Ortho Substituent Effects in Amide Hydrolysis of Maleanilic Acid Derivatives. Stabilization of Positive Charge Developed in the Transition State[1]

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The effects of ortho substituents on the rate of the hydrolysis of maleanilic acid derivatives have been studied. The rate is enhanced up to 55 times by polar substituents, while it is retarded greatly by nonpolar substituents. The acceleration by the polar ortho substituents is attributed to two structural aspects of the rate-controlling transition states: (1) the leaving nitrogen atom acquires a large partial positive charge in the transition state; (2) the rigid conformations of the *N*-alkyl and the phenyl portions of the transition state lock the polar ortho substituents in close proximity with the leaving nitrogen atom, so that the positive charge developed on the nitrogen atom is effectively stabilized by the polar substituents through hydrogen bonding or electrostatic interaction.

Stabilization of charges developed in the transition state through electrostatic interaction or hydrogen bonding has been proposed in enzymatic systems such as Asp-52 of lysozyme[2] or the charge-relay system of chymotrypsin.[3] Attempts to demonstrate these effects in small molecules have been made with varying degrees of success.[4-11] One of the major obstacles encountered in such studies is to obtain the frozen conformations required for the network of hydrogen bonds involving several functional groups simultaneously or required for efficient electrostatic interaction between two oppositely charged atoms. In order to achieve highly effective electrostatic interaction in an aqueous solution, additional factors such as sufficient charge development in the transition state and the removal of hydration shells surrounding the charged atoms are also required.

The hydrolysis of maleamic and maleanilic acid derivatives involves intramolecular nucleophilic catalysis by the proximal carboxylate group and proceeds quite rapidly for amide hydrolysis.[12-23] Thus, studies on the maleic acid derivatives have produced important mechanistic information on the reactions of amides and anilides as well as on the actions of proteases. The hydrolysis of ortho-substituted maleanilic acid derivatives **1a-1h** is accelerated

Scheme I

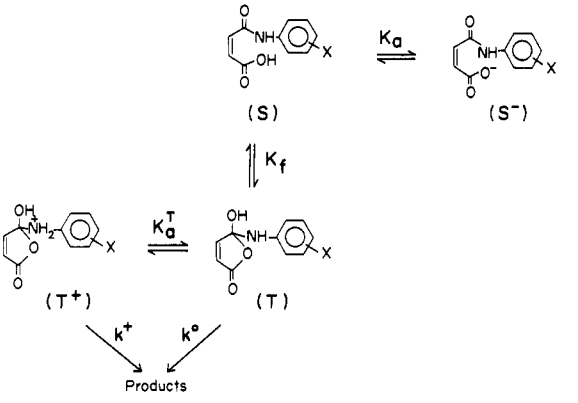
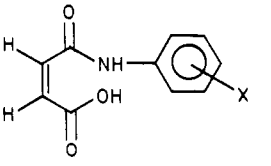


Table I. Parameter Values for the Hydrolysis of 1b-h at 50 °C

compd	$10^3 k_{H^+}$, M^{-1} s^{-1}	$10^4 k_W$, s^{-1}	pK_{AN}[a]
1b	18	4.5	2.22
1c	25	13	4.49
1d	6.0	1.5	2.62
1e	3.8	2.8	4.38
1f	3.0	2.4	4.37
1g	1.7	0.70	3.78
1h	0.087	0.050	4.05

[a] Taken from ref 24.



1a, X = *o*-COOH
b, X = *o*-$COCH_3$
c, X = *o*-OCH_3
d, X = *o*-Cl
e, X = *o*-CH_3
f, X = *o*-CH_2CH_3
g, X = *o*-C_6H_5
h, X = 2,6-(*i*-C_3H_7)$_2$
2a, X = *p*-COOH

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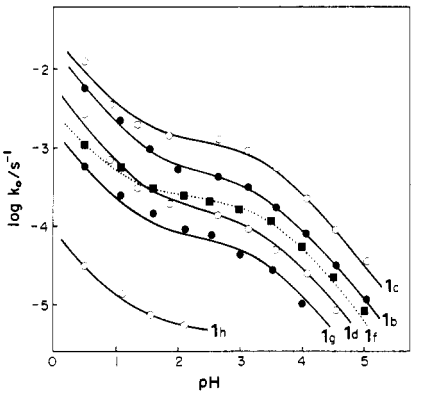


Figure 1. pH dependence of log k_0 for the hydrolysis of **1b–h** at 50 °C. The data points for **1e**, which are not included in this figure, lie slightly above those of **1f**.

by polar ortho substituents, as will be reported in this paper. The rate enhancement is believed to come from stabilization of the positive charge developed on the leaving nitrogen atom in the transition state by the polar ortho substituents.

