

in intramolecular solvation of the reaction center.^{8,9} The largest $\Delta\Delta S^*$ value for the *m*-NO₂ substituent again supports the argument that it is in this case that the

(8) M. L. Bender, *Chem. Rev.*, **60**, 53 (1960).

(9) R. E. Lovins, L. J. Andrews, and R. M. Keefer, *J. Am. Chem. Soc.*, **84**, 3959 (1962), and references therein.

"unmixing" of the solvent in forming the solvation shell of the transition state is greatest since the specificity of hydration persists to the highest ethanolic composition.

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[CONTRIBUTION FROM THE SCHOOL OF PHARMACY, UNIVERSITY OF WISCONSIN, MADISON 6, WIS.]

Facilitated Reversible Formation of Amides from Carboxylic Acids in Aqueous Solutions. Intermediate Production of Acid Anhydride

BY TAKERU HIGUCHI, TOMONORI MIKI, ASHOK C. SHAH, AND ALLEN K. HERD

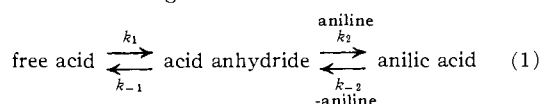
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The facilitative role of carboxylic grouping on formation and hydrolysis of certain amido acids is shown to be mediated by formation of acid anhydrides, which appears to be the rate-determining step. The mechanism is supported by (1) the pH-rate profile of conversion of succinic acid to succinic anhydride, (2) the pH profile of the reverse reaction, and (3) the independently determined pH profile of the equilibrium constant. The equilibrium concentration of the anhydride in water at 25° is estimated on this basis to be 2 parts per 10⁷ at 25° and 3 parts per 10⁶ at 95°. Citric acid, tartaric acid, and related di- and polycarboxylic acids are believed to react in similar fashion.

Introduction

In a recent preliminary communication¹ we have shown that certain dicarboxylic and polycarboxylic acids such as citric, tartaric, maleic, and succinic acids react relatively rapidly with aromatic amines in aqueous solution to form the corresponding amides. Detailed results of this and further studies elucidating the underlying mechanism responsible for the observed reaction are presented at this time.

Earlier rate measurements at 95° had suggested that the rate of amide formation in a solution of, for example, succinic acid and aniline was apparently second order, being proportional to the free acid and free amine concentrations. Measurements at higher free aniline concentrations and lower temperatures now indicate that under certain conditions the dependence on the amine may become substantially zero order. On this basis, the over-all reaction for a di- or polybasic acid with aniline, for example, is believed to proceed through the following series of reactions.



Data supporting this postulate and rate constants for the individual steps, both limited largely to the succinic acid-aniline interaction, are offered at this time.

Theoretical Considerations

Rate equations implicit in eq. 1 can be readily derived for certain fixed conditions. For the specific case consisting, for example, of a relatively low concentration of aniline in a large excess of succinate buffer we can formulate the relation as follows: Let A_0 represent the total initial concentration of aniline including both the protonated and the unprotonated species; A , the total aniline concentration at time t ; f_2 , the fraction of aniline in its free base form and equal to

$$\frac{K_a'/H^+}{1 + K_a'/H^+}$$

SH, the total concentration of the succinate buffer including the several ionized species; f_1 , the fraction of SH in the free acid form and equal to

$$\frac{1}{1 + K_{a1}/H^+ + K_{a1}K_{a2}/(H^+)^2}$$

$A_0 - A$, the total concentration of succinanic acid formed including both species; and f_{-2} , that fraction of the anilic acid in its free acid form and equal to $1/(1 + K_a/H^+)$. Since the concentration of the anhydride species would be expected to be always very small compared to those of other reactants and intermediates, it would be related to the concentrations of the primary reactants by

$$d[\text{anhyd.}]/dt = f_1 k_1 [\text{SH}] + f_{-2} k_{-2} (A_0 - A) - k_2 f_2 A [\text{anhyd.}] - k_{-1} [\text{anhyd.}] = 0$$

or

$$[\text{anhyd.}] = \frac{f_1 k_1 [\text{SH}] + f_{-2} k_{-2} (A_0 - A)}{k_{-1} + k_2 f_2 A}$$

If we assume that aniline is consumed only by the route indicated in (1), we have

$$dA/dt = f_{-2} k_{-2} (A_0 - A) - f_2 k_2 A [\text{anhyd.}]$$

Substituting this into the above, we have

$$\frac{dA}{dt} = \frac{k_{-1} f_{-2} k_{-2} (A_0 - A) - f_2 k_2 f_1 k_1 [\text{SH}] A}{k_{-1} + k_2 f_2 A} \quad (2)$$

$$= \frac{k_{-1} f_{-2} k_{-2}}{k_{-1} + k_2 f_2 A} \left[A_0 - A \left(1 + \frac{f_2 k_2 f_1 k_1 [\text{SH}]}{k_{-1} f_{-2} k_{-2}} \right) \right] \quad (3)$$

