The Intramolecular Facilitated Hydrolytic Rates of Methyl-Substituted Succinanilic Acids[1]

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Abstract: pH–rate profiles for hydrolysis of several methyl-substituted succinanilic acids have been determined. The cleavage rate of tetramethylsuccinanilic acid in particular evidenced the powerful cyclizing effect of alkyl substitution in promoting the intramolecular facilitative role of the free carboxyl group, the particular anilic acid hydrolyzing at 25° with a half-life of 15 min at pH 4. Data are presented for methyl-, 2,2-dimethyl-, *meso*-2,3-dimethyl-, *dl*-2,3-dimethyl-, trimethyl-, and tetramethylsuccinanilic acid together with those for succinamic acid and succinylamphetamine.

Hydrolytic cleavage of amides at room temperature under near-neutral conditions normally occurs rather slowly. Compounds such as, for example, acetamide and acetanilide exhibit half-lives of several hundred years at pH 5. Bender[3,4] has shown, however, that the case of phthalamic acid hydrolysis proceeds markedly faster, the increased rate being ascribed to intermediate formation of phthalic anhydride. More recently other amic acids[5,6] capable of forming cyclic anhydrides have been shown to cleave quite rapidly. In each instance the observed mechanism appeared to involve the free-acid form or its isoprotonic equivalence.

The present communication is concerned with results of attempts to determine the extent to which such intramolecular facilitations may be accentuated by selecting systems which particularly would be expected to favor such mechanisms. Since alkyl substitutions on the methylene backbone of succinic acid have been shown to facilitate formation of cyclic acid anhydrides,[7] the corresponding succinamic acids were selected for hydrolytic studies. The specific amides studied included succinanilic acid and its monomethyl, *meso*-dimethyl, 2,2-dimethyl, *rac*-2,3-dimethyl, trimethyl, and tetramethyl derivatives.

Experimental Section

Materials. The succinanilic acids were generally prepared according to the method described by Fieser[8]: succinanilic acid, mp 146°; methylsuccinanilic acid, mp 169°; 2,2-dimethylsuccinanilic acid, mp 190°; *rac*-2,3-dimethylsuccinanilic acid, mp 137°. The melting points were in good agreement with previously reported values.

Both trimethyl- and tetramethylsuccinanilic acids were found to be too unstable to permit isolation. In these instances the corresponding anils were prepared and hydrolyzed to the anilic, the resulting alkaline solution being sufficiently stable to be used as a stock solution for short periods. The ultraviolet spectra of these solutions corresponded to those expected of anilic acids.

Procedures. The rates of hydrolysis of the succinanilic acids were generally determined as follows.

Volumetric flasks (100 ml) containing approximately 150 mg of an anilic acid and 100 ml of preheated phosphate buffer at an ionic strength of 1 were placed in a thermostated oil bath. At intervals, 5-ml samples were withdrawn and diluted with 0.25 *M* disodium phosphate solution to a final volume of 25 ml, the resulting solution having a final pH of approximately 8 at which the hydrolytic reaction is effectively quenched.

The absorbances of the resulting solutions were measured at 240 mμ (λ_{max} for the anilic acids) and 280 mμ (λ_{max} for aniline), the spectra being essentially that expected of mixtures of aniline and amides. From the absorbances of the systems at infinite time the apparent first-order rate constant for each system was obtained from the usual semilog plots of pseudo-first-order reactions.

The hydrolysis of tetramethylsuccinanilic acid took place so rapidly that the above procedure proved unfeasible. The reaction in this instance was followed directly in the sample cell of a thermostated Cary 11 spectrophotometer. Approximately 20 μl of solution containing 4 mg of the succinanilic acid per milliliter of dilute sodium hydroxide was introduced into the cell followed by addition of 3 ml of an acidifying solution, the final system having an ionic strength of approximately 1. The change in absorbance was followed at 240 mμ and the apparent first-order rate constant was calculated as before.

Results and Discussion

The pH profile of the observed hydrolytic rate for tetramethylsuccinanilic acid is given in Figure 1. The behavior up to pH 7 is essentially that previously observed for the unsubstituted succinanilic acid[5] as shown in Figure 2, the plateau found between pH 2 and 4 again being probably attributable to intramolecular facilitative mechanisms participated in only by the uncharged, free acid. The major notable difference between the two amides in the neutral-to-acid regions is in the relative rate of hydrolysis, the persubstituted compound cleaving approximately 1200 times faster at 25° than the unsubstituted compound.

