with stirring, under nitrogen at 5 °C, 0.25 ml of methyl fluorosulfonate (3.1 mmol) over a period of 15 min. The oily precipitate was stirred at 4 °C overnight to give a white solid, which was washed repeatedly with dry ether and dried under vacuum over P_2O_5. The slightly hydroscopic material decomposed in the range 60–80 °C. The NMR spectrum in CD_3CN gave the following peaks relative to Me_4Si: δ 9.1 (1 H, s), 7.9–7.5 (7 H, m), and 4.0 (3 H, s). This material was used in the kinetic studies without further purification.

Kinetic Measurements. Pseudo-first-order rate constants were determined either spectrophotometrically at 260 nm using a Cary 14 spectrophotometer or by a pH stat method using a Radiometer apparatus which included a TTT60 titrator, PHM62 pH meter, ABU12T buret, and a REC61 servograph. Stock solutions of **3** were prepared in acetonitrile and were stable for weeks when stored at −15 °C. Stock solutions of **4** were prepared in acetonitrile and used the same day. Stock solutions of **2** were prepared in Me_2SO and were used within 2 h. No solvent was found for **2** which afforded solutions stable for more than 2 h. Typically, 10–50 μl of stock solution was used to initiate the reaction. In many pH-stat runs, **2** was added as a solid directly to the reaction vessel. Results using this technique compared well with results using fresh stock Me_2SO solutions. The concentration of substrates was approximately 3×10^{-2} M for the pH-stat runs and about 2.5×10^{-4} M for the spectrophotometric runs. The pseudo-first-order rate constants were fit to the appropriate rate expression using a nonlinear least-squares computer program.

Acknowledgment. Support from the Research Corporation is gratefully acknowledged.

References and Notes

(1) "The Enzymes", Vol. III, P. D. Boyer, Ed., Academic Press, New York, N. Y., 1971.
(2) E. T. Kaiser and B. L. Kaiser, *Acc. Chem. Res.*, **5**, 219 (1972).
(3) A. J. Kirby and A. R. Fersht, *Prog. Bioorg. Chem.*, **1**, 1 (1971).
(4) R. W. Wolfenden and W. P. Jencks, *J. Am. Chem. Soc.*, **83**, 4390 (1961).
(5) W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1272 (1959).
(6) J. W. Thanassi and T. C. Bruice, *J. Am. Chem. Soc.*, **88**, 747 (1966).
(7) M. Choi and E. R. Thornton, *J. Am. Chem. Soc.*, **96**, 1428 (1974).
(8) The $pK_a^{SH^+}$ for **2** should be about the same as the first pK_a for phthalic acid, 2.98.[9]
(9) W. R. Maxwell and J. R. Partington, *Trans. Faraday Soc.*, **33**, 670 (1937).
(10) T. C. Bruice and G. L. Schmir, *J. Am. Chem. Soc.*, **80**, 148 (1958).
(11) W. N. Lipscomb et al., *Brookhaven Symp. Biol.*, **21**, 24 (1968).
(12) M. Caplow and W. P. Jencks, *Biochemistry*, **1**, 883 (1962).

Mechanism of Decarboxylation of 1,3-Dimethylorotic Acid. A Model for Orotidine 5′-Phosphate Decarboxylase

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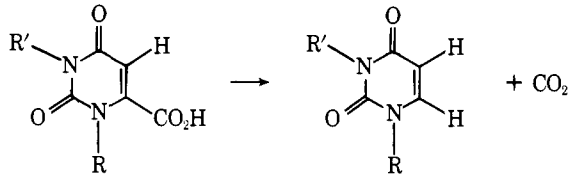
Abstract: The decarboxylation of 1,3-dimethylorotic acid (**1**) is shown to proceed by separate pH-determined pathways in sulfolane at 180–220 °C. Although a process involving ionization of **1** is the major pathway in the presence of excess base, decarboxylation is initiated by zwitterion formation in the neutral solvent. Measurements of the rate of loss of carbon dioxide from 6-carboxy-2,4-dimethoxypyrimidine (**5**) and 1-methyl-2,4-dimethoxypyrimidinium-6-carboxylate betaine (**7**) are used to estimate the equilibrium and rate constants for the zwitterionic pathway. Comparison of the rate constant for decarboxylation of **7** with k_{cat} for orotidine 5′-phosphate decarboxylase shows that the biological catalysis can be satisfactorily accounted for if the enzyme provides a site which displaces the equilibrium in favor of the zwitterionic form of orotidylic acid. It is also noted that the inhibitor 6-azauridine monophosphate, which has a greater affinity for the enzyme than does the substrate, provides a partial model for the intermediate formed on loss of carbon dioxide from the zwitterion.

The temperatures in excess of 180° which are required to promote decarboxylation of 1,3-dimethylorotic acid (**1**) to 1,3-dimethyluracil (**2**) are in sharp contrast to the ambient



1, R = R′ = CH_3 **2**, R = R′ = CH_3
3, R = *R*-5-P; R′ = H **4**, R = *R*-5-P; R′ = H

temperatures at which orotidine 5′-phosphate decarboxylase catalyzes the conversion of orotidylic acid (**3**) to uridylic acid (**4**). The enzymatic conversion of **3** to **4** is an essential step in nucleic acid biosynthesis and also appears to be biomechanistically novel. In virtually all known biochemical decarboxylations an intermediate is involved in which the electron pair remaining after breakage of the carbon–carbon bond can be stabilized by delocalization into a π orbital.[1] For decarboxylation of **3**, however, no such stabilization is apparent, and mechanisms involving intermediates with electron pairs in an sp^2 orbital orthogonal to the π system have been suggested.[2]

This report details our determination of the pathways of decarboxylation of **1**.[3] The understanding gained of that process provides a mechanism which can account for the catalytic action of the enzyme in the conversion of **3** to **4**.