Equation 3 can be simplified by inserting equilibrium values of the components

$$\frac{f_{-2} (A_0 - A_\infty)}{f_1 [\text{SH}] f_2 A_\infty} = \frac{k_1 k_2}{k_{-1} k_{-2}}$$

where the subscript ∞ refers to equilibrium conditions or

$$\frac{A_0}{A_\infty} - 1 = \frac{k_1 k_2}{k_{-1} k_{-2}} \frac{f_2 f_1 [\text{SH}]}{f_{-2}} \quad (4)$$

Substituting eq. 4 into 3, we find

$$\begin{aligned} \frac{dA}{dt} &= \frac{k_{-1} f_{-2} k_{-2}}{k_{-1} + k_2 f_2 A} \left[A_0 - \frac{A_0}{A_\infty} A \right] \\ &= \frac{k_{-1} f_{-2} k_{-2} A_0}{k_{-1} + k_2 f_2 A} \left[1 - \frac{A}{A_\infty} \right] \end{aligned}$$

On integration we have

$$\begin{aligned} \frac{(f_2 k_2 / k_{-1})(A_0 / A_\infty)(A_0 - A)}{(A_0 / A_\infty) + A_0 f_2 k_2 / k_{-1}} + \ln \frac{A_0 - A}{A - A_\infty} &= \\ \frac{f_{-2} k_{-2} (A_0 / A_\infty)^2 t}{(A_0 / A_\infty) + A_0 f_2 k_2 / k_{-1}} &= \quad (5) \end{aligned}$$

Results and Discussion

The loss of aniline in a reacting system typical of those considered here is shown as a function of time in Fig. 1. The plot shows the residual concentration of

(1) T. Higuchi and T. Miki, *J. Am. Chem. Soc.*, **83**, 3899 (1961).

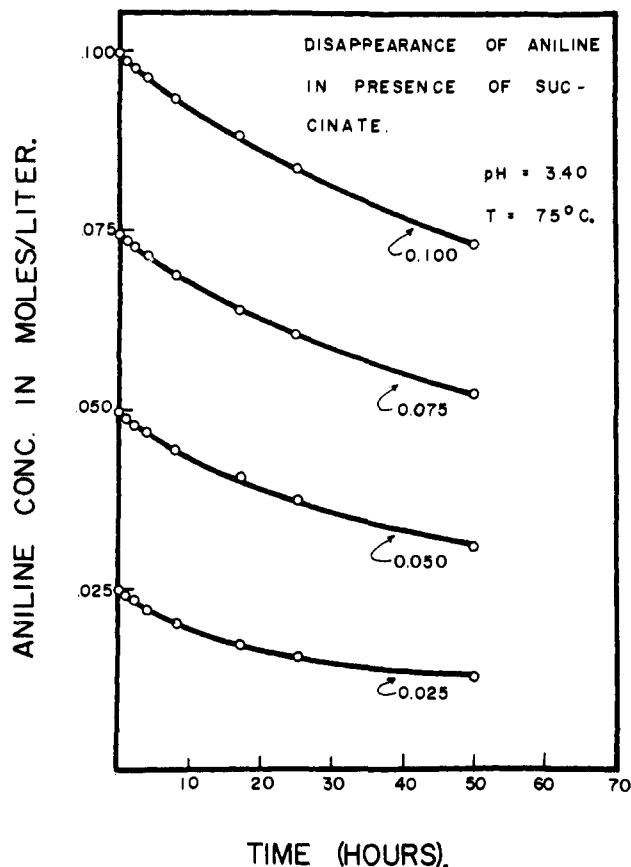


Fig. 1.—Residual aniline observed as function of time for the succinate-aniline interaction at pH 3.4 and at 75°.

