Available online at www.sciencedirect.com
ScienceDirect
Bioorganic Chemistry 35 (2007) 338–343
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

Carbanions from decarboxylation of orotate analogues: Stability and mechanistic implications

Fong Ying Yeoh, Roxanne R. Cuasito, Christina C. Capule, Freeman M. Wong, Weiming Wu *

Department of Chemistry and Biochemistry, San Francisco State University, San Francisco, CA 94132, USA

Received 9 January 2007
Available online 2 April 2007

Abstract

The pK_a's of the 6-CH groups of 1,3-dimethyluracil, *N*-methyl-2-pyridone, and *N*-methyl-4-pyridone were determined through their reactions with bases derived from carbon acids with known pK_a and the reactions of their corresponding carbanions with the carbon acids. No correlation between the stability of the carbanions and the rate of decarboxylation of corresponding carboxylic acids was found.

Keywords: pK_a; Carbanion; Uracil; Pyridone

1. Introduction

The decarboxylation of 1,3-dimethylorotic acid (**1**) has been studied as a model for the enzymatic decarboxylation catalyzed by orotidine-5′-monophosphate decarboxylase (ODCase) [1–11]. The availability of its two structural analogues, pyridone carboxylic acids **2** and **3** makes it possible to determine the factors important for reaction rate. Acids **1**–**3** decarboxylate at elevated temperature to yield uracil **4** and pyridones **5** and **6** (Scheme 1), respectively, as the only products. Despite their structural similarity, acids

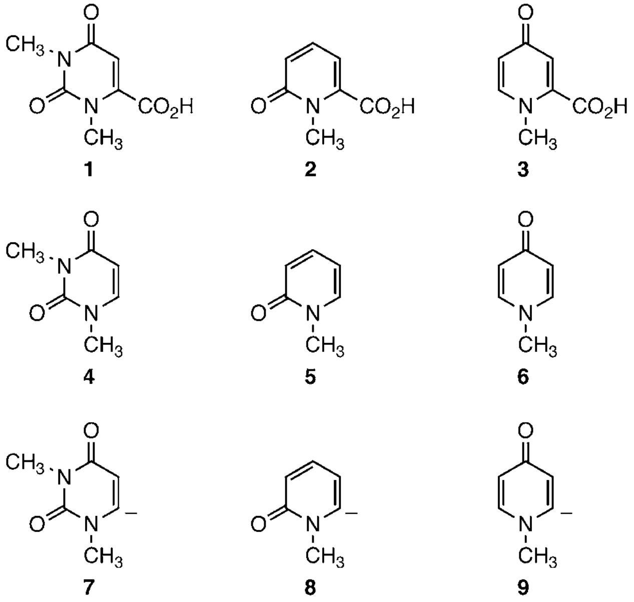
---

* Corresponding author. Fax: +1 415 338 2384.
E-mail address: wuw@sfsu.edu (W. Wu).

0045-2068/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2007.02.001

1–**3** decarboxylate at very different rates (Table 1) [1,7]. The rate of decarboxylation for acid **3** is approximately three orders of magnitude faster than those of either acid **1** or **2** [1,7]. This large difference in reaction rate raises the question of which structural feature is responsible for the facile decarboxylation of acid **3**.

Study of the stability of the corresponding carbanions **7**–**9** in the gas phase have found that carbanion **7** is 7 kcal/mol more stable than either carbanion **8** or **9** (Table 1) [6,7]. Therefore, there is a lack of correlation between the rate of decarboxylation and the gas-phase stability of resulting carbanions [7]. A two-step mechanism has been proposed in which an equilibrium leading to the formation of a zwitterionic structure such as **10** precedes the loss of CO_2 (Scheme 2) [1,7]. Since the zwitterionic structure is energetically unfavorable, i.e. equilibrium constant $K \ll 1$, the observed rate constant is simply the

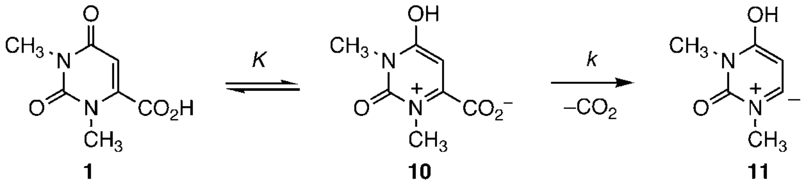


Scheme 1.

