Mechanism of the Hydrolysis of (Z)-4-Benzylidene-2-methyloxazolin-5-one or (Z)-4-Benzylidene-2-phenyloxazolin-5-one Derivatives

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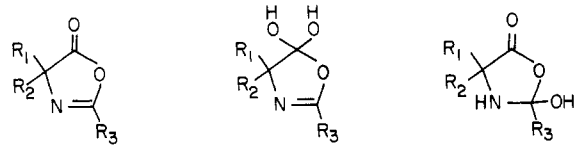
The methyl (**1a**) and the phenyl (**2**) derivatives of (*Z*)-4-benzylideneoxazolin-5-one were hydrolyzed at comparable rates under alkaline conditions. In contrast, the acidic hydrolysis of **2** was at least 10^5 times slower than that of **1a**. The Hammett ρ value obtained from the acidic hydrolysis of the phenyl substituted derivatives of **1a** was 0.20 ± 0.09 while that from the corresponding basic hydrolysis was 1.50 ± 0.11. Methanolysis of **1a** under basic conditions produced the methyl ester of (*Z*)-α-(acetylamino)cinnamic acid, and that under acidic conditions led to products consistent with the attack of methanol at the imine carbon. These results indicate that the alkaline hydrolysis of the oxazolin-5-one derivatives occurs through nucleophilic attack at the carbonyl carbon of the substrate and the acidic hydrolysis through that at the imine carbon.

The mechanism of nucleophilic reactions at acyl derivatives has been the subject of extensive investigation. In recent years, interests in this area have been focused on the determination of the detailed structure of the transition state.[1] In addition, catalysis by intramolecular functional groups[2] or metal ions[3] in transacylation reactions has been emphasized as models for hydroytic enzymes.

Oxazolin-5-ones (A), anhydrides of *N*-acyl amino acids, are acyl derivatives with an interesting structural feature; the presence of carbonyl and imine carbons. Thus, the carboxyl ester and the imidate ester linkages of A are the potential reaction sites for the attack by nucleophiles. Attack of a water molecule or hydroxide ion under alkaline or acidic conditions at the carbonyl carbon would produce tetrahedral intermediates related to B, while that at the imine carbon would lead to those related to C. Breakdown of B would be accompanied by ring opening at the C(acyl)–O bond, producing the corresponding *N*-acyl amino acid. Breakdown of C, however, could involve ring opening through the cleavage of either the C(alkyl)–O or the C(alkyl)–N bond.

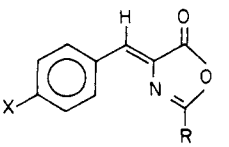


A B C

In the present study, kinetics of the hydrolysis of 2-methyl (**1a–f**) and 2-phenyl (**2**) derivatives of (*Z*)-4-benzylideneoxazolin-5-ones were measured under both acidic and alkaline conditions. The kinetic data, together with the products of methanolysis of **1a**, allowed identification of the reaction sites under the respective reaction conditions.

Experimental Section

Materials. Compounds **1a–f** were prepared according to the general procedure reported in the literature[4] by reacting the corresponding benzaldehydes with acetyl glycine in acetic anhydride in the presence of sodium acetate. **1a**: mp 151–152 °C (lit.[4] mp 148–150 °C). **1b**: mp 182–183 °C (lit.[5] mp 185–186 °C).



1a, R = CH_3; X = H
1b, R = CH_3; X = NO_2
1c, R = CH_3; X = CN
1d, R = CH_3; X = Cl
1e, R = CH_3; X = CH_3
1f, R = CH_3; X = OCH_3
2, R = C_6H_5; X = H