Results

At 206° in sulfolane, 1,3-dimethylorotic acid (**1**) evolves carbon dioxide quantitatively in 3 h, and 1,3-dimethyluracil (**2**), the only product observable by NMR, can be isolated in 79% yield. The rate of the reaction can be followed by observation of the C-6 proton by NMR, by titration of the residual acid, or by continuous monitoring of the carbon dioxide evolved. A rate constant of 7.6 ($\pm$0.2) $\times 10^{-4}$ s^{-1} is obtained by the three methods and the reaction is first order to more than 90% completion. In the presence of *N*,*N*-diethylaniline, the observed first-order rate constant rises to a plateau of 3.1 $\times 10^{-3}$ s^{-1} as the concentration of base increases, as shown in Figure 1a.[4] If the latter value is taken as the limiting rate for decarboxylation of the carboxylate anion of **1**, reaction in the absence of added base via the same intermediate would require that **1** be ca. 25% ionized in neat sulfolane. However, conductivity studies set an upper limit of 3% on the degree of ionization of **1** in sulfolane at 206°.[6] Figure 1b shows that a

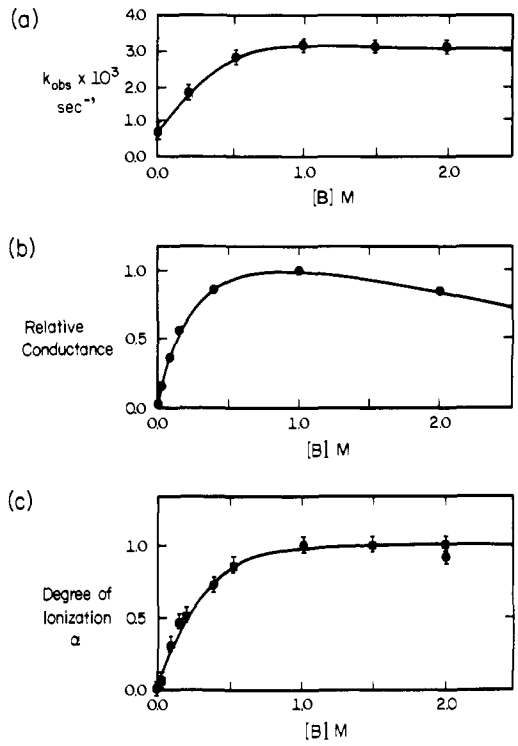


Figure 1. (a) First-order rate constant for the conversion of 0.03 M **1** to **2**. (b) Relative conductance of 0.05 M **1**. (c) The degree of ionization α as determined by conductance (●) and kinetic (■) data. All graphs are for *N,N*-diethylaniline [B] in sulfolane at 206°.

plot of the relative conductance vs. base concentration rises from essentially zero to a plateau. The conclusion which follows from these observations is that two mechanisms are operative for the decarboxylation of **1**. In the presence of base, reaction occurs via the carboxylate anion, but in neat sulfolane decarboxylation occurs through a neutral species stoichiometrically equivalent to **1**.

Both pathways provide the same net substitution. When carboxydeuterio-1,3-dimethylorotic acid is heated in sulfolane, or when the potassium salt of **1** is heated in sulfolane-*2,2,5,5-d*$_4$, 1,3-dimethyluracil-*6-d* is produced.[7] However, in the former case, an isotope effect is not observed; the ratio of rate constants for the decarboxylation of **1** and carboxydeuterio-1,3-dimethylorotic acid in neat sulfolane at 206° is 1.0 ± 0.1. Apparently symmetrical breakage of the oxygen–hydrogen bond is not important in the loss of carbon dioxide from the neutral species.[8,9]

In order to clarify the mechanisms of, and the structural requirements for, the conversion of **1** to **2**, decarboxylations of the model compounds **5**, **7**, **8**, **9**, and **10** were investigated under the conditions specified, with the results shown in Table I. The first-order rate constants are grouped according to whether reaction of a formally neutral species (k_n) or an anion (k_i) is involved. The betaine **7** is a rather unstable material obtained in a 7:1 mixture with a neutral ester. It has been characterized by NMR and its structure is established by its synthesis (Experimental Section). The significance of each case is considered in the following section.

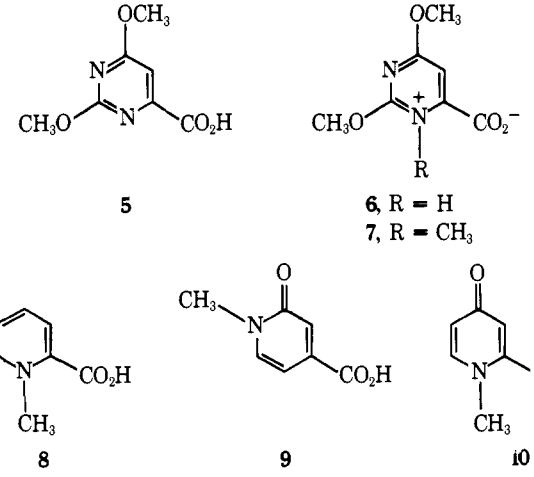
Discussion

The two mechanisms which we consider to be established by the above results as operative in the conversion of **1** to **2** are presented in Scheme I. The most straightforward process is the base-catalyzed formation of the carboxylate anion **13** which can undergo decarboxylation to **12**, which, in turn, accepts a proton to give **2**. In the presence of sufficient base to cause

Table I. First-Order Rate Constants for Decarboxylation in Sulfolane at 206°

Substrate	Rate constant, s^{-1}	
	Neutral (k_n)	Ionized (k_i)
1	7.6×10^{-4}	3.1×10^{-3} [a] 1.4×10^{-3} [b]
5	1.3×10^{-2}	N.R.[c]
7	4.3×10^{-4} (54.3°) 6.0×10^{-3} (69.8°) 5.0×10^{4} [d]	
8		1.2×10^{-3} [b]
9		N.R.[c]
10		1.7×10^{-3} (110.0°) 1.4×10^{-2} (138.5°) 3.2[d]

[a] The base is *N,N*-diethylaniline. [b] The solvent is isoquinoline. [c] The compound does not lose carbon dioxide even on heating to 300° in isoquinoline. [d] Extrapolated from the measured values by means of the Arrhenius equation.



