Flavin Trapping of Carbanion Intermediates as Catalyzed by Cyanide Ion and Cationic Micelle[1]

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Abstract: Benzaldehydes and benzoylformic acids which usually undergo benzoin-type condensation in the presence of cyanide ion were easily oxidized by flavin to the corresponding carboxylic acids under ambient reaction conditions (30–50 °C). Aliphatic substrates such as formaldehyde, butyraldehyde, and pyruvic acid were also oxidized by flavin under similar reaction conditions, although the yields were lower than those of aromatic substrates. The flavin oxidation was facilitated markedly by cationic micelles. Kinetic studies established that the reaction is zero order in flavin and first order in cyanide ion and substrate, indicating the rate-limiting formation of the carbanion intermediates from the cyanide adducts followed by the rapid oxidation by flavin. The results show that flavin is able to trap the transient carbanion intermediates to give the oxidation products. Hence, the reaction is readily diverted from condensation to oxidation. The catalytic role of the cationic micelles was accounted for by synergistic effects such as effective concentration of reactants and local reaction environments. The relevance of these reactions to biological systems and the application to organic synthesis are discussed.

The mechanisms by which flavoenzymes catalyze oxidation–reduction reactions have been of much concern.[2,3] Recently, it was proposed that several flavoenzymes such as L- and D-amino acid oxidases and lactate oxidase generate a carbanion intermediate during the course of the flavin oxidation.[4,5] The mechanism involves the proton abstraction from the substrates followed by the flavin oxidation of the carbanions. The proposal is also supported by model studies in nonenzymatic systems.[6]

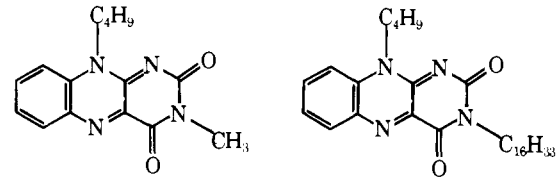
It occurred to us that the application of the concept—"flavin oxidation of carbanion"—to organic synthesis would be very useful. If the carbanion intermediates, the presence of which has been proposed for a great number of organic reactions (mainly for condensation reactions), are trapped instantaneously by flavin, one can easily change the reaction, for example, from the condensation reaction to the oxidation reaction. Flavin used for the purpose must be extremely reactive as an oxidizing agent in order to achieve the perfect trapping of the carbanions. Fortunately, we recently found that flavins bound to cationic micelles or to cationic polymers serve as excellent oxidizing agents of the carbanions.[7,8] For example, oxidation of nitroalkane carbanions by conventional flavins does not occur unless an electron-deficient isoalloxazine is used,[9] whereas the same reaction is easily mediated by the micelle- and polymer-bound flavins.[7,8] Thus, the flavin trapping of transient carbanions may be achieved by the use of these "activated" flavins.

Lapworth[10] established that cyanide ion catalysis of benzoin condensation proceeds via a carbanion intermediate, $PhC^{-}(CN)(OH)$. Franzen and Fikentscher[11] found later that the decarboxylation of α-keto acids yields similar carbanion intermediates and finally gives benzoin-type condensation products. We wish to report herein the first example of flavin trapping of the carbanion intermediates formed from the cyanide adducts of aldehydes and α-keto acids. On the whole these reactions (eq 1 and 2) are analogous to the actions of aldehyde oxidase and pyruvate dehydrogenase, respectively, both of which are known to require FAD as a cofactor.[2] It is noteworthy, in particular, that pyruvate dehydrogenase contains thiamin pyrophosphate as an additional cofactor,[2] the catalytic role of which can be partially imitated by cyanide ion.

$$\mathrm{RCHO} \xrightarrow{\text{flavin, } CN^-} \mathrm{RCOOH} \qquad (1)$$

$$\mathrm{RCOCOOH} \xrightarrow{\text{flavin, } CN^-} \mathrm{RCOOH} \qquad (2)$$

Flavins employed are illustrated below with their abbreviations (R = tetra-*O*-acetylribityl).



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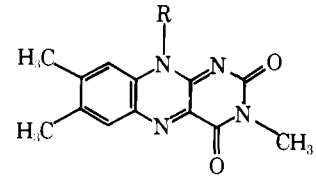

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Experimental Section

Materials. Formaldehyde, butyraldehyde, benzaldehyde, 4-chlorobenzaldehyde, 2,4-dichlorobenzaldehyde, and pyruvic acid were purchased from Wako Pure Chemical Industries, and all the aldehydes (except formaldehyde) were distilled under an N_2 stream before use. Surfactants were also available from Wako Pure Chemical Industries (for biochemical use) and used without further purification.