1c: mp 234–236 °C. **1d**: mp 143–145 °C. **1e**: mp 139–140 °C. **1f**: mp 113.5–114 °C (lit.[6] mp 114 °C). Compound **2** was prepared according to the literature, mp 167–168 °C (lit.[7] mp 165–166 °C). The *Z* configuration of the 4-benzylideneoxazolin-5-ones has been previously established.[8,9] α-(Acetylamino)cinnamic acid was prepared by hydrolyzing **1a** in boiling 2:1 acetone–water, mp 195–197 °C (lit.[4] mp 191–192 °C). α-(Benzoylamino)cinnamic acid was obtained by the hydrolysis of **2** in 10% NaOH solution heated on a steam bath, mp 240–240.5 °C (lit.[7] mp 224–236 °C). Methyl (*Z*)-α-(acetylamino)cinnamate was synthesized by the reaction of **1a** with sodium methoxide in methanol: mp 118–119 °C; ^{1}H NMR δ 2.2 (singlet, 3 H, acetyl), 3.9 (singlet, 3 H, methoxy). Satisfactory results of elemental analysis (C, H, N) were obtained for **1c–e** and methyl (*Z*)-α-(acetylamino)cinnamate. Acetone and methanol were purified according to the literature[10] before being used in kinetic studies. Water was distilled, deionized, and then used in kinetic measurements.

Kinetic Measurements. Reaction rates were measured with a Beckman Model 5260 or 25 UV/vis spectrophotometer by observing the decrease in absorbance in the 330–370-nm region. Temperature (25 °C) was controlled to within ±0.1 °C with a Haake E52 circulator. The reactions were carried out with initially added acetone (3–40% (v/v)), and the evaporation of acetone during kinetic measurements was prevented by sealing the cuvettes tightly with serum caps. Kinetics were performed at an ionic strength of 1.0 which was adjusted with sodium chloride. Buffers used were chloroacetate (pH 2.5–3), formate (pH 3–4.5), acetate (pH 4.5–5.5), borate (pH 7.7–9.6), and carbonate (pH 10–11.3). pH measurements of the acetone-containing buffer solutions were carried out with a Fisher Accumet Model 525 pH meter. Pseudo-first-order kinetics were observed up to at least three half-lives for relatively fast reactions, for which the pseudo-first-order rate constants were calculated with the infinity absorbance values measured. For reactions whose half-lives were greater than 2 h, pseudo-first-order rate constants were calculated

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Table I. Rate Parameters for the Hydrolysis of 1a and 2[a,b]

compd	solvent	$k_H$app	$k_H$corr	k_{OH}app	k_{OH}corr
1a	3% (v/v) acetone	162 ± 10	162 ± 10	(1.33 ± 0.12)10^4	(1.56 ± 0.14)10^4
1a	40% (v/v) acetone	70.8 ± 5.0	57.5 ± 4.0	(1.20 ± 0.10)10^3	(1.17 ± 0.10)10^4
2	40% (v/v) acetone	no reactn	no reactn	(3.46 ± 0.30)10^2	(3.38 ± 0.30)10^3

[a] At ionic strength 1.0 which was maintained with sodium chloride and at 25 °C. [b] Unit for the rate parameters are M^{-1} min^{-1}.

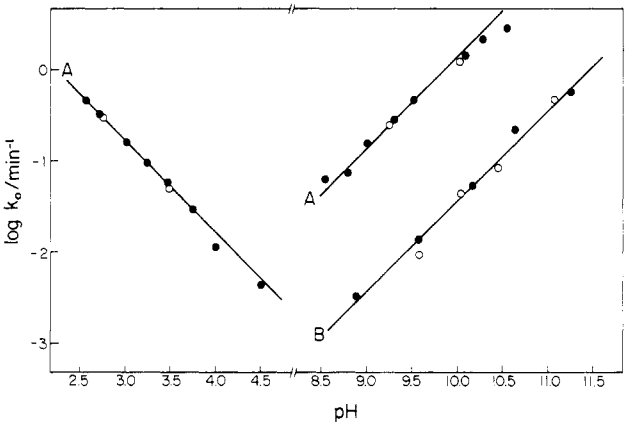


Figure 1. Kinetic data for the hydrolysis of **1a** (line A) in the presence of 3% (v/v) acetone and for the hydrolysis of **2** (line B) in the presence of 40% (v/v) acetone. Buffer concentration was either 0.01 M (○) or 0.05 M (●).

by the Guggenheim method or by the initial rate method.