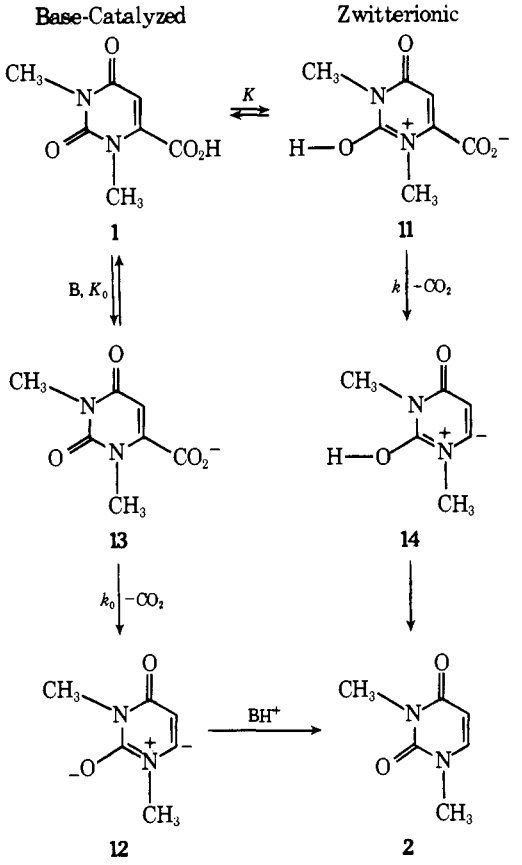
5 **6**, R = H **7**, R = CH_3

8 **9** **10**

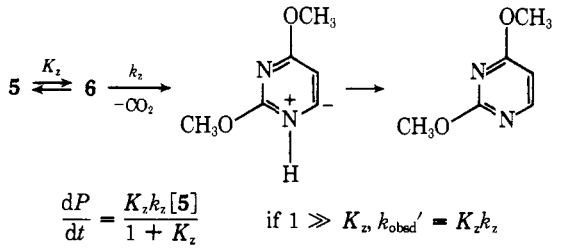
essentially complete ionization of **1**, the reaction proceeds by the base-catalyzed process only. The intermediate produced on loss of carbon dioxide is **12**, a dipole stabilized carbanion which finds analogy in the intermediates postulated in base-catalyzed hydrogen–deuterium exchanges of the pyridones and pyrimidones.[10,11] The importance of dipole stabilization in the transition state for decarboxylation is illustrated by the fact that the carboxylate anions of **1**, **8**, and **10**, which can form dipole-stabilized carbanions by loss of carbon dioxide, react readily in isoquinoline at 206°. On the other hand, the carboxylate anions of **5** and **9**, which cannot form such species, do not decarboxylate under the same conditions (Table I).

The observed first-order rate constant for the decarboxylation of **1** by the base-catalyzed process (k_{obsd}) is equal to αk_0, where k_0 is the rate constant for loss of carbon dioxide from the carboxylate anion and α is the fraction of ionization of **1**.[5,12] The shape of the curve in Figure 1a is consistent with an increase in α to essentially unity as the concentration of base increases. The value of α as a function of base concentration can be calculated from k_{obsd}, k_0, and k_0', where k_0' is the observed first-order rate constant for reaction of the neutral species. The expression is $\alpha = (k_{obsd} - k_0')/(k_0 - k_0')$, where k_0 is 3.1×10^{-3} s^{-1} and k_0' is 7.6×10^{-4} s^{-1}. An independent value of α as a function of base concentration can be obtained from the conductance data if the limiting conductance at high base concentration is assumed to represent complete ionization.[13] The close correspondence of the base-dependent degree of ionization as measured in these two different ways is shown

Scheme I



Scheme II



in Figure 1c and provides further support for the ionization mechanism in Scheme I.[14]

The mechanism shown in Scheme I for the decarboxylation of formally neutral **1** involves initial formation of the zwitterion **11,** which rapidly loses carbon dioxide to give **14,** which leads to **2** by proton transfers. The intermediacy of the zwitterion is consistent with the lack of a deuterium kinetic isotope effect on the reaction and is supported by the following analysis of the mechanism of decarboxylation of **5.**

Since the carboxylate anion of **5** does not lose carbon dioxide, its decarboxylation is postulated to occur via the zwitterion **6,** as shown in Scheme II, in analogy to the mechanism of decarboxylation of the 2-picolinic acid.[15] Kinetic analysis for this sequence, which appears after the scheme, shows that the observed first-order rate constant k_{obsd}' is composed of two terms: K_z, the equilibrium constant for zwitterion formation, and k_z, the rate constant for loss of carbon dioxide from **6**.[3,16] The ratio of the observed first-order rate constants for the decarboxylation of **1** and **5** is equal to $Kk:K_zk_z$ from Schemes I and II, respectively. If it is assumed that $k:k_z$ will have a ratio of ca. one, and if the ratio $K:K_z$ is estimated as 10^{-3} from the ratio of basicities of 1-methyl-2-pyridone and 2-methoxypyridine,[17] an estimated first-order rate constant of 1.3×10^{-5} s^{-1} is obtained for the reaction of **1** by the zwitterionic mechanism. Although this estimated rate constant is clearly an approximation,[18] it is sufficiently close to the observed first-order rate constant for the decarboxylation of **1** of 7.6×10^{-4} s^{-1} to support the proposed mechanism.

An implicit assumption in this analysis is that the proton positions are as indicated for **6** and **11.** Uracil and its N-methylated derivatives are reported to be mostly monoprotonated on the C-4 oxygen,[18a,19] although ca. 3% protonation occurs at the C-2 oxygen of uracil.[19b] The carboxylate at C-6 could reasonably promote protonation as shown for **11,** but we do not have evidence on that point. Even if protonation of **1** occurred at the C-4 oxygen, a positive charge would be induced at N-1 and decarboxylation would still be promoted, although the quantitative comparison with **5** would then be more problematical.

The relative values of the observed first-order rate constants for decarboxylation of **1** at 206° in sulfolane, tetraglyme, and 1 M sulfuric acid are 1.0:1.3:0.36.[20] Since the observed rate constant for the reaction is equal to the product of an equilibrium constant and a rate constant, and since both the equilibrium constant for formation[10,21] of **11** and the rate constant for decarboxylation[10,22] of **11** might be expected to vary by orders of magnitude with these solvent changes, the lack of a significant solvent effect is considered to reflect compensatory changes. Apparently, as solvent polarity increases, the equilibrium constant for zwitterion formation increases, but the rate constant for decarboxylation of the zwitterion decreases correspondingly. On the other hand, 5-haloorotic acids undergo decarboxylation at lower temperatures than the *N*-methyl or unsubstituted acids.[23] In those cases, the overall rate is apparently influenced by inductive stabilization of negative charge either in the carboxylate, in the zwitterion, or in the transition state for loss of carbon dioxide.