The degree of facilitation produced by evidently sterically favored free carboxyl group is truly remarkable. At room temperature the amide function in tetramethylsuccinanilic acid is cleaved with a half-life of less than 15 min at pH 2.5 and no more than 30 min at pH 5.0. This compares with half-lives of more than 2 and 300 years, respectively, at the two pH values for acetanilide, the hydrolytic rates of other amides such as acetamide and benzamide being of the same order of magnitude.

Experimentally observed rates of carboxyl-facilitated hydrolysis of variously substituted succinanilic acids determined at 25 and 60° are shown in Table I. These

(1) This work was supported by grants from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

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Table I. Intramolecular Rate Constants for Anilic Acids

Compound	At 25° (× 10^7, sec^{-1})	At 60° (× 10^5, sec^{-1})	E_A, kcal/mole	ΔS, eu
Succinanilic acid	6.5	2.1	19.5	−23.7
Methylsuccinanilic acid	13.1	5.55	21.1	−16.8
meso-2,3-Dimethylsuccinanilic acid	41.2	11.8	18.9	−21.5
2,2-Dimethylsuccinanilic acid	76.2	24.3	19.5	−19.0
rac-2,3-Dimethylsuccinanilic acid	178	49.2	18.7	−19.4
Trimethylsuccinanilic acid	2250	273	17.4	−20.0
Tetramethylsuccinanilic acid	8150	805	12.9	−31.3

intramolecular rate constants correspond essentially to the first-order constant for these systems in the pH range 2.5–3.0. Actual runs were not always carried out at 25 and 60° but at generally four convenient temperatures and the values listed were read from Arrhenius-type plots, some of these being shown in Figure 3. The apparent heats of activation calculated from the slopes of these lines are also given in the table together with estimates of apparent entropies of activation following Eyring.

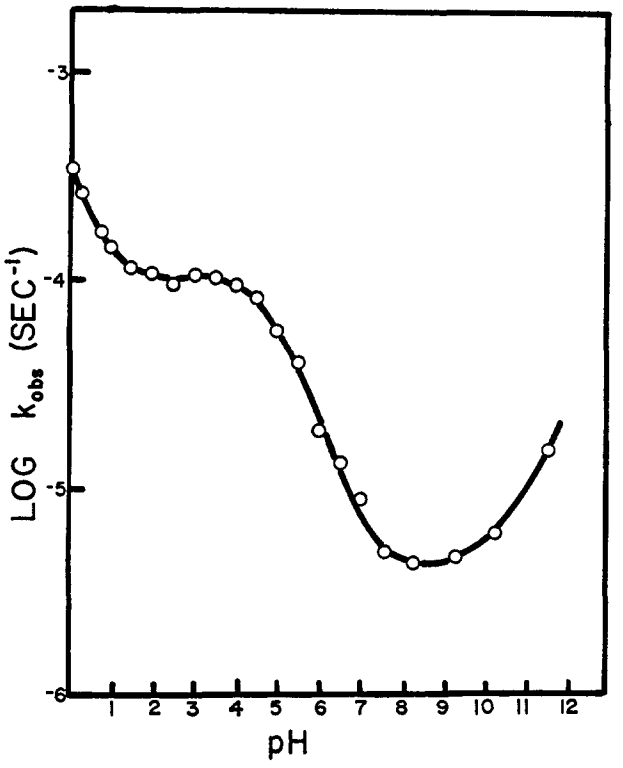


Figure 1. Plot showing rate of disappearance of tetramethylsuccinanilic acid at 25.5° as a function of pH in 0.25 *M* phosphate buffer.

It is evident from the table that the degree of facilitation is strongly influenced by alkyl substitution as expected. Every increase in substitution going from succinanilic acid to tetramethylsuccinanilic acid seemed to have produced very roughly equivalent effects, the introduction of the first methyl apparently having the least influence. The fourfold difference in the rates between the asymmetric and the *meso* form of 2,3-dimethyl-substituted anilic acids is apparently a direct measure of the two systems' ability to cyclize.