Acidic Methanolysis of 1a. After **1a** (0.4 g) was incubated in 30 mL of 0.05 M methanolic HCl for 3 h, the mixture was neutralized with a 0.2 M methanol solution of sodium methoxide. The solid material which was obtained after evaporation of methanol was washed with acetone and then with methanol. The ^{1}H NMR (the singlet at δ 4.1 was the only signal except those of phenyl protons) and IR spectra of the product were very similar to those of authentic sodium phenylpyruvate (Aldrich). For two separately prepared samples, the number of carbon atoms per nitrogen atom in the product was 43 and 23 when calculated from the results of elemental analysis.

Results and Discussion

The hydrolysis of **1a** was kinetically studied at various pHs in the presence of 3% (v/v) acetone. Acetone was added to dissolve **1a** (1×10^{-4} M) in water. Buffer effects were examined by measuring the rate in the presence of either 0.01 M or 0.05 M buffer. The results are illustrated in Figure 1. In order to dissolve **2** (1×10^{-4} M) in water, 40% (v/v) acetone was needed. The hydrolysis of **1a** was, therefore, also examined in the presence of 40% (v/v) acetone in order to compare its results with those of **2**.

Rate data for the hydrolysis of **2**, measured in the presence of 40% (v/v) acetone with either 0.01 M or 0.05 M buffer, are illustrated in Figure 1. No reaction was detected for **2** at acidic pHs.

As illustrated in Figure 1, rate data obtained with both 0.05 M and 0.01 M buffer are located on the same lines, indicating the absence of general acid or base catalysis by the added buffer species. The straight lines drawn in Figure 1 have the slope of either 1.00 (at basic pHs) or −1.00 (at acidic pHs). Thus, the pseudo-first-order rate constants are proportional to either hydroxide or hydronium ion concentration (eq 1 and 2).

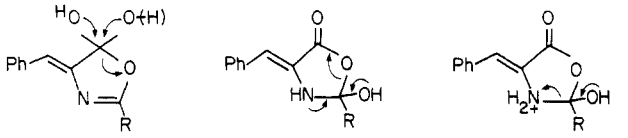
$$k_0 = k_{OH}[OH^-] = k_{OH}K_w/[H^+] \text{ (at pH } >8) \quad (1)$$

$$k_0 = k_H[H^+] \text{ (at pH } <5) \quad (2)$$

pH values were measured directly with a pH meter in the presence of added acetone. In order to calculate second-order rate constants (k_H and k_{OH}) from the pH profiles, it should be clarified whether the pH values reflect the correct hydronium concentrations even in the presence of acetone. In addition, K_w values in the acetone–water mixtures are needed for calculating hydroxide concentration and, subsequently, k_{OH} values. At ionic strength 1.0, the pH of 0.02 M HCl was higher by 0.09 in the presence of 40% (v/v) acetone than in water and was the same in the presence of 3% (v/v) acetone and in water. Thus, it appears that hydronium ion has smaller activity in the presence of acetone but the difference is not large. At an ionic strength of 1.0, the pH of 5 mM NaOH was greater by 0.07 in the presence of 3% (v/v) acetone and by 0.99 in the presence of 40% (v/v) acetone than that in water. The greater pH in the presence of acetone reflects the decreased activity of hydronium ion and, to a greater extent, the decreased K_w.

The values of $k_H$app and k_{OH}app were calculated by assuming that $[H^+] = 10^{-pH}$ and $K_w = 1 \times 10^{-14}$ M^2 and are summarized in Table I. These values were corrected for the pH differences in the acetone-containing water, and the consequently obtained values of $k_H$corr and k_{OH}corr are also included in Table I. An increase in the acetone content from 3% to 40% results in ca. a 10-fold decrease in k_{OH}app for **1a**. The rate difference, however, becomes much smaller after correction for the pH differences.

Quantitative production of (*Z*)-α-(acetylamino)cinnamic acid and (*Z*)-α-(benzoylamino)cinnamic acid during the alkaline and acidic hydrolysis of **1a** and during the alkaline hydrolysis of **2**, respectively, was evidenced by the UV spectra of the product solutions and by the large-scale synthesis of the cinnamic acids under the respective conditions. These products can be formed either by the nucleophilic addition of water at the carbonyl group and the subsequent C–O cleavage (i.e., D) or by that at the imine group followed by C–O cleavage (i.e., E). The products, however, rule out the possibility of the cleavage of C–N bond (F) after the nucleophilic addition at the imine group.