An important question is whether the processes outlined in Scheme I can provide understanding of the action of orotidine 5′-phosphate decarboxylase (EC 4.1.1.23).[24] The conversion of **3** to **4** catalyzed by this enzyme appears to follow the classical enzyme-substrate mechanism, although there is evidence for bimodal behavior at low substrate concentrations.[25b] The active site of orotidine 5′-phosphate decarboxylase remains undefined, although no cofactors or metal ions seem required for activity.[24,25] The fact that thiamine is not a cofactor is significant, since a process of nucleophilic addition to C-6[26a] followed by a decarboxylation analogous to that of the α-keto oxidases[26b] can readily be envisioned. On the other hand, addition of a thiol function which could also provide stabilization for a subsequent decarboxylation cannot be ruled out, although the addition of lauryl mercaptan did not produce any rate enhancement for the decarboxylation of **1.** The possibility of trace metal ion catalysis is also difficult to preclude for the enzymatic reaction. In the nonenzymatic reaction, it was found that Cu^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Cr^{3+}, and Hg^{2+} either had no effect or induced a slight retardation on the decarboxylation of **1.**

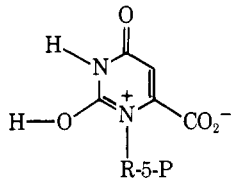
If it is assumed that the enzyme has a molecular weight of 51 000[24] and one active site, the published values of V_{max} can be used to calculate k_{cat}, the first-order rate constant for the conversion of the enzyme–substrate complex to products. That value, which is obviously provisional, is the first entry in Table II. Comparison of k_{cat} with the extrapolated rate constant for decarboxylation of **1** at 37°, which is the third entry in Table II, shows a rate enhancement for the enzyme-catalyzed reaction of more than 10^{12}. While that comparison is for different substrates and could be in error by as much as a few orders of magnitude, the difference in rates between the catalyzed and uncatalyzed reaction is clearly enormous. The second entry in Table II is the extrapolated first-order rate constant for decarboxylation of the betaine **7** at 37°. The fact that this rate constant can account for 10 of the 12 powers of 10 difference between the nonenzymatic reaction of **1** and the enzymatic reaction of **3** strongly suggests that the primary function of the

Table II. First-Order Rate Constants for the Decarboxylation of Orotic Acid Derivatives at 37°

Substrate	Rate constant, s^{-1}	Relative rate
3	2×10^{-3} [a]	4×10^{12}
7	2×10^{-5} [b]	4×10^{10}
1	5×10^{-14} [c]	1

[a] From values of 2.5×10^{-3} and 1.7×10^{-3} s^{-1} for water solution with orotidine 5′-phosphate decarboxylase from two different sources, ref 25. [b] Extrapolated from measurements in sulfolane at 54 and 70 °C. [c] Extrapolated from measurements in sulfolane at 206 and 226 °C.

enzyme could be to increase the concentration of the zwitterion of **3**.[27] Specifically, we propose that the enzyme functions by providing a binding site which greatly displaces the equilibrium between the uncharged and zwitterionic form of orotidylic acid in favor of the zwitterion (**15**). The transition state for the



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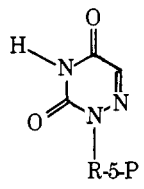

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subsequent decarboxylation would then resemble the intermediate **14** in Scheme I. In fact, 6-azauridine monophosphate (**16**), which resembles **14** in possessing a pair of electrons in a formally sp^2 orbital at C-6, is bound by orotidine 5′-phosphate decarboxylase approximately 10 times more strongly than is the substrate orotidine monophosphate, in accord with the tenets of "transition state analogue" binding.[25a,28,29] In view of the current interest in compounds which inhibit orotidine 5′-phosphate decarboxylase,[25a,29,30] it appears that the proposed mechanism of enzyme action should be useful as a rational basis for further investigation of both the enzyme and its inhibitors.

Experimental Section[31]

Solvents. *N,N*-Diethylaniline and isoquinoline were purified by vacuum distillation from calcium hydride. Sulfolane[32] and tetraglyme[33] were purified by previously reported procedures. Sulfolane-d_4 with 84% deuterium incorporation at the α hydrogens (NMR) was prepared by exchange with deuterium oxide catalyzed by potassium hydroxide.

1,3-Dimethylorotic acid (1) was prepared by a two-step procedure. Methyl orotate[20a] was allowed to react with methyl iodide in dimethyl sulfoxide containing carbonate to give 53% methyl 1,3-dimethylorotate: mp 77–78 °C; NMR (Me_2SO-d_6) δ 6.2 (s, 1, H_5), 3.9 (s, 3, CH_3O), 3.4 (s, 3, CH_3N), 3.2 (s, 3, CH_3N). Hydrolysis of this ester with equimolar potassium hydroxide in water at room temperature gave 65% **1**: mp 153–154 °C, mmp 151–153 °C (lit.[20a,34] 148–151 °C); NMR (D_2O) δ 6.0 (s, 1, H_5), (s, 3, CH_3N), 3.2 (s, 3, CH_5N); NMR (sulfolane) δ 10.2 (s, 1, acid); 6.2 (s, 1, H_5); the region above δ 4 was obscured by the solvent; ir (KBr) 3200–2000, 1726, 1697, 1654 cm^{-1}; mass spectrum (70 eV) *m/e* (rel intensity) 184 (50), 155 (5), 154 (3), 140 (2), 139 (2), 127 (5), 109 (3), 99 (3), 83 (10), 82 (100), 81 (6).

Anal. Calcd for $C_7H_8N_2O_4$: C, 45.65; H, 4.38; N, 15.21. Found: C, 45.46; H, 4.26; N, 15.45.

1,3-Dimethylorotic acid-*d* was prepared by repetitive exchange with deuterium oxide, mp 152–154 °C, NMR (sulfolane) δ 6.2 (s, 1, H_5); no absorption due to the carboxylic acid hydrogen could be observed, and the region above δ 4 is obscured by the solvent.

Anal. Calcd for $C_7H_7DN_2O_4$: C, 45.41; H(D), 4.89; N, 15.13; D, 12.5 atom %. Found: C, 45.68; H(D), 4.66; N, 15.00; D, 12.15 atom %.

2,4-Dimethoxypyrimidine-6-carboxylic acid (5) was prepared in 65% yield by allowing 2,4-dichloro-6-carbomethoxypyrimidine and sodium methoxide to react in hot methanol. Purification by sublimation gave **5**: mp 164–166 °C (lit.[35] 165 °C); NMR (Me_2SO-d_6) δ 9.7 (br s, 1, acid), 7.0 (s, 1, H_5), 3.9 (s, 6, 2 OCH_3); ir (KBr) 3100–2800, 1713 cm^{-1}; mass spectrum (70 eV) *m/e* (rel intensity) 184 (100), 183 (64), 169 (5), 165 (8), 155 (24), 154 (128), 151 (5), 140 (48), 139 (40), 138 (30), 137 (8), 136 (48), 126 (5), 125 (32), 124 (12), 121 (12), 111 (5), 110 (12), 109 (24), 108 (40), 96 (8), 95 (16), 93 (32), 82 (52), 81 (12).

