Tetrahedron Letters 52 (2011) 3960–3962

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Accelerated hydrolysis of α-halo and α-cyano pyridinium relative to uracil derivatives: a model for ODCase-catalyzed hydrolysis of 6-cyanoUMP

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ARTICLE INFO

Article history:
Received 8 April 2011
Accepted 23 May 2011
Available online 30 May 2011

Keywords:
Pyridinium
Uracil
Hydrolysis
ODCase
Aromatic nucleophilic substitution

ABSTRACT

α-Halo and α-cyano pyridiniums were found to undergo facile hydrolysis, in contrast to the sluggish reactions of corresponding uracils. The greatly enhanced rates found with pyridinium compounds have indicated a possible source of the rate acceleration seen in the hydrolysis of 6-cyanouridine 5′-monophosphate catalyzed by orotidine 5′-monophosphate decarboxylase.

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We have recently examined the acidity of α-proton of methoxy-substituted pyrimidinium **1** and pyridinium **2** and **3** as models for the proposed intermediates in the decarboxylation of orotic acid and analogues (Fig. 1).[1] The acidities of these compounds were measured through the hydrogen–deuterium exchange reactions at the α-position. The acidity of the α-CH groups of these positively charged compounds were found to be surprisingly lower than that of neutral pyridones, in great contrast to the results in the gas phase where they are much more acidic than their neutral counterparts.[1–6] In order to understand the contribution of the methoxy group to the electronic structure of the pyridinium compounds, reactions of a series of pyridinium compounds with various electron-donating or -withdrawing groups at different positions need to be investigated.[7] During the examination of the hydrogen–deuterium exchange reaction of these pyridinium derivatives, it was observed that 2-chloro and 2-cyanopyridinium (**4** and **5**) underwent facile substitution to form pyridone **6**, resembling the conversion of 6-cyanouridine 5′-monophosphate (6-cyanoUMP, **7**) to β-D-ribofuranosylbarbiturate 5′-monophosphate (BMP, **8**) catalyzed by orotidine 5′-monophosphate decarboxylase (ODCase), as shown in Figure 1.[8] BMP **8** is a potent inhibitor of ODCase with a reported K_i of 8.8×10^{-12} M.[8] Using the half-life of about 20 h for the inactivation of ODCase by **7**, an inactivation rate constant of about 1×10^{-5} s^{-1} can be calculated for the enzymatic conversion of **7** to **8** at pH 7.5.[8] In the absence of ODCase, nucleotide **7** shows no reaction after 5 days under the same conditions.[8]

The hydrolysis of 6-cyanoUMP **7** represents an interesting twist in the catalytic activity of ODCase, whose physiological function is catalyzing the decarboxylation of orotidine 5′-monophosphate (OMP) to UMP. In this Letter, we discuss the great rate enhancement seen in the hydrolysis of pyridinium compounds and its relevance to the ODCase-catalyzed hydrolysis of 6-cyanoUMP.

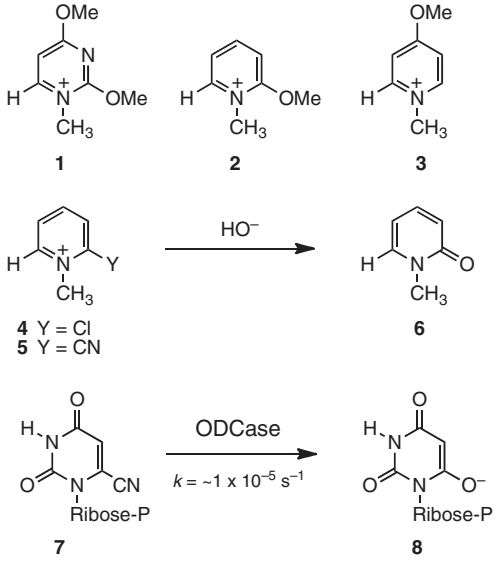

Figure 1. Structures and reactions of some pyridinium compounds and 6-cyanouridine-5′-monophosphate.