Benzoylformic acid and 4-chlorobenzoylformic acid were prepared by permanganate oxidation of the corresponding mandelic acids according to the method of Corson et al.:[12] benzoylformic acid, mp 57–61 °C (lit.[12] 58–61 °C); 4-chlorobenzoylformic acid, mp 61–63 °C (lit.[13] 61–62 °C). 4,4′-Dichlorobenzoin was prepared by benzoin condensation of 4-chlorobenzaldehyde:[14] mp 80–84 °C (lit.[15] 85–87 °C). 4,4′-Dichlorobenzil was obtained by treatment of 4,4′-dichlorobenzoin with $CuSO_4$:[16] mp 195–197 °C (lit.[16] 195–196 °C).

3-Methyl-10-butylisoalloxazine (**1**) was a gift from Professor F. Yoneda (for the preparation see ref 17). The preparation of 3-hexadecyl-10-butylisoalloxazine (**2**) and 3-methyltetra-*O*-acetylriboflavin (**3**) has been described.[18]

Methods of Flavin Oxidation. The flavin oxidations of aldehydes and α-keto acids were performed according to three different procedures.

Method A. An aqueous solution of CTAB (hexadecyltrimethylammonium bromide), KCN, and substrate was heated in the dark under aerobic conditions (the details of the reaction conditions are recorded in Tables I–III). The solution was brought to pH 12–13 by addition of 1 N KOH to hydrolyze acyl cyanides to carboxylic acids. The solution was then acidified with 4 N HCl to pH 1–2. When the yield was generally higher than about 10%, benzoic acids were re-

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Table I. Flavin Oxidation of 4-Chlorobenzaldehyde[a]

method	1, mM	KCN, mM	CTAB, mM	yield, % 4-ClC_6H_4COOH	4,4-dichlorobenzoin	4,4′-dichlorobenzil
A	0.212	0	0	0	0	0
A	0.212	0	2.0	0	0	0
A	0	7.5	0	2.4	72	2.1
A	0.212	7.5	0	22.0	8.6	0.6
A	0	7.5	2.0	47.5	2.7	0.3
A	0.212	7.5	2.0	74.2	0.8	0.1
C	0.212	7.5	0	19.6	48.0	0.7
C	0.212	7.5	2.0	27.2	45.3	1.2
C[b]	0.212	7.5	0	19.5	21.0	2.1

[a] 1 day at 30 °C in the dark. [b] Substrate is benzaldehyde.

Table II. TLC Study of the Reaction of Aliphatic Substrates

substrate		flavin, mM	KCN, mM	CTAB, mM	R_f product	R_f authentic sample
HCHO[a]	2	0.212	7.5	2.0	0.70	0.72 (HCOOH)
C_3H_7CHO[a]	2	0.212	7.5	2.0	0.78	0.74 (C_3H_7COOH)
CH_3COCO_2H[b]	3	4.5	169	4.1	0.54	0.56 (CH_3COOH)

[a] 48 h at 30 °C in the dark under aerobic conditions. The developing solvent used for paper chromatography is a mixture of ethanol–water–aqueous NH_3 (80:60:4). [b] 60 h at 50 °C in the dark under aerobic conditions. The developing solvent used for paper chromatography is a mixture of ethanol–water–aqueous NH_3 (16:12:0.8).

Table III. Oxidative Decarboxylation of 4-Chlorobenzoylformic Acid by Flavin[a]

method	3, mM	KCN, mM	CTAB, mM	yield, % 4-ClC_6H_4COOH	4,4′-dichlorobenzoin	4,4′-dichlorobenzil
A	0.179	0	0	0	0	0
A	0.179	0	2.0	0	0	0
A	0	7.5	0	3.0	16.0	0
A	0.179	7.5	0	29.6	2.3	0.1
A	0	7.5	2.0	47.0	0.2	0.1
A	0.179	7.5	2.0	57.0	0.1	0.1
B	0.650	7.5	0	15.7	2.7	0.9

[a] 2 days at 50 °C in the dark.

covered as precipitates. The precipitates were collected by suction, while the filtrate was extracted three times with chloroform. Concentration of the chloroform layer under reduced pressure afforded an oily material, which was analyzed by using high-pressure liquid chromatography. The yield of carboxylic acids summarized in Tables I and III is the sum of the precipitated and extracted material.

Method B. All the reaction solutions were prepared by using water twice distilled under an N_2 stream, and the same reaction as described in method A was carried out in the dark under N_2.