Anal. Calcd for $C_7H_8N_2O_4$: C, 45.65; H, 4.38; N, 15.21. Found: C, 45.88; H, 4.47; N, 15.38.

1-Methyl-2,4-dimethoxy-6-carbomethoxypyrimidinium Fluorosulfonate. To a stirred solution of 2.60 g (13.1 mmol) of 2,4-dimethoxy-6-carbomethoxypyrimidine[36] in 10 ml of ethylene dichloride under a nitrogen atmosphere was added 1.20 ml (14.8 mmol) of methyl fluorosulfonate. The reaction was heated at reflux (100°) for 2 h, then cooled to −20° to promote crystallization of the product. Vacuum filtration and air drying in a nitrogen atmosphere yielded 2.3 g (56%) of a white salt, 1-methyl-2,4-dimethoxy-6-carbomethoxypyrimidinium fluorosulfonate: NMR (CD_3CN) δ 7.1 (s, 1, H_5), 4.4 (s, 3, CH_3), 4.3 (s, 3, CH_3), 4.0 (s, 3, CH_3), 3.9 (s, 3, CH_3).

A structure proof, which establishes the location of the *N*-methyl, is based on its chemical degradation to 1-methyluracil. An aqueous solution of 0.9 M (4.5 mmol) of the salt at room temperature was allowed to stand for 4 days at pH 2. Filtration gave 0.70 g (3.9 mmol, 87%) of methyl 1-methylorotate: NMR (Me_2SO-d_6) δ 6.0 (s, 1, H_5), 3.8 (s, 3, CH_3O), 3.2 (s, 3, CH_3N). A solution of 0.45 g (2.5 mmol) of this ester was heated at reflux in 10% hydrochloric acid for 90 min. Upon cooling, 0.37 g (2.2 mmol, 88%) of 1-methylorotic acid was collected: mp 256–257 °C (lit.[37] 257 °C); NMR (Me_2SO-d_6) δ 5.9 (s, 1, H_5), 3.3 (s, 3, C H_3N) [lit.[37] (Me_2SO-d_6) δ 5.96 (s, 1, H_5), 3.32 (s, 3, CH_3N)]. In an argon atmosphere, 200 mg (1.2 mmol) of 1-methylorotic acid was heated to 280° for about 20 min until gas evolution ceased and 100 mg (67%) of a white solid had sublimed onto the walls of the test tube. The solid was collected and shown to be 1-methyluracil: mp 231–232 °C (lit.[38] 232 °C); NMR (Me_2SO-d_6) δ 7.6 (d, J = 8 Hz, 1, H_6), 5.5 (d, 1, H_5), 3.2 (s, 3, CH_3N). 1-Methyluracil has also been identified by x-ray crystallography,[39] and 3-methyluracil has a reported mp of 174–176 °C.[20a,38]

1-Methyl-2,4-dimethoxypyrimidinium-6-carboxylate Betaine (7) in a 7:1 Mixture with a Neutral Ester. To a solution of 312 mg (1.00 mmol) of 1-methyl-2,4-dimethoxy-6-carbomethoxypyrimidinium fluorosulfonate in 5 ml of deionized water was added 138 mg (1.00 mmol) of solid potassium carbonate in three portions. The reaction mixture was stirred for 5 min at room temperature, then passed through a 3 × 40 cm column of Amberlite MB-3 deionizing resin and collected in a single 100-ml aqueous fraction. The solution was lyophilized to a powder, which was then dissolved in methylene chloride and evaporated to dryness at 15–20 °C to yield a white, solid mixture of 1-methyl-2,4-dimethoxypyrimidinium-6-carboxylate betaine and an ester, either 1-methyl-4-methoxy-6-carbomethoxy-2-pyrimidone or 1-methyl-2-methoxy-6-carbomethoxy-4-pyrimidone. The betaine resulted from hydrolysis of the carbomethoxy group, whereas the ester is the product of hydrolysis of one of the ring methoxy groups: NMR (CD_3CN) δ 6.7 (s, betaine H_5), 6.3 (s, ester H_5), 4.4 to 3.4 (six overlapping methyl singlets). The correspondence between each compound and its respective NMR absorptions for H_5 was determined by heating the solution in the NMR tube for 10 min at 110°, then comparing the NMR spectra. The decarboxylation product of the betaine was identified by the H_6 and H_5 doublets at δ 7.7 and 5.9, respectively, with J_{56} = 7 Hz. The signal of the ester was unaltered. If the betaine is prepared in this manner, the composition of the product mixture varies from 1:1 to 7:1, betaine to ester. If the volume of the solution that is lyophilized is less than 70 ml, only the ester can be isolated. Upon standing in a dry nitrogen atmosphere at room temperature, the solid product isomerizes completely to the ester with a half-life of about 2 weeks. All kinetic experiments were carried out on a freshly prepared sample containing 7:1 betaine to ester, as determined by NMR.

***N*-Methyl-2-pyridone-6-carboxylic acid (8)** was prepared in 69% yield from methylammonium acetate and 2-pyrone-6-carboxylic acid:[40] mp 256–258 °C [lit.[41] (methanol) 247–248 °C]; NMR (Me_2SO-d_6) δ 8.1 (dd, 1, J_{34} = 7 Hz, J_{45} = 9 Hz, H_4), 7.5 (dd, 1, J_{35} = 1 Hz, H_3), 6.7 (dd, 1, H_5), 3.6 (s, 3, CH_3N); ir (KBr) 3100–1800, 1703, 1624 cm^{-1}; mass spectrum (70 eV), *m/e* (rel intensity) 153 (100), 125 (12), 124 (12), 109 (28), 108 (74), 107 (7), 106 (13), 95

Table III. Relative Conductance of 0.03 M 1,3-Dimethylorotic Acid in Sulfolane as a Function of *N,N*-Diethylaniline Concentration

N,N-Diethylaniline, M	Relative conductance			
	50°	100°	150°	206°[a]
0.00	0.01	0.03	0.01	0.01
0.02	1.62	0.71	0.43	0.06
0.09	1 81	1.07	0.72	0.28
0.15	1.63	1.13	0.82	0.44
0.40	1.39	1.16	1.01	0.72
1.00	1.00	1.00	1.00	1.00
2.00	0.69	0.75	0.79	0.85

[a] Extrapolated values with estimated error of ±0.1.