Table 1
Rate constants, proton affinities, and pK_a values

Acid	Rate constant at 206 °C (s^{-1})	Gas-phase stability of carbanions **7**–**9** (kcal/mol)[c]	pK_a of 6-CH (**4**–**6**) in water	pK_a of 6–CH (**4**–**6**) in DMSO[f]
1	7.5×10^{-4}[a]; 1.6×10^{-3}[b]	370	34[d]; 30[e]	29 ± 1
2	1.2×10^{-3}[a]; 1.3×10^{-3}[b]	377	32[e]	31 ± 1
3	0.32[a]; 1.6[b]	377	32[e]	$\geqslant 32$

[a] Rate constants in sulfolane reported in Ref. [7].
[b] Rate constants in isoquinoline reported in Ref. [7].
[c] Proton affinities of **7**–**9** reported in Ref. [7].
[d] Reported in Ref. [12].
[e] Reported in Ref. [13].
[f] Present study.



$$dP/dt = Kk[\mathbf{1}]/(K+1) \quad \text{When } K \ll 1,\ k_{ob} = Kk$$

Scheme 2.

product of the K and the rate constant k for the second step, loss of CO_2 [1,7]. In this mechanism, the stability of the zwitterionic structure relative to the acid and thus its equilibrium population, not the stability of the carbanion, plays a major role in determining the reaction rate. It has been shown that the zwitterionic structure derived from acid **3** is much more stable than those from acids **1** and **2** [7].

However, reactivity in the gas phase can be very different from that in the condensed phase because solvation can have a great impact on stability, especially that of ions. The pK_a values of the 6-CH groups of compounds **4**–**6** in water were determined in order to estimate the stability of carbanions **7**–**9** in aqueous medium [12,13]. Unfortunately, the pK_a values determined for uracil **4** in two separate studies were not consistent as shown in Table 1 [12,13]. In this study, we report the estimated pK_a values determined in DMSO, a solvent with similar properties to that employed in kinetic measurement of the decarboxylation reactions, sulfolane.

2. Materials and methods

Chemicals. Acid **1** was prepared through the methylation of orotic acid (from Sigma) as reported by Curran and Angier [14]. The same method was followed to prepare **2** from 2-pyridone-6-carboxylic acid (from Aldrich). Acid **3** was prepared from 4-pyrone-2-carboxylic acid (from TCI America) as reported by Beak and Siegel [1]. The identity and purity of the synthesized compounds was verified by their ^1H NMR spectra, mass spectra, and melting points.

Carboxylic acids were dissolved in one mole equivalent of a 0.02 N NaOH solution. The solutions were evaporated to dryness to yield the sodium carboxylates.

Triphenylmethane, diphenylmethane, 1,2,3,4,5-pentamethylcyclopentadiene, 2- and 4-benzylpyridine, and butyllithium in hexanes are commercially available from Sigma–Aldrich. 9-Phenylxanthene is prepared from 9-phenylxanthene-9-ol (from Aldrich) as reported [15].

Method A: Generation and reaction of carbanions. A carboxylate (5 mg) was mixed with an indicator (5 mg) in DMSO (0.5 mL) in a sealed tube. The mixture was heated at 230 °C for 40 min. Any color change was recorded.

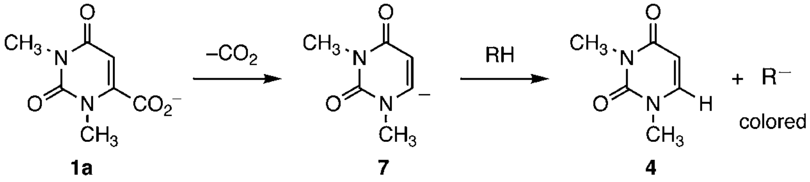
Method B: Reaction of indicator carbanions with uracil and pyridones (***4***–***6***). To a solution of an indicator (5 mg) in DMSO (0.5 mL) was added one or two drops of butyllithium in hexanes (~0.5 M) and the lithium salt of the resulting carbanion separated as a colored solid. A solution of uracil **4** or pyridones **5**–**6** (5 mg) in DMSO (0.5 mL) was then added to the mixture with stirring and the color of the mixture was recorded.