Method C. The reaction was performed in an ampule with a side arm. A solution containing KCN and CTAB was placed in the bottom of the ampule, while the solution containing flavin and CTAB was deposited in the side arm. The ampule was degassed carefully by thawing and freezing and sealed under reduced pressure. After equilibration of the ampule to the desired temperature, the content of the side arm was rapidly mixed with the solution in the bottom. The reaction was stopped by breaking the ampule in a 4 N HCl solution. Workup was as in method A.

Product Analyses. The aromatic products such as benzoic acids, benzoins, and benzils were analyzed by using high-pressure liquid chromatography (Shimadzu LC-2), and their yields were determined by comparing the integrated intensity of the peaks with that of the authentic samples. On the other hand, aliphatic carboxylic acids were identified qualitatively by paper chromatography[19] (the details of the analysis are recorded in Table II).

Kinetics. The kinetic measurements of the flavin oxidation of aldehydes and α-keto acids were carried out at 30 °C (for aldehydes) or at 50 °C (for α-keto acids) in 3 vol % aqueous ethanol containing 0.05 M KCN and 0.005 M boric acid (pH 10.5) under anaerobic conditions (N_2). A typical reaction was as follows. A 2.5-mL aqueous solution containing CTAB and KCN was placed in a Thunburg cuvette, and 0.5 mL of aqueous ethanol solution containing flavin and substrate was deposited in a side arm of the cell. Both solutions were degassed for 20 min by bubbling N_2 through them and, after closing, the cuvette was equilibrated to the desired temperature. The content of the side arm was rapidly mixed with the micellar KCN solution and the reaction was followed at the characteristic absorption maxima of flavins (433 nm for **1**, 440 nm for **2**, and 447 nm for **3**) on a Hitachi 200 spectrophotometer.

Results

Flavin Oxidation of Aldehydes to Carboxylic Acids. The product analysis of 4-chlorobenzaldehyde oxidation is summarized in Table I. In the absence of KCN, neither oxidation nor benzoin condensation occurred, which was ascertained by high-pressure liquid chromatography. The main product in the presence of KCN was, of course, 4,4′-dichlorobenzoin (72% yield), but a small amount of 4-chlorobenzoic acid was also detected (2.4% yield). On the other hand, the addition of **1** (20 mol % of 4-chlorobenzaldehyde) strikingly suppressed the formation of 4,4′-dichlorobenzoin and simultaneously enhanced the yield of 4-chlorobenzoic acid (22%). The addition of CTAB above the critical micelle concentration (9.0×10^{-4} M at 25 °C and 4.0×10^{-4} M under the present reaction condition at 30 °C and $\mu = 0.05$) caused a further increase in the oxidation product. The results establish that (1) the existence of cyanide ion is a primary prerequisite to the oxidation, (2) **1** dramatically diverts the reaction from the condensation to the oxidation, and (3) the CTAB micelle provides a pertinent reaction environment for the oxidation. The low yield of 4,4′-dichlorobenzil is attributable to the further oxidation of 4,4′-dichlorobenzoin.[4]

Interestingly, the micellar KCN solution provided 4-chlorobenzoic acid in relatively high yield (47.5%) even in the absence of **1**, and the benzoin condensation product was recovered

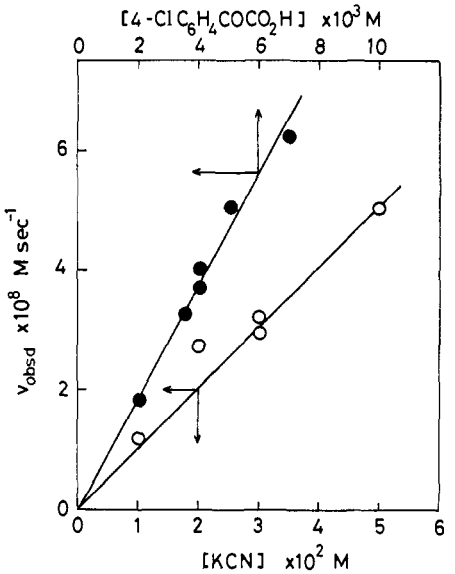


Figure 1. Observed reaction rates for oxidative decarboxylation of 4-chlorobenzoylformic acid plotted as a function of [KCN] and [4-chlorobenzoylformic acid]. [3] = 2.0×10^{-5} M, [CTAB] = 9.75×10^{-3} M. (○): the concentrations of 4-chlorobenzoylformic acid (=5.00×10^{-3} M) and [KCN] + [KCl] (=5.00×10^{-2} M) were maintained constant while varying the concentration of KCN. (●): the concentration of KCN (=5.00×10^{-2} M) was maintained constant while varying the concentration of 4-chlorobenzoylformic acid.