(11), 81 (10), 80 (18), 79 (9), 78 (6).

Anal. Calcd for $C_7H_7NO_3$: C, 54.90; H, 4.61; N, 9.15. Found: C, 54.81; H, 4.63; N, 9.22.

***N*-Methyl-2-pyridone-4-carboxylic acid (9)** was prepared in 40% yield by basic ferricyanide oxidation of *N*-methyl-4-carbomethoxypyridinium iodide:[42] mp 260–263 °C [lit.[42a] (methanol) 254 °C]; NMR (Me_2SO-d_6) δ 13.3 (br s, 1, RCO_2H), 7.8 (d, 1, J_{56} = 7 Hz, H_6), 6.8 (d, 1, J_{35} = 2 Hz, H_3), 6.5 (dd, 1, H_5), 3.4 (s, 3, CH_3N); ir (KBr) 2700–1900, 1708, 1647 cm^{-1}; mass spectrum (70 eV) *m/e* (rel intensity) 153 (100), 125 (20), 108 (47), 81 (5), 80 (9).

Anal. Calcd for $C_7H_7NO_3$: C, 54.90; H, 4.61; N, 9.15. Found: C, 55.19; H, 4.56; N, 9.36.

***N*-Methyl-4-pyridone-2-carboxylic acid (10)** was prepared in 34% yield from 4-pyrone-2-carboxylic acid[40] and 40% aqueous methylamine by a procedure similar to that used for 1,3,5-trimethyl-4-pyridone.[11a] The product was precipitated by concentration of the aqueous reaction solution and purified by treatment with Darco and recrystallization from water: mp 192–193 °C dec; NMR (D_2O) δ 8.2 (d, 1, J_{56} = 7 Hz, H_6), 7.2 (d, 1, J_{35} = 2 Hz, H_3), 7.0 (dd, 1, H_5); 4.1 (s, 3, CH_3N); ir (KBr) 2600–2200, 1690, 1630 cm^{-1}; mass spectrum (70 eV) *m/e* (rel intensity) 153 (50), 125 (5), 110 (5), 109 (100), 108 (23), 82 (7), 81 (19), 80 (15).

Anal. Calcd for $C_7H_7NO_3$: C, 54.90; H, 4.61; N, 9.15. Found: C, 54.83; H, 4.57; N, 9.07.

Quantitative Decarboxylation Reactions. Precisely 1.00 mmol of carboxylic acid was added to 3 ml of solvent over which a stream of nitrogen was passed. The nitrogen was bubbled through two saturated barium hydroxide solutions in series. The reaction mixture was heated at 206° for 3 h with a continuous nitrogen purge. The barium carbonate precipitate was collected by filtration, washed with deionized water, acetone, and chloroform. The filtrate was dried at 105°, weighed, washed with 10% hydrochloric acid, oven dried, and reweighed. Results were consistently accurate to within ±2%. Carbon dioxide yields are 99.5% (**1**), 99.4% (**5**), 98.6% (**8**), 0.4% (**9**), and 100.5% (**10**), in sulfolane. No detectable carbon dioxide evolution is observed when **5** is heated in isoquinoline.

Decarboxylation in Sulfolane-d_4. A suspension of 22 mg (0.1 mmol) of potassium 1,3-dimethylorotate in 0.6 ml of sulfolane-d_4 (84% isotopically pure) in a sealed NMR tube was heated at 220° for 30 min: NMR δ 7.3 (d, 0.17, J_{56} = 8 Hz, H_6), 5.6 (large singlet superimposed on a small doublet, 1.0, H_5). The calculated deuterium incorporation at C-6 is 83%.

Conductivity Measurements. All conductance experiments were carried out under a nitrogen atmosphere in a cell constructed of Pyrex glass and unplatinized platinum electrodes. All leads in contact with the solution in the cell were platinum. The inverse resistivity of each solution was recorded directly from a Radiometer conductivity meter, CDM2C. Readings were shown to be precise within 5% by reported measurement. The cell temperature was maintained to ±1° in the range of 50–150 °C. Relative conductances are presented in Table III.

Kinetic Measurements. The rate of evolution of carbon dioxide was followed manometrically with apparatus analogous to that of Martin and Timberlake.[43] The constant-temperature bath was held to within ±1° in the range of 230°. Each reaction was followed through approximately 8 half-lives to obtain the infinity pressure reading. Data were collected over the first 3 half-lives. The first order rate constants were calculated from a least-squares analysis of the slope of a graph of ln ($P - P_t$) vs. time.[44] The correlation coefficient for each kinetic plot was 0.999 or greater.[45] All data analysis was carried out with a Digital Corp. PDP-8E computer and typical data are supplied in Table IV. In an individual run the observed first-order rate constants typically have a standard deviation of approximately 5%.

Table IV. First-Order Rate Analysis of a Typical Set of Decarboxylation Data for 1,3-Dimethylorotic Acid in Sulfolane at 206°

Reading, P	Time, min	Log ($P - P_t$), first-order
2.0	0.0	1.857
7.3	1.4	1.824
13.4	3.6	1.783
19.9	6.0	1.733
25.4	8.4	1.686
30.6	11.0	1.638
35.7	13.8	1.583
39.5	16.6	1.538
45.5	20.2	1.455
51.3	25.8	1.356
54.8	30.0	1.284
60.3	37.6	1.137
64.5	45.6	0.978
74.0	∞	