Although **1a** and **2** are hydrolyzed at comparable rates under alkaline conditions, acidic hydrolysis was observed only for **1a**. For **2**, no reaction was observed spectrophotometrically for several hours at pH 1. Thus, k_H is smaller for **2** than for **1a** by at least 10^5 times. This difference is not easily explained in terms of the intermediacy of D. However, if the acidic hydrolysis occurs through the addition of a water molecule at the imine group, protonation of the imine nitrogen would be an important factor (Scheme I). The Schiff bases derived from aromatic carbonyl compounds are less basic by 2–3 pK units compared with those derived from aliphatic ones.[11,12] A similar

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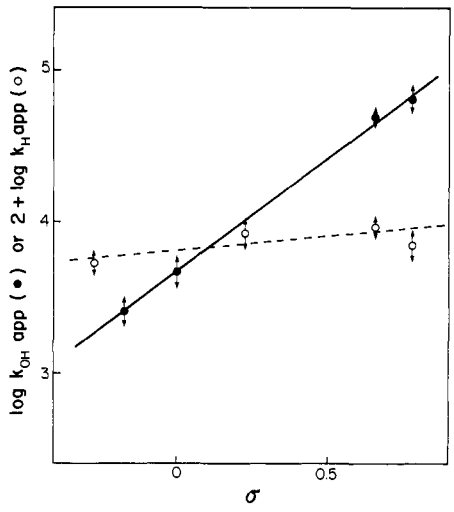
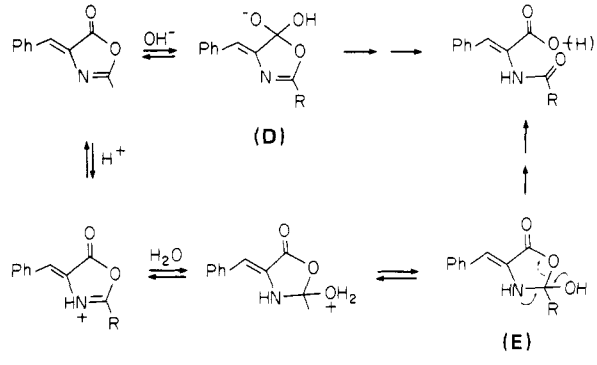


Figure 2. Hammett plots for $k_H$app (○) and k_{OH}app (●). The compounds studied for the construction of the solid line are **1e**, **1a**, **1c**, and **1b** (from left), and those of the dotted line are **1f**, **1d**, **1c**, and **1b** (from left).

Scheme I



pK difference might be present between the nitrogen atoms of **2** and **1a**. This can be related to the slower acidic hydrolysis of **2**. However, it is difficult to account for the large rate difference (>10^5-fold) in terms of the basicity of the imine nitrogen alone. This is because the conjugate acid of **2**, once protonated, could react much more readily than that of **1a**, if only electronic effects are considered.

Examination of a space-filling model revealed that the attack of water at the imine carbon could be sterically hindered by the phenyl group attached to the imine carbon. The steric effect can be the major factor for the very slow acidic hydrolysis of **2**. Similar rates for the alkaline hydrolysis of **1a** and **2** are consistent with the attack of hydroxide ion at the carbonyl carbon, since the attack at the imine carbon might be accompanied by large steric retardation for **2**.

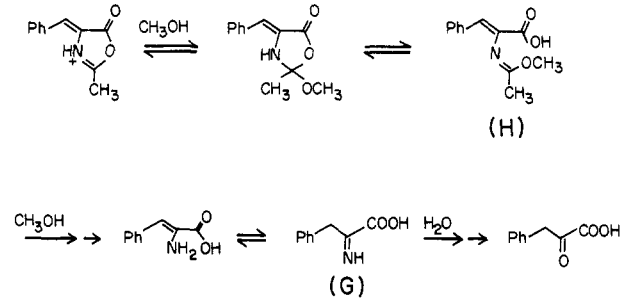
The effects of the phenyl substituents in the derivatives of **1a** were examined by measuring the rates of acidic and alkaline hydrolysis of **1a–f**. Because of the limited solubility of the compounds in water, the reactions were carried out in the presence of 30% (v/v) acetone. For each compound, $k_H$app and k_{OH}app were calculated from the pH profile of k_0. Hammett plots for $k_H$app and k_{OH}app are illustrated in Figure 2. The parameter values were not corrected for the pH readings, since the ρ value is not affected by the correction. The ρ values obtained by the weighted linear regression of the data in Figure 2 are summarized in Table II, together with the ρ values reported for the alkaline hydrolysis of *trans*-cinnamoyl derivatives.