3. Results and discussion

The identical pK_a values of the 6-CH groups of pyridones **5** and **6** in water (Table 1) have confirmed that the stability of carbanions **8** and **9** are similar in at least one condensed phase, following the same trend as in the gas phase [6,7,13]. This result has further demonstrated that there is a lack of correlation between the stability of the carbanion and the rate of the decarboxylation (see Table 1). However, the values from two separate studies on uracil **4** do not agree with each other very well [12,13]. The two studies were carried out using the same kinetic method but under somewhat different conditions due to the chemical reactivity of **4** [13].

In the gas-phase study, the carbanions were generated in the mass spectrometer through the decarboxylation of corresponding carboxylates and subsequently reacted with a series of acids to bracket the proton affinities [6,7]. We thought the same strategy could be utilized to probe the pK_a values of the 6-CH groups of **4**–**6**, and thus the stability of the carbanions **7**–**9** in the condensed phase. The trapping of carbanion **7** with benzyl bromide has been reported [5]. Furthermore, this strategy has been employed to estimate the pK_a values of the 6-CH group of uracil **4** to be less than 30 [16]. In this study, the experiments were carried out in DMSO because it has the same property as the solvent utilized in the kinetic measurement of the decarboxylation reactions, sulfolane [1,7]. Unfortunately, sulfolane itself is not suitable as a solvent in this study because it readily protonates the resultant carbanions **7**–**9** due to the acidity of its α-protons [1].

When carboxylates such as **1a** (Scheme 3) is heated, decarboxylation will result in the formation of carbanions (**7** in the case of **1a**). The subsequent reactions of **7** with deutero-sulfolane, cyclopentadiene, and triphenylmethane have been examined previously [1,16]. These studies put an upper limit of the pK_a of the 6-CH group of uracil **4** at 30, that of triphenylmethane. We have chosen a series of carbon acids with known pK_a as indicators (Table 2) [17]. These indicators are chosen for their pK_a, potential for the conjugate base (i.e. the carbanion) to be colored, and availability. The experiments were carried out as shown in Scheme 3. The carboxylates were heated in the presence of an indicator in DMSO. If the carbanion generated is a strong enough base relative to indicator RH, deprotonation of RH will occur to give R^-, and as a result, a color change will be observed. On the contrary, no color change will be observed when the carbanion produced is not a strong enough base to deprotonate the indicator. The pK_a values of the indicator can then be utilized to bracket that of the conjugate acids of the carbanions [18]. The pK_a of DMSO is 35 so that protonation of the carbanions by the solvent is not an issue [17]. The reported preferential deprotonation of the methyl groups in pyridone **5** makes it impractical to generate carbanions **7**–**9** through the deprotonation of compounds **4**–**6** by a strong base such as butyllithium [19].



Scheme 3.

Table 2
Reactions between carbanions and indicators in DMSO[a]

Indicators	pK_a[b]	Carboxylate from acid **1**	Carboxylate from acid **2**	Carboxylate from acid **3**
Pentamethylcyclopentadiene	26.1	Yellow	—	—
9-Phenylxanthene	27.9	Red	—	—
4-Benzylpyridine	26.7	Red	—	—
2-Benzylpyridine	28.2	Red	—	—
Triphenylmethane	30.6	No change	Red	Red
Diphenylmethane	32.2	No change	No change	Red

[a] Reactions carried out at 230 °C.

[b] Taken from Ref. [17].

As seen in Table 2, carbanion **7** was able to deprotonate a series of carbon acids but failed to react with triphenylmethane and diphenylmethane. The pK_a of the 6-CH group of uracil **4** was then bracketed to be 29 ± 1. Both carbanions **8** and **9** reacted with triphenylmethane but only carbanion **9** was able to react with diphenylmethane. The pK_a values of the 6-CH groups of pyridones **5** and **6** were estimated to be 31 ± 1 and $\geqslant 32$, respectively. These results were listed in Table 1 for convenient comparisons with results from other media.

The stability of the carbanions **7–9** was also examined through the interactions of uracil **4** and pyridones **5–6** with carbanions derived from the indicators. In this study, the colored carbanions from the indicators were generated by their reactions with butyllithium. Uracil **4** and pyridones **5–6** was then added to the colored mixture. If the indicator carbanion is a strong enough base to deprotonate the uracil or pyridone, the color will be quenched. Results from this complementary study were summarized in Table 3 and good agreement between the two studies was found.