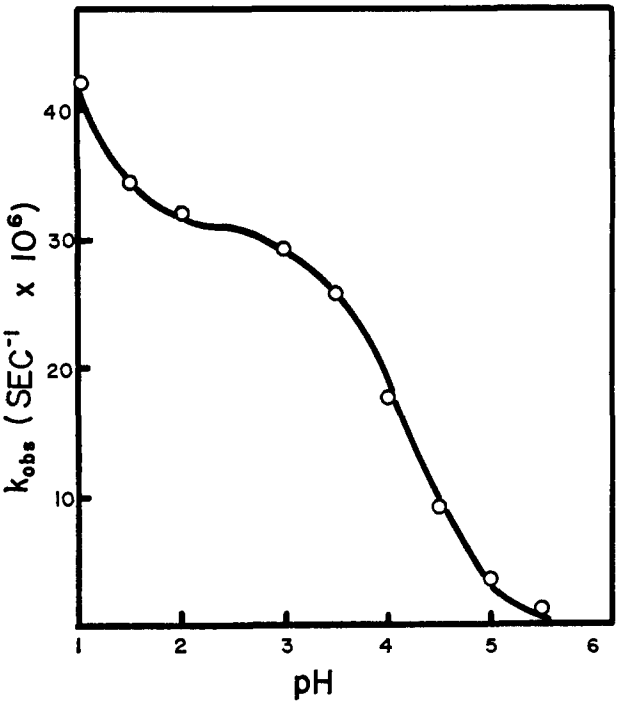


Figure 2. pH–rate profile for hydrolysis of unsubstituted succinanilic acid at 65°. The smooth curve corresponds to the rate profile expected on the basis of uncatalyzed and proton-catalyzed reaction of only the free-acid form.

The pH–rate profiles of hydrolysis of the succinanilic acids show rapid increase in rate in the very acid range as is evident, for example, in Figure 1 below pH 1. The apparent second-order rate constants of the proton-dependent reaction of the unsubstituted and the tetrasubstituted anilic acids calculated from data in this range are shown listed in Table II along with those of other common amides. The data strongly suggest that the proton-induced hydrolysis of the amide derived from the unsubstituted acid is essentially normal but that of the substituted anilic acid may reflect some degree of internal facilitation. The observed 1000-fold greater rate in the latter instance is difficult to rationalize on any other basis. Bender[4] has also reported faster rate of acid-catalyzed hydrolysis of phthalamic acid as compared to benzamide also shown in the table.

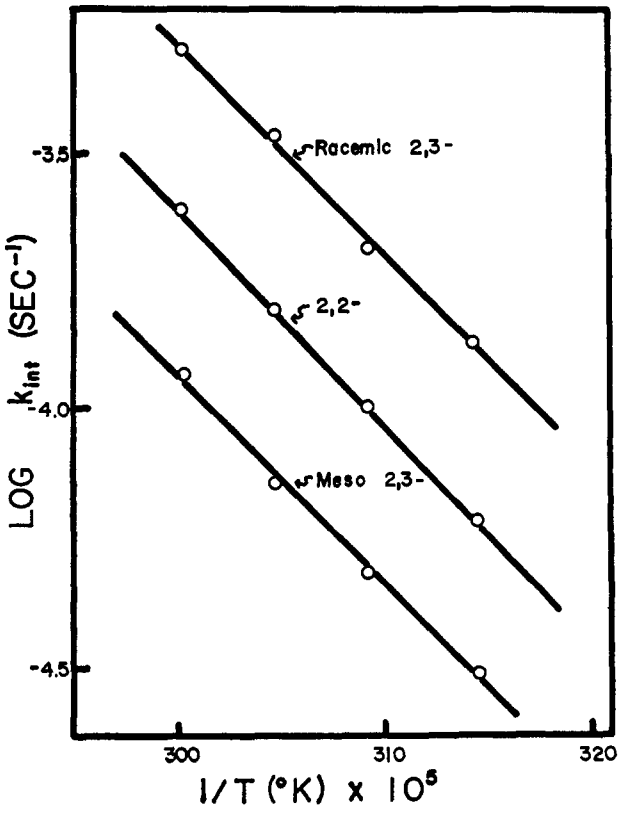
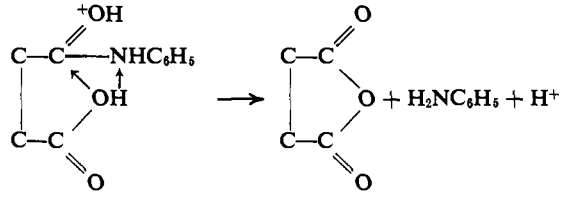


Figure 3. Arrhenius-type plots for the three dimethyl-substituted succinanilic acids.