Table II. Hammett Reaction Coefficients for the Hydrolysis of 1a–f and *trans*-Cinnamoyl Derivatives[a]

compd[b]	hydrolysis	solvent	ρ
1a–f	acidic	30% (v/v) acetone	0.20 ± 0.09
1a–f	alkaline	30% (v/v) acetone	1.50 ± 0.11
O-trans-cinnamoyl-L-β-phenyllactic acids[c]	alkaline	no organic solvents	0.92 ± 0.07
trans-cinnamoyl azides[d]	alkaline	0.8% (v/v) acetonitrile	0.98 ± 0.01
trans-cinnamoyl azides[d]	alkaline	50% (v/v) acetone	1.29 ± 0.07
p-nitrophenyl *trans*-cinnamates[d]	alkaline	50% (v/v) acetone	1.27 ± 0.07

[a] At 25 °C. Ionic strength was 0.55 for *O-trans*-cinnamoyl-L-β-phenyllactic acids and 1.0 for other compounds. [b] For *trans*-cinnamoyl derivatives, the benzene ring of the cinnamoyl portion was substituted for the structure–reactivity study. [c] Reference 13. [d] Reference 14.

Scheme II



The small ρ value for the acidic hydrolysis of the derivatives of **1a** is consistent with the mechanism of Scheme I. Electron-withdrawing substituents will retard the protonation of the imine nitrogen but will promote the water attack at the protonated substrate. The small ρ value for $k_H$app indicates that these two contradicting effects are nearly balanced. The ρ value observed for the alkaline hydrolysis of the derivatives of **1a** is similar to those observed for the alkaline hydrolysis of *trans*-cinnamoyl derivatives. This provides further support for the attack of hydroxide ion at the carbonyl carbon of **1a**.

Methanolysis products obtained with **1a** under either acidic or basic conditions were examined. Methyl (*Z*)-α-(acetylamino)cinnamate was quantitatively formed from the basic methanolysis (0.05 M sodium methoxide) of **1a**, as indicated by the UV spectra of the product solutions and by the large-scale synthesis of the methyl ester under similar conditions.

The reaction of **1a** with acidic methanol (0.05 M HCl) did not produce the methyl ester as checked by thin-layer chromatography, although the ester was stable under the experimental conditions. The product isolated after acidic methanolysis of **1a**, as indicated in the Experimental Section, is best described as sodium phenylpyruvate contaminated by the sodium salt of β-phenyl-α-iminopropionic acid (G). Although pure G was not isolated apparently due to its instability, the absence of acetyl or methoxy signals in the ^{1}H NMR spectra of the product clearly demonstrates that the carbonyl carbon is not the reaction site in the acidic methanolysis of **1a**. Addition of methanol at the protonated imine group of **1a** would lead to imidate ester

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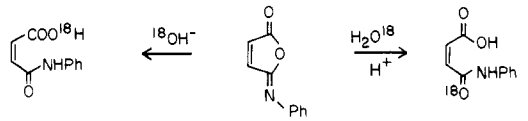
Scheme III



H (Scheme II), which should be further solvolyzed to G. Hydrolysis of G to phenylpyruvic acid is attributable to contact with trace water either in the methanolysis step or in the workup process. In acidic hydrolysis, attack of water at the imine carbon of protonated **1a** would produce (*Z*)-α-(acetylamino)cinnamic acid in the place of H, and this product is not easily converted to G.