Results

Part of rate data for **1b–h**, maleanilic acid derivatives without any ionizing substituent, are illustrated in Figure 1. The pseudo-first-order rate constants (k_0) obtained by extrapolation to zero buffer concentrations are plotted against pH in this figure. Analysis of the pH dependence of k_0 was made according to the mechanism (Scheme I) determined by Kluger and Lam.[18-20] In this mechanism, both T and T^+ are converted to products upon reaction with water. Thus, the predominant reaction path at pH <2 (acid path) is the water attack at the hydroxyl hydrogen of T^+ with simultaneous cleavage of the acyl–nitrogen bond.[18,19] General bases can also attack the hydroxyl hydrogen of T^+, leading to apparent general-acid catalysis.[19] At pH >2, the predominant path is the reaction of water with T (water path), which proceeds through a solvent-mediated proton transfer between the hydroxyl and the amino groups with the concerted cleavage of the O–H and the C–N bonds.[18,20]

The rate expressions of Scheme I are shown in eq 1–3.

$$k_0 = (k_{H^+}[H^+] + k_W)/(1 + K_a/[H^+]) \quad (1)$$

$$k_{H^+} = k^+K_f/K_a^T \quad (2)$$

$$k_W = k^0K_f \quad (3)$$

Here, k_{H^+} and k_W are the pseudo-first-order rate constants for the acid path and the water path, respectively. The values of k_{H^+} and k_W for **1b–h** obtained by analysis with eq 1 are summarized in Table I. The pK_a values of the carboxyl groups calculated from the rate data are 3.35–3.45, being very close to those (3.40)[18] measured kinetically with meta- or para-substituted maleanilic acid derivatives.

The pH dependences of k_0 for **1a** and **2a** are illustrated in Figures 2 and 3, respectively. The bell-shaped curve for **1a** at pH >2 indicates the ionization of two functional groups. The decrease in k_0 for **2a** at pH >2 occurs over a much wider pH range than expected from the ionization of a single group. These pH profiles are consistent with Scheme II, which includes the ionization of the substituents. In this scheme, the productive reaction paths involve T^- in addition to T and T^+. The rate expressions derived

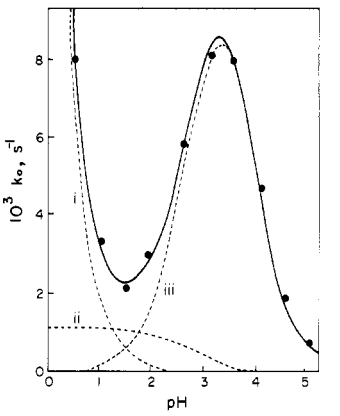


Figure 2. pH dependence of k_0 for the hydrolysis of **1a** at 50 °C.

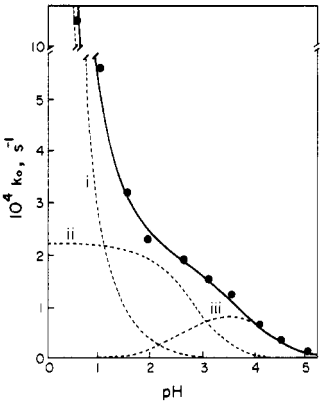
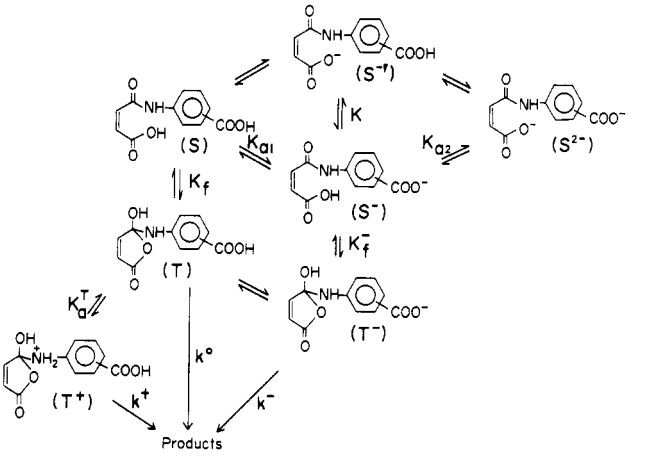


Figure 3. pH dependence of k_0 for the hydrolysis of **2a** at 50 °C.