A mechanism analogous to that proposed earlier by Bender for hydrolysis of uncharged phthalamic acid seems to be logically applicable for the proton-assisted system. Thus



the carboxylic group in this instance attacking the protonated amide function. It is evident that the steric contribution would be equally effective for both modes of intramolecular facilitation.

Table II. Specific Proton-Catalyzed Hydrolytic Rate Constants

Compound	Temp, °C	k_{H^+}, l./mole sec	Ref
Acetanilide	25	2.5×10^{-6}	*a*
Succinanilic acid	25	1.37×10^{-6}	
Tetramethyl-succinanilic acid	25	2430×10^{-6}	
Acetamide	65	206×10^{-6}	*b*
Succinamic acid	69.3	291×10^{-6}	*c*
Benzamide	48.7	3.1×10^{-6}	*c*
Phthalamic acid	47.3	540×10^{-6}	1

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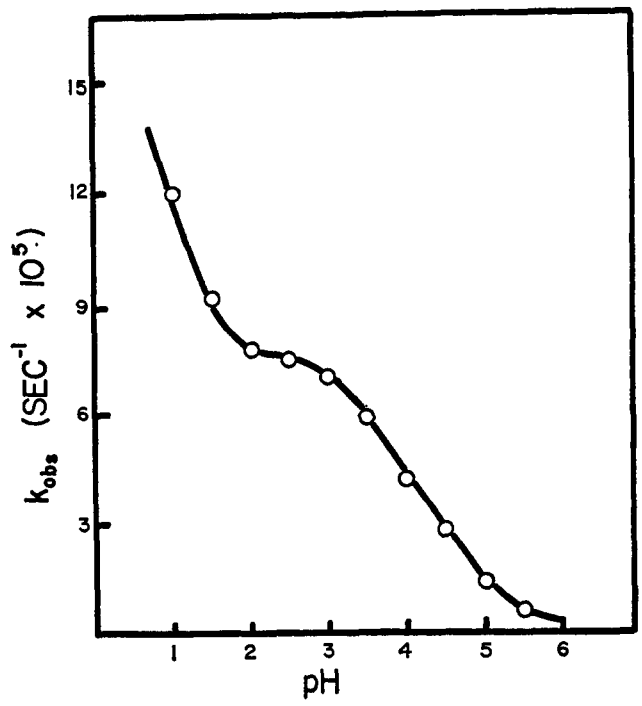

Figure 4. pH–rate profile for hydrolysis of succinamic acid at 69.3° in 0.25 *M* phosphate buffer.

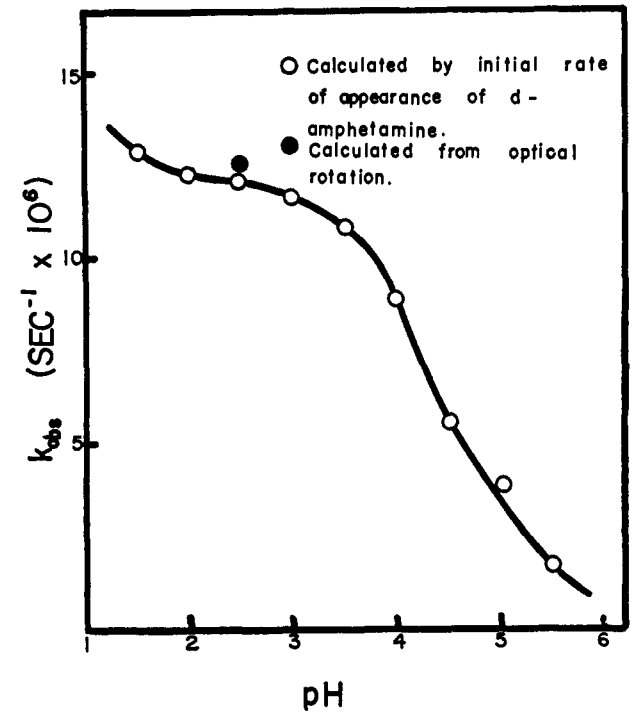

Figure 5. pH–rate profile of hydrolysis of the (*d*-amphetamine) monoamide of succinic acid at 75° in 0.25 *M* phosphate buffer.