In summary, lack of the reactivity of **2** under acidic conditions, results of the Hammett plots obtained for **1a-f**, and methanolysis products of **1a** indicate that the carbonyl carbon of the oxazolin-5-one derivatives is the site of nucleophilic attack under basic conditions, while the imine carbon is the site under acidic conditions. The hydrolysis of *N*-phenylmaleisoimide may be cited as an analogue of the present reaction. As summarized in Scheme III, the carbonyl carbon and the imine carbon were the reaction sites under basic and acidic conditions, respectively, in the hydrolysis of *N*-phenylmaleisoimide.[15]

Acknowledgment. This work was supported by the Basic Science Research Institute Program (1984), Ministry of Education, Republic of Korea.

Registry No. (*Z*)-**1a**, 38879-46-8; (*Z*)-**1b**, 66949-13-1; (*Z*)-**1c**, 94929-80-3; (*Z*)-**1d**, 93634-55-0; (*Z*)-**1e**, 93634-54-9; (*Z*)-**1f**, 71198-72-6; (*Z*)-**2**, 17606-70-1; PhCHO, 100-52-7; *p*-$NO_2C_6H_4CHO$, 555-16-8; *p*-NCC_6H_4CHO, 105-07-7; *p*-ClC_6H_4CHO, 104-88-1; *p*-MeC_6H_4CHO, 104-87-0; *p*-$MeOC_6H_4CHO$, 123-11-5; (*Z*)-PhCH=C(CO_2H)NHAc, 55065-02-6; (*Z*)-PhCH=C(CO_2H)-NHC(O)Ph, 26348-47-0; (*Z*)-PhCH=C(CO_2Me)NHAc, 60676-51-9; *N*-acetylglycine, 543-24-8.

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Elimination Reactions of α-Substituted Thymines Derived from Tautomeric Heterocyclic Thiols and Selenols

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Tautomeric heterocyclic thiols are readily alkylated by 5-(halomethyl)uracils, giving both S- and N-substituted products. S derivatives such as **19** undergo rapid elimination of thiolate anion in base, whereas the isomeric 6-substituted uracil derivatives such as **3** show no elimination. Kinetic and ^{13}C NMR studies are consistent with an elimination mechanism involving heterocyclic quinone methide intermediates, which can arise from the 5-substituted uracil derivatives but not from the 6-substituted series. The proposed mechanism is further supported by studies of the pH dependency of the elimination reaction and of the effect of substitution in the uracil ring (see Table I and Scheme II). N-Substituted (thione) derivatives such as **36** also undergo base-catalyzed elimination, but at rates some 10^5 to 10^6 times slower than those for the corresponding S derivatives when the uracil is unsubstituted on nitrogen. The high sensitivity of elimination rate to changes in the leaving group atom is attributed to a transition state in which the connecting methylene group has considerable carbocation character (see Scheme VI). Analogous derivatives (such as **42**) of tautomeric heterocyclic selenols have also been prepared, and their elimination kinetics further support this interpretation.

The importance of uracil and its derivatives as constituents of nucleic acids and related biological systems has led to extensive studies of the chemistry of pyrimidine derivatives bearing oxygen functions.[1] One interesting aspect of this chemistry concerns the relationship between uracil, thymine, and 5-(hydroxymethyl)uracil (**23**) derivatives[2] (Scheme IV). Kinetic studies[3,4] of the solvolysis of esters and *p*-nitrophenyl ethers derived from **23** suggest involvement of heterocyclic quinone methide species **20** and **21**; intermediacy of **20** has also been invoked[5] to explain the reaction of the corresponding Mannich bases[6] with aniline. A number of 5-(thiomethyl)uracil derivatives have been described,[7] but the possibility of solvolysis involving quinone methide species in this series appears not to have been addressed. Our interest in protective groups for heterocyclic thiols and photographically useful materials[8] led us to investigate the chemistry of the thioethers derived from alkylation by 5-(chloromethyl)uracils. We report here our findings, which not only demonstrate that such thioethers undergo base-catalyzed elimination of thiolate anions but also provide strong evidence for a mechanism involving heterocyclic quinone methide species.

Results and Discussion

Thioethers Derived from 1-Phenyl-1,2,3,4-tetrazole-5-thiol and 5- and 6-(Chloromethyl)uracils. 5-

† Principle Author. Deceased January 19, 1984.

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