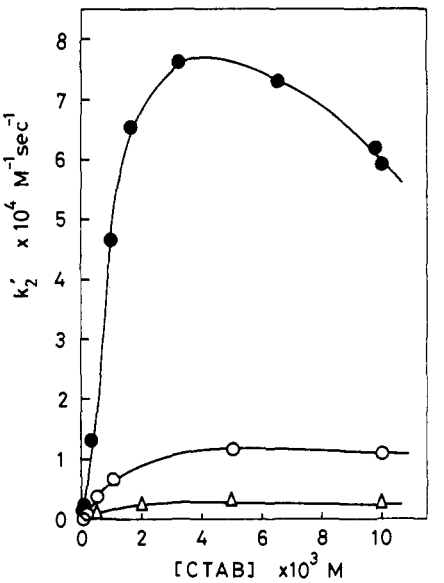


Figure 2. Apparent second-order rate constants for flavin oxidation of aldehydes plotted as a function of the concentration of CTAB, 30 °C, pH 10.5 ([KCN] = 0.05 M, [H_3BO_3] = 0.005 M), 3 vol % ethanol, [1] = 2.0×10^{-5} M, [substrate] = 5.00×10^{-3} M. (●), 2,4-dichlorobenzaldehyde; (○), 4-chlorobenzaldehyde; (△), benzaldehyde.

only as a minor product in 2.7% yield. This result suggests that molecular oxygen is able to act as an oxidizing agent in the CTAB micellar system.[20,21] As noted, little oxidation occurs in the aqueous KCN solution. The difference may be thus rationalized in terms of the activition of the carbanion intermediate by the cationic micelles.[7] In order to distinguish the O_2 oxidation from the flavin oxidation, the reaction was performed under anaerobic conditions (method C in Table I). If **1** is not recycled by reoxidation, the yield of 4-chlorobenzoic acid cannot exceed the mole ratio of **1** to 4-chlorobenzaldehyde (i.e., 20%). Table I reveals that the yield in the absence of the CTAB micelle (19.6%) satisfies this condition, but in the CTAB micelle the yield slightly exceeded the expected amount (yield 27.2%), indicating the occurrence of turnover of **1** and/or direct oxidation of the carbanion intermediate by a trace of oxygen.

The product analysis of aliphatic aldehydes was performed qualitatively with paper chromatography (Table II). Although the chromatogram showed the formation of the corresponding carboxylic acids, starting aldehydes were still detected even after 48 h. Thus, the oxidation of aliphatic aldehydes proceeded quite slowly relative to that of aromatic aldehydes.

Oxidative Decarboxylation of α-Keto Acids. Results of the product analysis are summarized in Table III. Under aerobic conditions, **3** diverted the reaction from benzoin-type condensation to oxidation. The importance of the cyanide ion and the catalytic role of the CTAB micelle are also seen in Table III. Hence, the mechanism for the oxidative decarboxylation may be similar to that depicted for the flavin oxidation of aldehydes.

In the anaerobic reaction without the CTAB micelle (method B), the yield of 4-chlorobenzoic acid was again lower (15.7%) than that in the similar aerobic reaction (29.6%). The yield did not exceed the mole ratio of **3** to 4-chlorobenzoylformic acid (i.e., 26%). This result suggests that **3** acts as the sole oxidant in the anaerobic reaction medium.

Pyruvic acid also underwent oxidative decarboxylation. The product was analyzed with paper chromatography (Table II). Only two spots were observable: one was pyruvic acid while the other was acetic acid. Hence, the conversion of pyruvic acid to acetic acid appears clear without accumulation of other byproducts.

Kinetics of Micellar Catalysis. Oxidation of aromatic substrates exhibited zero-order kinetics with respect to flavin followed by a relatively slow first-order process. For example, oxidation of 4-chlorobenzaldehyde by **1** with the CTAB micelle and CN^- followed zero-order kinetics up to 95% conversion, and up to 80% conversion in the absence of the CTAB micelle. Introduction of oxygen into the reaction cell regenerated the oxidized flavin quantitatively, and after several minutes the zero-order disappearance of flavin started again. The reaction rate calculated from the initial slope of the zero-order decrease was first order in CN^- ([KCN] = $(1-5) \times 10^{-2}$ M) and aromatic substrate ([substrate] = $(1-7) \times 10^{-3}$ M). Typical examples of the plots are shown in Figure 1.

The kinetic behavior for the oxidation of aliphatic substrates was somewhat complicated. Since the rate of the oxidation was relatively slow, the hydrolytic decomposition of flavin could not be neglected. The reaction order is thus difficult to judge precisely. The reaction rates were estimated by subtracting the hydrolysis rate in the absence of substrates from the initial rate in the presence of substrates. A detailed examination of the kinetic order was not carried out for the aliphatic substrates.