The apparent rise in the reaction rate above pH 7 for tetramethylsuccinanilic acid as shown in Figure 1 is not ascribable to higher hydrolytic rate but to increased rate of attainment of equilibrium with the corresponding imide. Analysis of the reaction products of pH 8 and higher showed very little aniline present but a significant amount of the corresponding imide. Equilibrium mixtures (24-hr samples) showed the approximate distribution given in Table III. The anil in the pH 8.0

Table III

Species	Mole % present — pH 8.00	pH 11.55
Anilic acid	3.5	90.2
Anil	94.8	9.3
Aniline	0.0	0.0

system largely precipitated from the solution, the value shown representing the sum of the amounts extracted from the solution phase and that in the precipitate. These results suggest that tetramethylsuccinanilic acid is unstable in aqueous solution, the amide hydrolyzing to yield protonated aniline in acidic solutions and forming the imide in neutral solutions. Only under highly alkaline conditions such that the effective concentration of the free acid is very low will the anilic acid be expected to be moderately stable, slow hydrolysis to dianion, however, probably taking place.

Although the preceding data have been limited to hydrolysis of anilides there appears to be no reason to feel that essentially similar situation does not exist for other amides. Comparable data for unsubstituted succinamic acid are shown in Figure 4 and for succinylamphetamine in Figure 5. In the latter instance the rate was followed by extraction of the amine formed, the rate at pH 2.5 being also checked polarimetrically. These compounds appear to cleave in essentially the same way and at rates roughly within the same order of magnitude as succinanilic acid.

Discussion

Because of the magnitude of the catalytic effect produced by favorably located carboxyl group in promoting amide formation and hydrolysis it is certainly attractive to consider the possibility of similar mechanism being directly or indirectly responsible for the catalytic properties of enzymes. The ease with which normally resistant amides can be cleaved under extremely mild conditions under the influence of favorably located carboxyl is suggestive. Enzymes behave as they do presumably because of their highly efficient structural organization. It does not require too great a stretch of imagination to picture one or more free carboxyls in these biocatalysts operating from sterically favorable position on peptide linkages.

Kinetics of Hydrolysis of Hydroxy and Methoxy Derivatives of N-Benzylidene-2-aminopropane

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Abstract: The kinetics of hydrolysis of *o*-, *m*-, and *p*-hydroxy-N-benzylidene-2-aminopropane, and of their methoxy analogs, has been investigated in the pH range from 0 to 14, at 30°, by means of ultraviolet spectrophotometry. Dissociation constants have been determined. The pH–rate profile is very similar to ones previously reported for similar compounds, except for a different behavior of the *o*- and *p*-hydroxy derivatives in the pH region where they predominantly exist as free bases, as well as for the three hydroxy compounds when they are converted into the anionic form. The pK_1 values for the addition of a proton to the *o*-hydroxy and *p*-hydroxy derivatives are very different from the corresponding values for the methoxy analogs, while the pK_1 of *m*-hydroxy and *m*-methoxy are identical. The pK_2 value for the formation of the anion of the *p*-hydroxy derivative is inconsistent with pK values of phenol derivatives, while the *m*-hydroxy compound seems to be normal in that respect. The possibility of a tautomeric equilibrium in strongly polar solvents is suggested by examination of the ultraviolet absorption spectra. This hypothesis allows a coherent interpretation of the totality of the kinetic data.

The hydrolysis reaction of Schiff bases has been extensively studied, especially as regards to the effect of substitution on the benzylidene ring in the case of alkyl aromatic molecules.[1-8] We have been led to investigate the effect of hydroxy substituents in a systematic study of stability of chelating compounds.[9-11] In order to help in the elucidation of some singularities observed in the spectral and kinetic behavior of the *o*- and *p*-hydroxy-N-benzylidene-2-aminopropanes, we have compared them to their methoxy analogs.

Experimental Section

Kinetic measurements were carried out spectrophotometrically with a Perkin-Elmer 350 spectrophotometer equipped with a cell holder thermostated at 30 ± 0.1°. In all kinetic runs, ionic strength was maintained at 0.10 by addition of KCl. Acetate, phosphate, borate, and carbonate buffers were used in their appropriate pH range.[12] All reactions were conducted in doubly distilled water containing 2% Spectrograde methanol for solublity. Spectra were scanned continuously from 400 to 200 mμ (or part of this range) at fixed time intervals (0.5 to 5 min), and the zero-time

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