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doi:10.1016/j.tetlet.2011.05.108

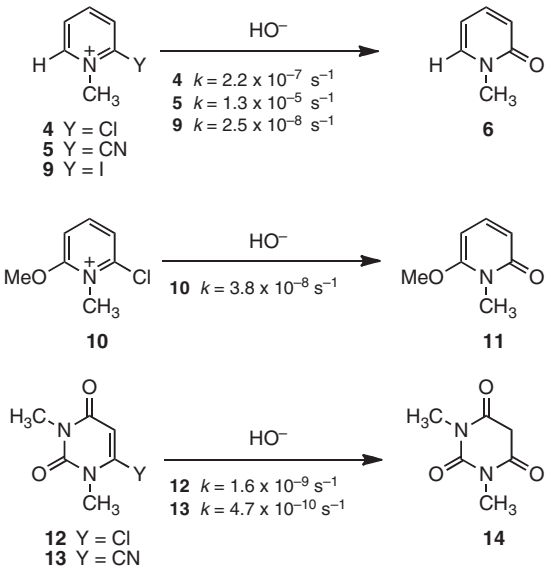

Figure 2. Reactions of some pyridinium compounds and their pseudo first-order rate constants at pH 7.5.

To estimate the rate of the uncatalyzed reaction, the rates of the hydrolysis reactions of uracil derivatives **12** and **13** in NaOD/D_2O were measured (Fig. 2). 6-Cyanouracil **13** was prepared from the commercially available chlorouracil **12** as reported.[9] The hydrolysis reactions were studied using NMR spectroscopy to follow the disappearance of H-5 through integration of the aromatic peaks. The reaction kinetics were found to be second-order overall and have a first-order dependence on the concentration of the substrate as well as NaOD. The pseudo first-order reaction rates measured at $[OD^-] = 0.1$ M for the uracil derivatives were corrected using the secondary solvent deuterium isotope effect of $k_{DO}/k_{HO} = 1.46$ as reported[1,3,10] and extrapolated to values at pH 7.5. The pseudo first-order rate constants for uracils **12** and **13** at pH 7.5 thus calculated were 1.6×10^{-9} and $4.7 \times 10^{-10}\,s^{-1}$, respectively. It is estimated that ODCase accelerates the hydrolysis reaction by a factor of 2×10^4 as is evident from the comparison of the rate constants measured for **7** and **13**.

On the other hand, the reaction of pyridinium **4** and **5** with the hydroxide ion was found to be facile at pH 10.0. The pseudo first-order rate constants (at pH 7.5) for the reactions of **4** and **5** with hydroxide ion were calculated to be 2.2×10^{-7} and $1.3 \times 10^{-5}\,s^{-1}$, respectively. It is obvious that the pyridinium moiety provides large rate acceleration in the substitution reaction at the α-position. The rate constant of the hydrolysis of cyanouracil **5** is essentially identical to that measured for the ODCase-catalyzed hydrolysis of cyanoUMP **7**. We have further investigated the reactions of two other pyridinium derivatives to determine the factors affecting the reaction rate. Iodopyridinium **9** was studied to examine the steric effect of the leaving group. Methoxypyridinium **10** was chosen because of the ability of the methoxy group to donate electrons and thus alter the electronic structure of the pyridinium ring. The pyridinium compounds were readily prepared through the methylation of corresponding pyridines.[7,11,12] In all cases, the products were identified by NMR. Pyridone **6** and barbituric acid derivative **14** are commercially available and the NMR spectra of pyridone **11** have been reported.[13] The reactions of **4** and **5** were followed by measuring the concentration of reaction product pyridone **6** through its UV absorbance at 310 nm. The reactions of other compounds were investigated in D_2O using NMR spectroscopy.

From the comparison of the pseudo first-order rate constants, it is clear that the positive charge in pyridinium greatly increase the reaction rates. Mechanisms for the reaction of α-substituted uracils and pyridiniums are proposed in Figure 3. The α-carbon is electron-deficient due to the electron-withdrawing ability of the positively charged nitrogen in the pyridinium compounds or the enone group in the uracils. The mechanism involves nucleophilic attack on the α-carbon to form the tetrahedral intermediate followed by the departure of the leaving group, in contrast to those proposed for the enzymatic reaction.[8] Substitution reactions at carbon-6 of the uracils have been reported and have been utilized to synthesize uracil derivatives.[8,9]