The plots of apparent second-order rate constant, k_2' ($=v_{obsd}/[CN^-][substrate]$), vs. CTAB concentration are illustrated in Figures 2 and 3. Examination of these plots reveals that (1) the oxidation of aromatic aldehydes occurs slowly in the nonmicellar system, while the nonmicellar oxidation of aromatic α-keto acids is scarcely observable; (2) in the CTAB micelle the rates of the oxidation are remarkably enhanced (except for 2,4-dichlorobenzoylformic acid); the rate increase relative to the nonmicellar system is 10^2–10^4-fold; (3) the sigmoid curve of k_2' vs. CTAB concentration shows that the effects of the typical cationic detergent are associated with micelle formation; (4) the hydrophobicity of flavin is not an important factor in the oxidation of aromatic aldehydes; (5) the order of reactivity for aromatic aldehydes is 2,4-dichlorobenzaldehyde > 4-chlorobenzaldehyde > benzaldehyde, whereas that for aromatic α-keto acids is 4-chlorobenzoylformic acid > benzoylformic acid > 2,4-dichlorobenzoylformic acid (no reaction). On the other hand, the reaction was unaf-

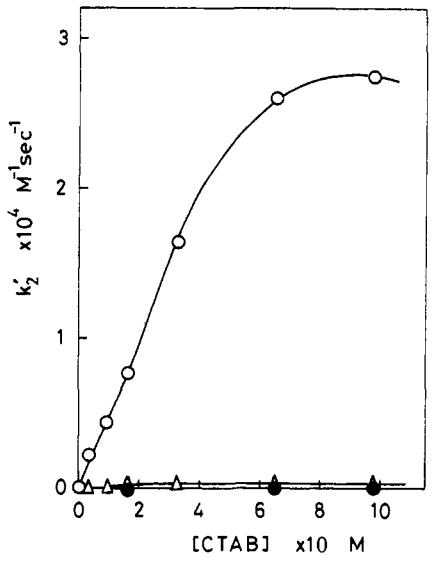


Figure 3. Apparent second-order rate constants for oxidative decarboxylation of α-keto acids plotted as a function of the concentration of CTAB, 50 °C, pH 10.5 ([KCN] = 0.05 M, [H_3BO_3] = 0.005 M), 3 vol % ethanol, [3] = 2.0 × 10^{-5} M, [substrate] = 5.00 × 10^{-3} M. (●), 2,4-dichlorobenzoylformic acid; (○), 4-chlorobenzoylformic acid; (△), benzoylformic acid.

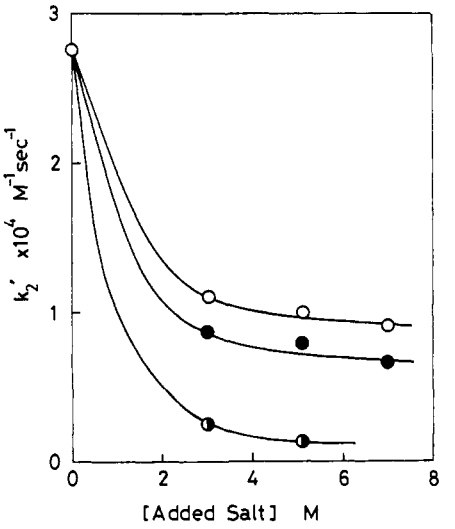


Figure 4. Apparent second-order rate constants for oxidative decarboxylation of 4-chlorobenzoylformic acid plotted as a function of salt concentrations. The reaction conditions at [added salt] = 0 are 50 °C, pH 10.5 ([KCN] = 0.05 M, [H_3BO_3] = 0.005 M), 3 vol % ethanol, [3] = 2.0 × 10^{-5} M, [4-chlorobenzoylformic acid] = 5.00 × 10^{-3} M, [CTAB] = 9.75 × 10^{-3} M. (○), KCl; (●), KBr; (◑), sodium benzenesulfonate.

fected by the anionic (sodium dodecyl sulfate) and the nonionic (Brij-35) surfactant micelles.