Scheme II



are given in eq 4 and 5. Here, k_H^+ and k_W are as defined by eq 2 and 3.

$$k_0 = \frac{(k_{H^+}[H^+] + k_W)\dfrac{[H^+]}{K_{a1}(1+K)} + \dfrac{k_W^-}{(1+K)}}{\dfrac{[H^+]}{K_{a1}(1+K)} + 1 + \dfrac{K_{a2}}{[H^+](1+K)}} \quad (4)$$

$$k_W^- = k^-K_f^- \quad (5)$$

Parameter values obtained by analysis of the rate data for **1a** and **2a** according to eq 4 are summarized in Table II. Values of k_W^- are estimated from the experimental values of $k_W^-/(1 + K)$, with $(1 + K)$ being calculated from the kinetically determined values of p$K_{a2}/(1 + K)$ and the

Table II. Parameter Values for the Hydrolysis of 1a and 2a at 50 °C

parameter	1a	2a
$10^3k_{H^+}$, M^{-1} s^{-1}	22	3.5
10^4k_W, s^{-1}	11	2.2
$10^4k_W^-/(1 + K)$, s^{-1}	140	1.3
$10^4k_W^-$, s^{-1}	310	5.2
$pK_{a1}(1 + K)$	2.85	2.90
$pK_{a2}/(1 + K)$	3.80	4.00
pK_{AN} [a]	2.1 [c]	2.45 [d]
pK_{AN}^- [b]	3.8 [c]	3.79 [d]

[a] pK_a of anilinium ion with *o*-COOH or *p*-COOH substituent. [b] pK_a of anilinium ion with *o*-COO^- or *p*-COO^- substituent. [c] Calculated from the ionization constants reported in ref 25. [d] Taken from ref 25.

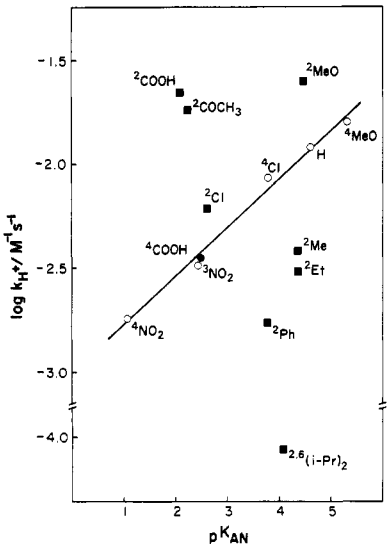


Figure 4. Plot of log k_{H^+} against pK_{AN} for the hydrolysis of various maleanilic acid derivatives: ○, unsubstituted and meta- or para-substituted derivatives reported in the literature;[18] ●, para-substituted derivatives investigated in the present study; ■, ortho-substituted derivatives investigated in the present study. Conditions of the kinetic measurements in the present study are identical with those employed for the open circle data points.

normal pK_a (3.4) of the maleic carboxyl group. The solid line of Figure 2 or 3 is drawn as the sum of dotted curves i–iii, which represent reaction paths of T^+, T, and T^-, respectively. The bell shape of the pH profile above pH 2 for **1a** is the result of the much faster reaction of T^- compared with T.

The values of log k_{H^+} and log k_W for the hydrolysis of various maleanilic acid derivatives are plotted against the corresponding pK_{AN} (the pK_a of the conjugate acid of the leaving aniline moiety) values in Figures 4 and 5. Parameter k_W^- represents the water path in the hydrolysis of maleanilic acid derivatives with anionic carboxylate substituent, and log k_W^- is plotted in Figure 5 together with log k_W.

Discussion

A linear free energy relationship is manifested by meta- or para-substituted maleanilic acid derivatives in the plots of log k_{H^+} and log k_W against pK_{AN}. The slope (β_{LG}) of such a linear line reflects the effectiveness of substituents in stabilizing the excessive partial charge developed on the leaving atom in the rate-controlling transition state.[26,27] Thus, the β_{LG} values of Figures 4 (slope 0.23) and 5 (slope

