuming that the specific activity of the pure enzyme is 410 μmol of CO₂ min⁻¹ mg⁻¹ of enzyme.[4] [b] Carbon isotope effects were measured using 0.2 M sodium acetate buffer, pH 5.25, 25 °C, by our published procedure.[8]