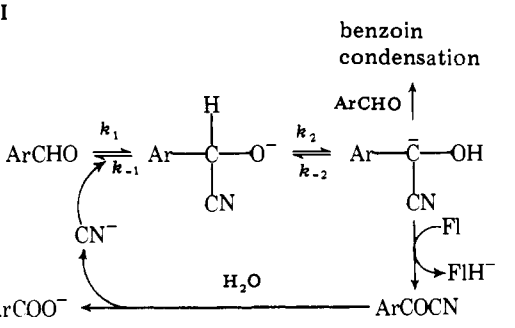
The effect of ionic strength on the oxidative decarboxylation of 4-chlorobenzoylformic acid in the CTAB micelle is shown in Figure 4. The k_2' values were suppressed significantly with increasing salt concentration, reaching a constant value which corresponded to $\frac{1}{3}$–$\frac{1}{20}$ of that obtained under the controlled conditions.

The apparent second-order rate constants at a given CTAB concentration (10 mM) are summarized in Table IV. Examination of the values in parentheses, which indicate the percentage recovery of flavin by oxygen reoxidation, reveals that flavin oxidation of aromatic substrates proceeds almost quantitatively, whereas that of aliphatic substrates competes with alkaline hydrolysis of the isoalloxazine ring. Table IV also indicates that, in the oxidation of aliphatic substrates such as butyraldehyde and formaldehyde, **2** (hydrophobic isoalloxazine) behaves as a more efficient oxidizing agent than **1**. The result contrasts with the previous observation that the hydrophobicity of the flavin has little influence on the oxidation of aromatic substrates. Speculating on the hydrophobicity of substrates, the partition of aliphatic substrates to the micellar phase must be smaller than that of aromatic substrates. The result implicates that the micelle effect is observed when either substrate or flavin, or both, is bound to the micellar phase.

Discussion

The kinetic and product analysis data indicate that (1) the reaction is first order in substrate and cyanide ion and zero order in flavin; (2) flavin (especially in the presence of the CTAB micelle) is able to divert the reaction from benzoin-type condensation to flavin oxidation; (3) electron-withdrawing substituent(s) facilitates the oxidation reaction. There are several precedents for the zero-order disappearance of flavin.[3,6,20-22] These systems involve commonly a slow, rate-limiting formation of a reactive intermediate followed by the rapid flavin-mediated oxidation of the intermediate. The present results, together with the previous information on benzoin-type condensation reactions,[10,11] suggest that deprotonation and decarboxylation of the cyanide adducts are rate limiting and that the subsequent flavin-mediated oxidation occurs as a trapping step. These results are summarized by

Scheme I



Schemes I and II for the oxidation of aldehydes and the oxidative decarboxylation of α-keto acids, respectively. Since the benzoin condensation reaction is second order in aldehyde,[23] the condensation process must be involved (at least partially) in the rate-limiting step. This result is consistent with the condensation being much slower than the oxidation, allowing for the oxidative "trapping" by flavin. Assuming the occurrence of flavin trapping and [CN^-] ≫ [substrate], the rate equation for the zero-order decrease in the initial stage is given by

$$v_{obsd} = k_2K[CN^-][\text{substrate}]/(1 + K[CN^-]) \quad (3)$$

where $K = k_1/k_{-1}$. Equation 3 clearly indicates that v_{obsd} is independent of the flavin concentration. Equation 3 also predicts that plots of v_{obsd} vs. cyanide ion concentration may ex-

Scheme II

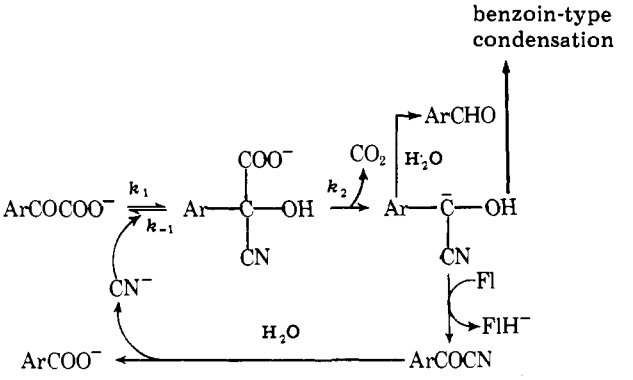


Table IV. Apparent Second-Order Rate Constants (k_2') in the Presence and Absence of the CTAB Micelle[a]

substrate	mM	$k_2' \times 10^6$, M^{-1} s^{-1}			
		1 or **3**[c]	**1** + CTAB	**2** + CTAB	**3** + CTAB
$2,4\text{-}Cl_2C_6H_3CHO$	5.0	11.3 (100%)	592 (100%)		
$4\text{-}ClC_6H_4CHO$	5.0	0.49 (100%)	107 (100%)	126 (100%)	
C_6H_5CHO	20.0	0.10 (100%)[b]	25.3 (100%)	16.8 (100%)	
C_3H_7CHO	34.0	0.01 (12%)	0.23 (4%)	0.57 (47%)	
HCHO	100	<0.001 (0%)	<0.001 (0%)	0.04 (12%)	
$2,4\text{-}Cl_2C_6H_3COCO_2H$	2.5	<0.1 (0%)			<0.1 (0%)
$4\text{-}ClC_6H_4COCO_2H$	5.0	<0.1 (0%)			275 (100%)
$C_6H_5COCO_2H$	10.0	<0.01 (0%)			1.4 (92%)
CH_3COCO_2H	30.0	<0.005 (0%)			0.05 (27%)