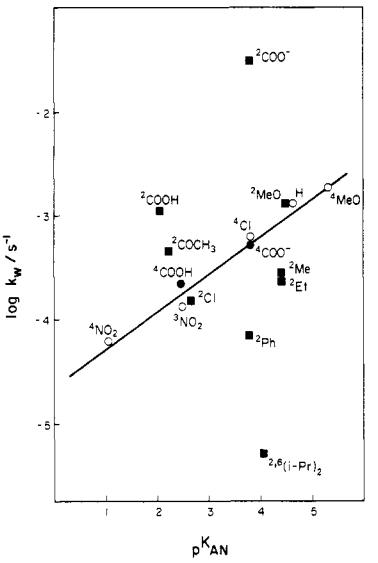
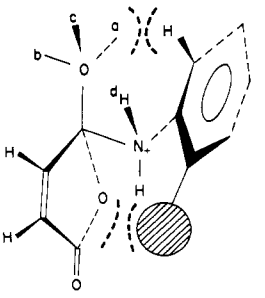


Figure 5. Plot of log k_W against pK_{AN} for the hydrolysis of various maleanilic acid derivatives. For the explanation of the symbols, see Figure 4.

0.36) indicate that the excessive positive charge developed on the leaving nitrogen atom in the rate-controlling transition state compared with the ground state is about 0.3 both in the acid and in the water paths. Since the amide nitrogen in the ground state has a δ_+ of about 0.6[26] due to resonance, a δ_+ value of about 0.9 can be assigned to the leaving nitrogen in the transition state.

Nucleophilic reactions on acyl derivatives are generally retarded when bulky substituents are introduced into the vicinity of the reaction center (on the acyl portion, the leaving group, or the nucleophile),[26,28,29] unless these substituents play catalytic roles or induce steric compression between the reacting groups. This is because the rate-controlling transition state is more crowded than the ground state. The rate retardation caused by 2,6-diisopropyl, *o*-phenyl, *o*-ethyl, and *o*-methyl groups is in accordance with the increased crowdedness in the transition state. However, the derivatives containing *o*-carboxylate, *o*-carboxyl, and *o*-acetyl substituents (and, although to much lesser extents, those with *o*-methoxy and *o*-chloro substituents) are hydrolyzed faster than expected from the linear lines.

As the leaving nitrogen atom is almost fully protonated in the rate-controlling transition state, the substituent effects on the transition state would be almost identical with those on T^+. Based on the space-filling and the Dreiding models, the most stable conformation of T^+ can be represented by structure I. The nonbonded interaction



I

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between the lactone and the benzene rings is minimized in this conformation. When located on the side of the hydroxyl group instead of the lactone ring, the ortho substituent suffers from a severe steric interaction with the hydroxyl group.[30] As the ortho substituent becomes bulkier, the nonbonded interaction between the ortho substituent and the lactone ring becomes greater. This can be related to the rate retardation caused by nonpolar ortho substituents. In the presence of carbonyl ortho substituents, the most stable conformation involves a close contact between the carbonyl oxygen and the anilinium hydrogen. Attempts to move them apart by rotation around the C(carbonyl)–C(Ph) or the N–C(Ph) single bond led to an increased nonbonded interaction either between the ortho substituent and the lactone ring or between the *o*-hydrogen and the hydroxyl oxygen. Therefore, the rigid conformations of the *N*-alkyl group and the phenyl portion of $T^{\pm}$ appear to lock the polar ortho substituent into close proximity with the protonated nitrogen. This would result in effective stabilization of the positive charge developed on the nitrogen by the polar ortho substituents through hydrogen bonding or electrostatic interaction.[31,32]

For acetal hydrolysis, a rate enhancement of 22 due to electrostatic stabilization of a carbonium ion by a neighboring carboxylate group has been proposed as being near maximal in water.[8] In this regard, the rate enhancement of about 55 observed in the present study (k_W of *o*-COO^-) is very significant.[33]

Experimental Section

Maleanilic Acid Derivatives. Compounds **1a–h** and **2a** were prepared by the reaction of the corresponding aniline (1 g) with maleic anhydride (1 g) in 30 mL of ether at room temperature for 1–3 h. Crystals formed were filtered, washed with ether, and recrystallized from acetone–hexane or ethanol–hexane. Melting points are 193–194 °C for **1a**, 124–125 °C for **1b**, 147–148 °C for **1c**, 135–136 °C for **1d**, 121–122 °C for **1e**, 104–105 °C for **1f**, 169–170 °C for **1g**, 184–186 °C for **1h**, and 227–228 °C for **2a**. Elemental analysis (C, H, N) on the compounds gave satisfactory results.