The results on the stability of carbanions **7–9** obtained in different media (such the gas phase, water, and DMSO) and with different methods (thermodynamic or kinetic) have all demonstrated a lack of correlation between their stability and the rate of decarboxylation of their corresponding acids. The current result from DMSO is especially noteworthy because DMSO shares the same properties as sulfolane, the solvent utilized for measuring the rates of decarboxylation. It is obvious that the stability of the carbanion has little impact on the rate of decarboxylation. On the other hand, the two-step mechanism shown in Scheme 2 envisions an important role for the relative stability of zwitterionic intermediates and is able to account for the large difference in the reaction rates seen in the decarboxylation of orotic acid analogues.

Table 3
Reactions of indicator carbanions with uracil **4** and pyridones **5–6** in DMSO[a]

Indicators	Color of anions	pK_a[b]	Uracil **4**	Pyridone **5**	Pyridone **6**
4-Benzylpyridine	Red	26.7	Red	—	—
2-Benzylpyridine	Red	28.2	Red	—	—
Triphenylmethane	Red	30.6	Quenched	Red	Red
Diphenylmethane	Orange	32.2	—	Quenched	Quenched

[a] Reactions carried out at room temperature.

[b] Taken from Ref. [17].

In summary, the pK_a of the 6-CH groups of the decarboxylation products of orotic acid analogues **1**–**3** in DMSO and thus the stability of carbanions **7**–**9** have been determined. The study has further demonstrated a lack of correlation between the rates of decarboxylation of orotic acid analogues and the stability of the resultant carbanions. It remains to be seen whether the same conclusion can be applied to the reaction catalyzed by ODCase.

Acknowledgments

This investigation was supported by the National Institutes of Health, MBRS SCORE Program—Grant #5 S06 GM52588. C.C.C. was supported by a MBRS RISE scholarship. We also thank Professor Scott Gronert at SFSU for helpful discussions. We thank one of the reviewers for suggesting Method B.

References

[1] P. Beak, B. Siegel, J. Am. Chem. Soc. 98 (1976) 3601–3606.
[2] A. Radzicka, R. Wolfenden, Science 267 (1995) 90–93.
[3] J.K. Lee, K.N. Houk, Science 276 (1997) 942–945.
[4] W. Wu, A. Ley-Han, F.M. Wong, T.J. Austin, S.M. Miller, Bioorg. Med. Chem. Lett. 7 (1997) 2623–2628.
[5] M.P. Nakanishi, W. Wu, Tetrahedron Lett. 39 (1998) 6271–6272.
[6] S. Gronert, W.Y. Feng, F. Chew, W. Wu, Int. J. Mass Spectrom. 195/196 (2000) 251–258.
[7] W.Y. Feng, T.J. Austin, F. Chew, S. Gronert, W. Wu, Biochemistry 39 (2000) 1778–1783.
[8] D.L. Shem, S. Gronert, W. Wu, Bioorg. Chem. 32 (2004) 76–81.
[9] R.B. Silverman, M.P. Groziak, J. Am. Chem. Soc. 104 (1982) 6434–6439.
[10] D.A. Singleton, S.R. Merrigan, B.J. Kim, P. Beak, L.M. Phillips, J.K. Lee, J. Am. Chem. Soc. 122 (2000) 3296–3300.
[11] F.M. Wong, W. Wu, Bioorg. Chem. 34 (2006) 99–104.
[12] A. Sievers, R. Wolfenden, J. Am. Chem. Soc. 124 (2002) 13986–13987.
[13] F.M. Wong, C.C. Capule, W. Wu, Org. Lett. 8 (2006) 6019–6022.
[14] W.V. Curran, R.B. Angier, J. Org. Chem. 31 (1966) 201–205.
[15] H.E. Ungnade, E.F. Kline, E.W. Crandall, J. Am. Chem. Soc. 75 (1953) 3333–3336.
[16] B. Siegel, Ph.D. Thesis. University of Illinois at Urbana/Champaign, 1974.
[17] F.G. Bordwell, Acc. Chem. Res. 21 (1988) 456–463.
[18] D.J. Cram, Fundamentals of Carbanion Chemistry, Academic Press, New York, 1965, Chapter I.
[19] P. Meghani, J.A. Joule, J. Chem. Soc. Perkin Trans. I (1988) 1–8.