[a] [CTAB] = 10.0×10^{-3} M. The reaction temperature for aldehydes is 30 °C and for α-keto acids 50 °C. The value in parentheses indicates the percentage of reduced flavin. [b] 10 vol % acetonitrile. [c] **1** was used for the oxidation of aldehydes and **3** was used for the oxidative decarboxylation of α-keto acids.

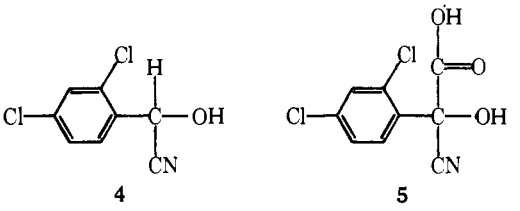
hibit a saturation phenomenon at higher cyanide ion concentrations. The plots of v_{obsd} vs. $[CN^-]$ in Figure 1 show, however, that v_{obsd} is first order in cyanide ion under the present conditions. Since $K[CN^-]$ should be less than unity, the apparent second-order rate constant (k_2') must be identical with k_2K in eq 3. Therefore, the k_2' values listed in the tables and the figures are affected by both the rate-limiting step and the equilibrium constant for the cyanide addition. Thus, two distinct factors must be considered to account for the rate enhancement.

As shown in Schemes I and II, cyanide ion acts as a catalyst to facilitate the formation of the carbanion. Since the local concentration of cyanide adducts must be enriched on the CTAB micellar surface,[24] one of the micellar effects would stem from enhancement in the equilibrium formation of the adducts.

The rate-limiting step for the oxidation of aldehydes is deprotonation from the cyanide adducts. In a previous study of this series,[20] we reported that the CTAB micelle accelerates proton abstraction from the carbon acids owing to the enhanced OH^- ion concentration around the micelle surface. Importantly, the catalytic efficiency of the micelles is suppressed with increasing salt concentrations, which is attributed to the competitive binding of anions and OH^- onto a site of the cationic head group of the surfactant.[25,26] The present system also features a high concentration of cyanide ion, which also would exclude OH^- from the micelle surface. Speculating on the fact that the rate augmentation of 100–600-fold is brought forward by the CTAB micelle, the occurrence of such salt inhibition by KCN is rather unlikely. The discrepancy may be rationalized in terms of the basicity of the cyanide ion (pK_a = 9.17[27]) which would be strong enough as a general base to abstract a proton from the carbon acids.

The micellar effect on decarboxylation has been well documented by Bunton and co-workers.[28] Cationic micelles serve as the most efficient catalysts. For example, the rate constant for the decarboxylation of 6-nitrobenzisoxazole-3-carboxylate in the presence of the CTAB micelle is greater by a factor of 95 than that for the nonmicellar decarboxylation.[28] The origin of the rate enhancement was discussed in detail by several groups who noted two noteworthy micellar effects:[28-30] one is related to the delocalized transition state being stabilized by the micellar ionic environment and the other to the carboxylate ion activated by the hydrophobic environment of micelles. Table IV indicates that decarboxylation of the cyanide adducts does not occur in the nonmicellar system, whereas in the presence of the CTAB micelle the decarboxylation rate is enhanced as much as that of the oxidation of the corresponding aldehydes. One may conclude, therefore, that the marked rate acceleration is brought about by synergistic effects, such as effective concentrations of reactants and OH^-, and localized environmental factors.

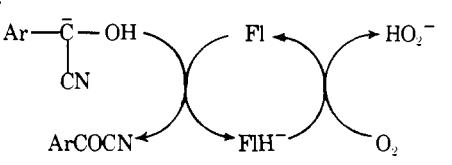
In the oxidation of aromatic aldehydes, the introduction of electron-withdrawing substituent(s) (e.g., Cl) into the aromatic ring obviously accelerates the reaction. This is consistent with the proposal that deprotonation from the cyanide adducts is rate limiting. In the oxidative decarboxylation, however, 2,4-dichlorobenzoylformic acid is unreactive even in the micellar system. The difference may be rationalized in terms of the steric effect. CPK model building indicates that the cyanide adduct of 2,4-dichlorobenzaldehyde (**4**) does not suffer any steric hindrance. This is consistent with the fact that the benzoin condensation of 2-chlorobenzaldehyde involving a similar carbanionic intermediate gives 2,2′-dichlorobenzoin in high yield.[31] In contrast, the cyanide adduct of 2,4-dichlorobenzoylformic acid (**5**), in which the hydrogen of **4** is replaced by