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References and Notes

(1) (a) T. C. Bruice and S. Benkovic, "Bioorganic Mechanisms", Vol. 2, W. A. Benjamin, New York, N.Y., 1966, pp 188–194; (b) M. L. Bender, "Mechanisms of Homogeneous Catalysis from Protons to Proteins", Wiley-Interscience, New York, N.Y., 1971, pp 165–175, 586–594.
(2) P. Beak and R. N. Watson, *Tetrahedron*, **27**, 953 (1971).
(3) P. Beak and B. Siegel, *J. Am. Chem. Soc.*, **95**, 7919 (1973), provides a preliminary report of some of this work.
(4) Similar results are observed with isoquinoline in sulfolane and dicyclohexylamine in tetraglyme.[5] The decarboxylation of **1** does not appear to be catalyzed by acid.[5]
(5) Details are available: B. Siegel, Ph.D. Thesis, University of Illinois, 1974. Available from University Microfilms, Ann Arbor, Mich. 48106.
(6) Sulfolane is a very poor solvent for ionization of acids. J. Coetzee and R. Bertozzi, *Anal. Chem.*, **45**, 1064 (1973).
(7) Controls establish that 1,3-dimethyluracil-*6-d* is not produced by exchange of the product under these conditions.[5]
(8) The maximum isotope effect for symmetrical breakage of the OD bond at 206° would be 4.4: K. Wiberg, *Chem. Rev.*, **55**, 713 (1955); F. Westheimer, *ibid.*, **61**, 265 (1961).
(9) Deuterium isotope effects on decarboxylation of some β-aryl-β,γ-olefinic acids at 187° are reported to vary from 1.84 to 3.00 as a function of the substituent in the aromatic ring. On the other hand, a similar study of β-aryl-β-keto acids at 50° shows a variation from 0.85 to 2.85 for the same substituents: C. Swain, R. Bader, R. E. Steve, and R. Griffin, *J. Am. Chem. Soc.*, **83**, 1951 (1961); D. Bigley and J. Thurman, *J. Chem. Soc. B*, 436 (1968).
(10) P. Beak and R. Farney, *J. Am. Chem. Soc.*, **95**, 4771 (1973).
(11) (a) P. Beak and J. Bonham, *J. Am. Chem. Soc.*, **87**, 3365 (1965); (b) P. Beak and E. M. Monroe, *J. Org. Chem.*, **34**, 589 (1969). The difference in the rates of decarboxylation of **8** and **10** is in the same direction as the relative rates of exchange of the parent pyridones at C-6. (c) J. A. Rabi and J. J. Fox, *J. Am. Chem. Soc.*, **95**, 1628 (1973).
(12) In this analysis [**1**] and [**11**] are kinetically equivalent and α is [**13**]/([**13**] + [**1**]).
(13) D. MacInnes, "The Principles of Electrochemistry", Reinhold, New York, N.Y., 1939, Chapter 3.
(14) Kinetic analysis of the reactions in Scheme I allows an analytical determination of the extent to which the carboxylate anion might contribute to the decarboxylation in pure sulfolane. For the base-catalyzed mechanism in Scheme I, it can be derived that $k_{obsd} = [(k_0 - k_0')K_a]^{1/2}[(k_0 - k_{obsd})/C_0]^{1/2} + k_0'$, where C_0 is the initial concentration of **1**. A plot of k_{obsd} vs. $[(k_0 - k_{obsd})/C_0]^{1/2}$ over the concentration range of 0.01 to 0.25 M **1** gives an intercept of 7.6 (±0.5) × 10^{-4} s^{-1}. This value may be used to calculate the fraction of ionization (α) over this concentration range as 0.00 ± 0.02, or not distinguishable from zero.[5]
(15) (a) For an analogy see P. Haake and J. Mantecon, *J. Am. Chem. Soc.*, **86**, 5230 (1964); J. Zoltewicz, C. Smith, and J. Meger, *Tetrahedron*, **24**, 2269 (1968); H. Quast and E. Schmitt, *Justus Liebigs Ann. Chem.*, **732**, 43 (1970); G. E. Dunn, G. K. Lee, and H. Thimm, *Can. J. Chem.*, **50**, 3017 (1972); R. J. Moser and E. V. Brown, *J. Org. Chem.*, **37**, 3938 (1972); R. G. Button and