The proposed mechanism can account for the difference in reactivity between the uracil and pyridinium compounds and amongst the pyridinium molecules with different substituents. It is evident that the increased electrophilicity of the positively charged

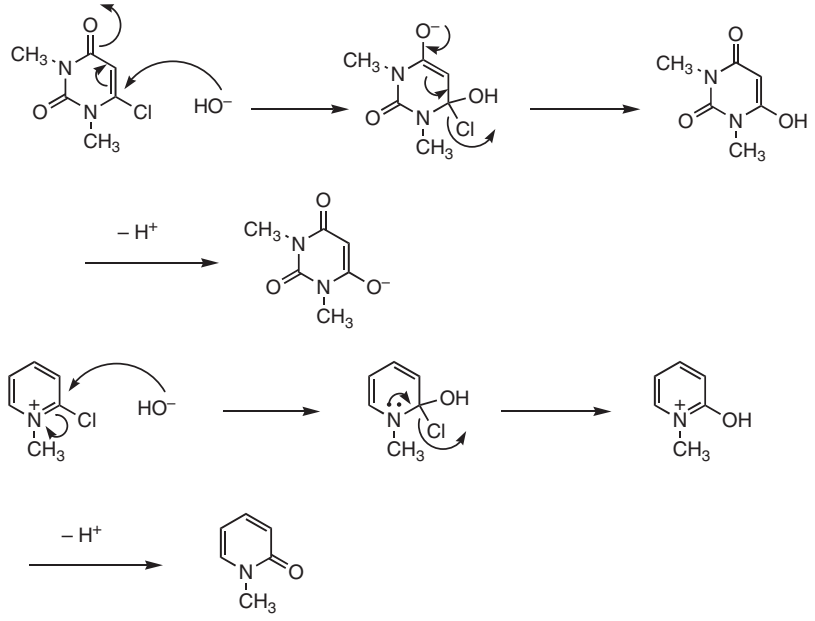

Figure 3. Proposed mechanisms for the substitution reaction of uracil and pyridinium compounds with hydroxide.

pyridinium compounds has a large impact on the reactivity. Comparison of pyridinium **5** and uracil **13** reveals rate acceleration of more than 20,000-fold, although comparison of the rate constants measured for **4** and **12** shows a much smaller effect. Methoxy substitution at the *o*-position, however, can contribute electron density to the pyridinium ring through resonance and thus reduce the extent of rate enhancement. 6-Methoxypyridinium **10** is thus sixfold less reactive than pyridinium **4**. The iodo compound is less reactive than the chloro compound presumably due to a combination of its lower electronegativity and larger size. If the radical mechanism (one of the mechanisms[8] proposed for the enzymatic reaction) were operating for the model reactions studied here, the iodo substrate will be the more reactive. These results are consistent with the 'element effect' observed in the nucleophilic aromatic substitution reactions of 2,4-dinitrobezene derivatives.[14,15] The results have also suggested that the first step in the proposed mechanism is likely the rate-determining step.

The results obtained are consistent with the idea that the pyrimidine structure is polarized in the active site of ODCase, although the outright formation of a zwitterionic intermediate[16,17] is unlikely due to the lack of basic residues adjacent to O-2 and O-4 as revealed by crystallographic studies.[18–21] Deuterium exchange studies on UMP have demonstrated the existence of a stabilized carbanion intermediate in the enzymatic reaction.[5,22] It has been proposed that a pre-organized polar environment at the active site due to its charge distribution stabilizes the carbanionic intermediate through dipole interactions.[3,23,24] It is conceivable such dipole interactions may alter the electron density distribution on the uracil moiety to facilitate the nucleophilic attack at carbon-6 in the hydrolysis of cyanoUMP **7**. The results suggest that this feature of the active site of ODCase can also explain its unexpected ability to catalyze the hydrolysis reaction of 6-cyanoUMP **7**.

Acknowledgments

This investigation was supported by the National Institutes of Health, MBRS SCORE Program—Grant S06 GM52588 and by a grant from the Center for Computing for Life Sciences at SFSU. The NMR facility was funded by the National Science Foundation (DUE-9451624 and DBI 0521342). S.H. was supported by the Summer Research Fellowship from the Department of Chemistry and Biochemistry. In addition, we are indebted to Professor James Keeffe at SFSU for helpful discussions.

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