a carboxyl group, exhibits considerable steric hindrance between the ortho chlorine and the carboxyl group. The product analysis indicated that 2,4-dichlorobenzoylformic acid is recovered quantitatively after treatment with cyanide ion in the presence of the CTAB micelle and that no benzoin-type condensation occurs. As expected, the kinetics and the product analysis show that the steric hindrance is apparently significant enough to block nucleophilic attack of cyanide ion on the α-carbonyl group.

The product analysis data in Tables I and III suggest that molecular oxygen is unable to oxidize the carbanion intermediates in the nonmicellar system, whereas it becomes an excellent oxidizing agent of the intermediates in the micellar system. Undoubtedly, oxidation in the absence of the micelle proceeds according to the so-called ping-pong mechanism (Scheme III).[32] On the other hand, one must consider two mechanisms for the micellar system: the direct oxidation by molecular oxygen and the flavin oxidation involving the ping-pong mechanism. Although it is difficult to estimate quantitatively the relative contribution of the two pathways from the experimental data, we believe that the ping-pong mechanism takes place in preference to the direct oxidation.

Scheme III



Why molecular oxygen could oxidize the carbanions only in the micellar system is of considerable interest. We recently found that the flavin oxidation of nitroalkane carbanions, which does not proceed in nonenzymatic systems unless an electron-deficient isoalloxazine is used,[9] occurs readily with flavins bound to cationic micelles or to cationic polyelectrolytes.[7,8] It was concluded that the reaction is facilitated by the activation of the adsorbed carbanions on the cationic micelles and not by the shift of the redox potential of the flavins.[7,8,33] Therefore, the behavior of molecular oxygen may be described as follows: the carbanion, $R\bar{C}(CN)(OH)$, possesses the more positive redox potential than molecular oxygen being in a simple aqueous medium. In the cationic micellar system the opposite situation becomes true owing to the negative shift of the redox potential of the micelle-bound carbanion. The detailed mechanism of the micellar activation of the carbanion has been discussed elsewhere.[7,8,34]

In conclusion, the present work establishes that the combination of cyanide ion and cationic micelle remarkably facilitates the flavin oxidation of aldehydes and α-keto acids. Also significant is that the pattern of the flavin-mediated reaction is altered readily by changes in the reaction conditions. Although the results of the present study do not stimulate further understanding of the enzymatic mechanism, they do provide useful information concerning the interaction of flavins and carbanions in enzyme and model systems.

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Free, Hydrogen-Bonded, and Cation-Stabilized Carbanions α to a Cyano Group in a Cyclobutane Ring[1]

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Abstract: The carbanion α to the cyano group of 3-alkoxycyclobutanecarbonitrile (**3**) was prepared by three different methods: (a) addition of MeO^- in MeOH and *i*-PrO^- in *i*-PrOH to bicyclobutanecarbonitrile (**2**); (b) deprotonation of the cis and trans isomers of **3** in MeO^-–MeOH and in *i*-PrO^-–*i*-PrOH in the presence of crown ether; (c) deprotonation of **3** under ion-pairing conditions (*i*-PrONa–*i*-PrOH). The different product distribution (cis–trans ratio) obtained in each case indicates that each method yields a different type of carbanion. In method (a), a "free" carbanion whose inversion rate is faster than the reorganization of the surrounding solvent molecules is obtained. The observed cis–trans ratio of 3.5 reflects the relative ease of protonation of an equatorial position as compared to protonation of an axial one. Method (b) yields a hydrogen-bonded carbanion which exhibits some retention of configuration, while the paired sodium cation in method (c) induces equivalent amounts of retention and inversion. In the latter case, the inversion is accompanied by some isoinversion. A near-unity H/D kinetic isotope effect in the deprotonation reactions points to a preequilibrium formation of the carbanion with the subsequent step being rate limiting. The elimination of HCl from 3-chlorocyclobutanecarbonitrile under non-ion-pairing conditions is intrepreted accordingly as a monomolecular elimination from the hydrogen-bonded conjugated base ($E1cB_{hb}$).

Unless they are heavily substituted by electron-withdrawing groups, carbanions are usually encountered as short-lived intermediates. As the direct observation of a carbanion is rarely feasible, its nature is usually inferred from the rate, type, and

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