P. J. Taylor, *J. Chem. Soc., Perkin Trans. 2*, 557 (1973). (b) An alternative possibility is that proton transfer to nitrogen is concerted with the loss of carbon dioxide. Such a concerted transfer is structurally not possible for **1**.
(16) If the value of k_z for **5** at 206° is estimated at 5×10^4 s^{-1} (from an extrapolation for **7**, Table I), K_z for **5** may then be calculated to be 2.6×10^{-7}.
(17) A. R. Katritzky, Ed., "Physical Methods in Heterocyclic Chemistry", Vol. I, Academic Press, New York, N.Y., 1963, pp 63–108. The pK_a's are 1-methyl-2-pyridone, 0.32, and 2-methoxypyridine, 3.28. The use of aqueous pK_a data is justified by studies which show that weak oxygen and nitrogen bases have approximately the same relative pK_a's in water and sulfolane: E. M. Arnett and C. Douty, *J. Am. Chem. Soc.*, **86**, 409 (1964).
(18) (a) The pK_a of 1,3-dimethyluracil is −2.63: G. D. Frederick and C. D. Poulter, *J. Am. Chem. Soc.*, **97**, 1797 (1975). (b) 2-Methoxypyrimidine appears to have a pK_a < 1: A. Albert and H. N. Phillips, *J. Chem. Soc.*, 1294 (1956).
(19) (a) C. D. Poulter and R. Anderson, *Tetrahedron Lett.*, 3923 (1972), and references cited therein; (b) C. D. Poulter and G. D. Frederick *ibid.*, 2171 (1975).
(20) (a) In 1.0 M sulfuric acid no more than 7% of **1** should be ionized to **13**; the pK_a of **1** is 0.97 and 1.0 M sulfuric acid has an H_0 value of −0.25: J. Fox, N. Young, and I. Wempen, *Biochem. Biophys. Acta*, **23**, 295 (1957); M. Paul and F. Long, *Chem. Rev.*, **57**, 13 (1957). (b) Less than 1% of **1** should be protonated if the pK_b of **1** is approximately −1.7, that of 1-methyl-2-pyridone-5-carboxylic acid (ref 17). Accordingly, the rate obtained in 1.0 M sulfuric acid is considered to be that of the neutral species.
(21) R. Green and H. Tong, *J. Am. Chem. Soc.*, **78**, 4896 (1956); H. Stephenson and H. Spooner, *ibid.*, **79**, 2050 (1957).
(22) D. Kemp and K. Paul, *J. Am. Chem. Soc.*, **92**, 2553 (1970).
(23) J. Filip and F. Vysata, *J. Labelled. Compd*, **5**, 295 (1970).
(24) I. Lieberman, A. Kornberg, and E. Simms, *J. Am. Chem. Soc.*, **76**, 2844 (1954); K. Unezy, T. Amaga, A. Yoshimoto, and K. Tomita, *J. Biochem. (Tokyo)*, **70**, 249 (1971), and references cited therein.
(25) (a) S. Appel, *J. Biol. Chem.*, **243**, 392 (1968); (b) J. Fyfe, R. Miller, and T. Krenitsky, *ibid.*, **248**, 380 (1973).
(26) (a) R. W. Erickson and E. G. Sander, *J. Am. Chem. Soc.*, **94**, 2086 (1972); Y. Wataya, H. Hayatsu, and Y. Kawazoe, *ibid.*, **94**, 8927 (1972), and references cited therein; (b) J. Crosby and G. E. Llenhard, *ibid.*, **92**, 5707 (1970); ref 1a, pp 214–223; ref 1b, pp 154–155.
(27) The assumption of this comparison is that k_{cal} is rate limiting. The possibility that other processes are rate limiting cannot be excluded: W. W. Cleland, *Acc. Chem. Res.*, **8**, 145 (1975).
(28) R. Wolfenden, *Acc. Chem. Res.*, **5**, 10 (1972); W. P. Jencks, "Catalysis in Chemistry and Enzymology", McGraw-Hill, New York, N.Y., 1969, p 300.
(29) C. C. Cheng and B. Roth, *Prog. Med. Chem.*, **7**, 285 (1970).
(30) A. Conn, W. Creasy, and P. Calabrrest, *Cancer Res.*, **27**, 618 (1967).
(31) Uncorrected melting points were determined in open capillaries in a Thomas Hoover melting point apparatus. NMR and ir spectra were recorded on a Varian Associates T-60 spectrometer and a Perkin-Elmer 521 spectrophotometer, respectively, by Mr. R. Thrift and associates or by the authors. Mass spectra were determined on a Varian Associates MAT CH-5 spectrometer by Mr. J. Wrona. Satisfactory elemental analyses, which were performed by Mr. J. Nemeth and associates, were obtained for all compounds used in the kinetic and conductance experiments. Temperatures were measured with National Bureau of Standards calibrated thermometers.
(32) J. Coetzee, J. Simon, and R. Bertozzi, *Anal. Chem.*, **41**, 766 (1969).
(33) A. Vogel, *J. Chem. Soc.*, 616 (1948).
(34) W. Corran and R. Angler, *J. Org. Chem.*, **31**, 201 (1966).
(35) W. Klotzer, *Monatsh. Chem.*, **87**, 527 (1956).
(36) H. Gershan, *J. Org. Chem.*, **27**, 3507 (1962).
(37) R. Warrener and E. Cain, *Aust. J. Chem.*, **24**, 785 (1971).
(38) D. Brown, E. Hoerger, and S. Mason, *J. Chem. Soc.*, 211 (1955).
(39) H. Sobell and K. Tomita, *Acta Crystallogr.*, **17**, 122 (1964); D. Green, F. Mathews, and A. Rich, *J. Biol. Chem.*, **237**, 3573 (1962).
(40) K. Heyns and G. Vogelsang, *Chem. Ber.*, **87**, 1440 (1954).
(41) E. Spath and G. Foller, *Chem. Ber.*, **56**, 88 (1923).
(42) (a) M. Frank and W. Moches, *J. Org. Chem.*, **24**, 196 (1959); (b) J. Supniewski and M. Serafinowa, *Arch. Chem. Farm.*, **3**, 109 (1936).
(43) J. Martin and J. Timberlake, *J. Am. Chem. Soc.*, **92**, 987 (1970).
(44) W. Youden, "Statistical Manual for Chemists", Wiley, New York, N.Y., 1951, pp 40–45.
(45) H. Young, "Statistical Treatment of Experimental Data", McGraw-Hill, New York, N.Y., 1962, pp 126–131.

Kinetics and Mechanism of the Outer-Sphere Oxidation of Cyclohexanone by Tris(polypyridyl) Complexes of Iron(III) and Ruthenium(III)[1]

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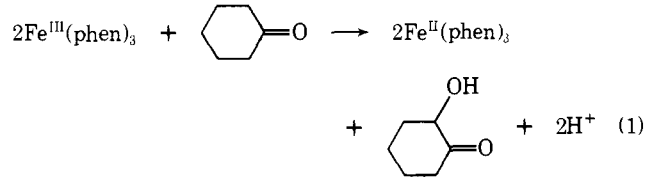
Abstract: Oxidation of cyclohexanone by a number of substituted 1,10-phenanthroline and 2,2′-bipyridyl complexes of Fe(III) and Ru(III) were found to be first order in cyclohexanone and first order in the metal complex in 1 M H_2SO_4. A linear correlation between the logarithms of the second-order rate constants and standard reduction potentials of the metal complexes were found. The slope of the linear free energy plot was that predicted by Marcus for outer-sphere electron transfer between a similar series of reactions. At low acidity and high [Fe(III)], a rate which was first order in [H^+], first order in [cyclohexanone], and zero order in [Fe(III)] was found and the rate constant closely resembled the acid-catalyzed enolization rate constant for cyclohexanone. These kinetics are consistent only with electron transfer from the enol rather than the ketone form of cyclohexanone. The data for the Ru(III) complexes fell on the same linear free-energy plot as the Fe(III) complexes, indicating the size of the d orbitals of the metal oxidant is not an important factor in determining the rate of oxidation. We interpret this result in terms of electron transfer via the periphery of the polypyridyl ring.

Mechanisms of oxidation of ketones by metal ions and complexes are divided into two classes, depending on whether the keto or the enol is oxidized in the rate-determining step. Oxidation of cyclohexanone by one-equivalent oxidants such as Co(III), Ce(IV), V(V), and Mn(III) were postulated to occur via the keto tautomer while two-equivalent oxidants, such as Hg(II), Tl(III), and Mn(VII), apparently oxidize the enol tautomer.[2] Relative rates of enolization and oxidation, isotope effects, and kinetic orders were used to differentiate between oxidation of the keto or the enol tautomer. More recently Mn(III) was postulated to attack the enol form of ketones in acetic acid medium.[3]

Littler reported that the oxidation of cyclohexanone by tris(1,10-phenanthroline)iron(III) (ferriin), occurred via an outer-sphere electron transfer from the ketone to the Fe(III). Stoichiometry of the oxidation in the absence of air is given by eq 1.[4] The rate-determining step of the reaction was postulated



(1)

to involve an outer-sphere electron transfer from cyclohexanone to the Fe(III) complex to generate a 2-oxocyclohexyl radical, R·. (eq 2), or a hydrogen atom abstraction followed