Kinetic Measurements. Reaction rates were measured with a Beckman Model 5260 UV/vis spectrophotometer by observing the decrease in amide absorbance in the 250–300-nm region, depending on the particular compound studied. Temperature was controlled to within ±0.1 °C with a Haake E52 circulator. The reactions were carried out at 50 °C, and evaporation of the solvent during kinetic measurements was prevented by sealing the cuvettes tightly with serum caps. Kinetics were performed at an ionic strength of 0.5 which was adjusted with sodium chloride. Buffers (0.1–0.5 M) used were chloroacetate (pH 2.5–3), formate (pH 3.5–4), and acetate (pH 4.5–5). pH measurements were carried out with a Fisher Accumet Model 525 pH meter at 50 °C, except at pH 0.5 which was calculated from the added concentration of hydrochloric acid. Water was distilled and deionized before being used in kinetic studies. Substrates were added to 3 mL of buffer solutions either in 25-μL portions in 0.01 N NaOH solution in which the hydrolysis is very slow or in acetonitrile (25 μL). The addition of 0.8% (v/v) acetonitrile to the reaction media did not affect the kinetic data appreciably. Concentrations of the substrates were (1–5) × 10^{-4} M. Pseudo-first-order kinetics were observed up to at least 3 half-lives for relatively fast reactions. For reactions whose half-lives are greater than 2 h, pseudo-first-order rate constants were calculated by the Guggenheim method or by the initial rate method. Identification of products was performed according to the literature.[18]

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Registry No. **1a**, 62530-49-8; **1b**, 86162-55-2; **1c**, 31460-26-1; **1d**, 53616-16-3; **1e**, 53616-19-6; **1f**, 86162-56-3; **1g**, 86162-57-4; **1h**, 86162-58-5; **2a**, 5432-04-2.

(30) The hydroxyl hydrogen can take either position a, b, or c. Position a, however, suffers from a 1,3-diaxial steric interaction with the benzene ring and appears to be the least preferred one. In the water-mediated proton-switch mechanism for the water path, the cyclic transition state must involve positions c and d regardless of the number of the water molecules participating in the proton switch, according to the molecular models. In the reaction of $T^{\pm}$, the water molecule would attack the hydroxyl hydrogen at position b. These statements, however, do not contradict Deslongchamps' stereoelectronic theory (Deslongchamps, P. *Tetrahedron*, **1975**, *31*, 2463).

(31) Although polar ortho substituents form hydrogen bonds with the amino group of the ortho-substituted anilines in nonpolar solvents (Lady, J. H.; Whetsel, K. B. *Spectrochim. Acta* **1965**, *21*. 1669), the intramolecular hydrogen bonds are disrupted in polar solvents producing intermolecular hydrogen bonds (Bekárek, V.; Kavâlek, J.; Socha, J.; Andrýsek, S. *Chem. Commun.* **1968**, 630). On the other hand, N-alkylation of the ortho-substituted anilines can bring significant hydrogen bonding or electrostatic interaction between the amino group and the polar ortho substituent even in polar solvents, as exemplified by the increased stability of the zwitterionic forms of *N*-alkylanthranilic acids (Uhlig, E.; Doering, K. *Chem. Ber.* **1964**, *97*, 1127. Tramer, A. *J. J. Mol. Struct.* **1969**, *4*, 313).

(32) For acetanilide and the majority of its phenyl-substituted derivatives, the amide and the benzene planes are twisted from coplanarity both in the crystalline state and in solution in order to avoid the nonbonded interaction between the two groups (Brown, C. J.; Corbridge, D. E. C. *Acta Crystallogr.* **1954**, *7*, 711. Stewart, W. E.; Siddall, T. H. *Chem. Rev.* **1970**, *70*, 517). On the other hand, the coplanarity of the two planes in acetanilide derivatives with polar ortho substituents can accommodate hydrogen bonds between the amide nitrogen and the ortho substituents in spite of the increased steric interaction, as exemplified by the crystal structure of *N*-acetylanthranilic acid (Mascarenhas, Y. P.; de Almeida, V. N.; Lechat, J. R. *Acta Crystallogr., Sect. B* **1980**, *B36*, 502). Such hydrogen bonds which still exist in nonpolar solvents, however, are disrupted in polar solvents with the formation of intermolecular hydrogen bonds (Rae, I. D. *Can. J. Chem.* **1968**, *46*, 2589. Sopchik, A. E.; Kingsbury, C. A. *J. Chem. Soc., Perkin Trans. 2* **1979**, 1058). The rate enhancement observed in the present study with polar ortho substituents, therefore, cannot be attributed to the intramolecular hydrogen bonding and the consequent crowdedness in the ground state.

(33) The actual rate enhancement due to the stabilization of the positive charge becomes much greater than 55 when the unfavorable steric effect caused by the bulkiness of the ortho substituents is considered.