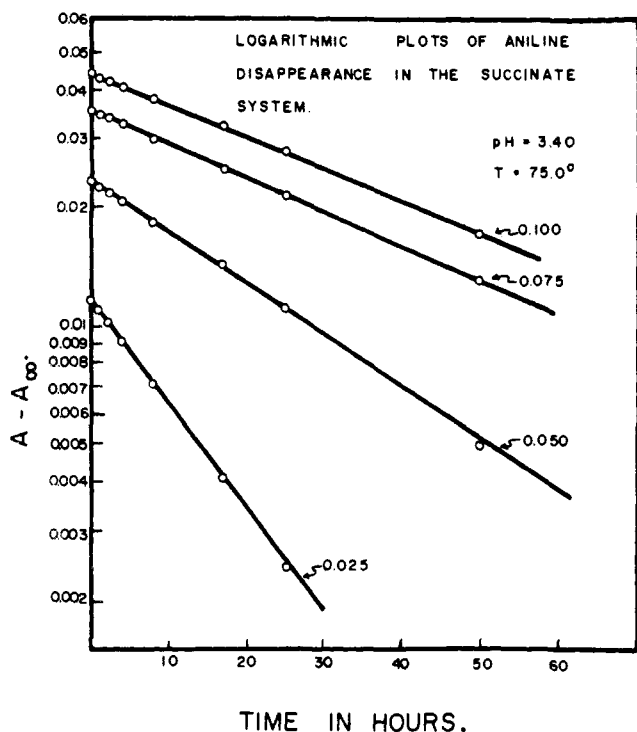


Fig. 2.—Plots showing that the rate of disappearance of aniline in presence of 0.5 M succinate is apparently proportional to its concentration above that at equilibrium for any given run.

aniline in a solution consisting initially of 0.025 to 0.100 M aniline in a 0.5 M pH 3.40 succinate buffer at 75°. As would be expected on the basis of eq. 1 the reaction does not go to completion but to equilibrium states con-

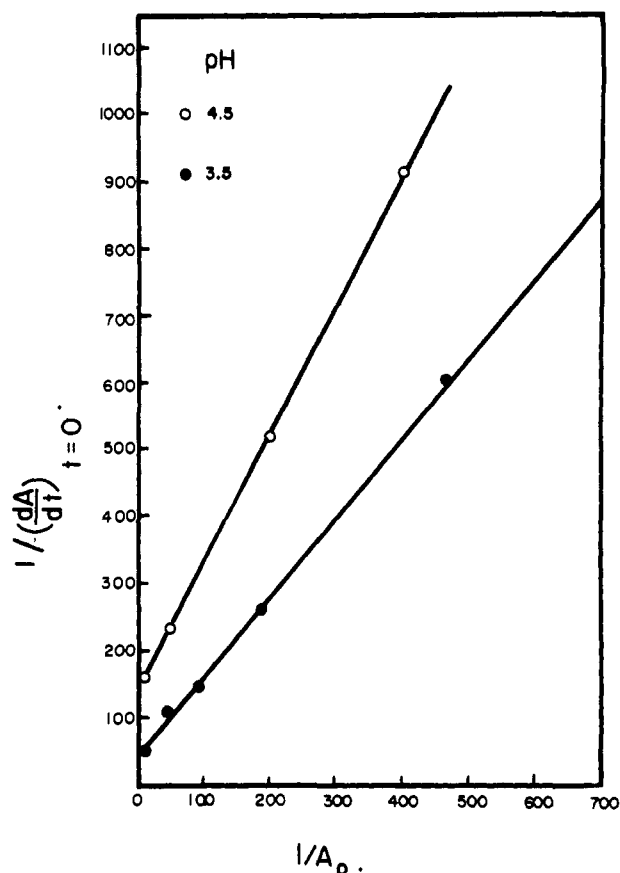


Fig. 3.—Plots of reciprocal initial rate of aniline disappearance in hr. l.⁻¹ mole⁻¹ against reciprocal initial aniline concentration in l. mole⁻¹ for aniline in presence of aqueous 0.5 M citrate buffer at pH 3.5 and 4.5 and at 75°.

taining substantial concentrations of the free amine. The $A - A_{\infty}$ values when plotted semilog fashion against time appear to yield linear plots as shown in Fig. 2. It is apparent even from these, however, that the approach to the equilibrium state is not of the first-order nature since the several lines representing different initial aniline concentrations have obviously different slopes.

Several of the rate constants shown in eq. 1 can be conveniently obtained from eq. 2 and initial rates of aniline disappearance if the postulated mechanism is correct. At $t = 0$, eq. 2 becomes

$$-\left(\frac{dA}{dt}\right)_{t=0} = \frac{f_2 k_2 f_1 k_1 [\text{SH}] A_0}{k_1 + k_2 f_2 A_0}$$

OR

$$-\left(\frac{1}{(dA/dt)}\right)_{t=0} = \frac{1}{k_1 f_1 [\text{SH}]} + \frac{k_{-1}}{k_2 k_1 f_1 f_2 [\text{SH}] A_0} \quad (6)$$

According to the above reciprocal relationship, a plot of the reciprocal of the initial rate of amine consumption against the reciprocal of the initial amine concentration will yield a straight line whose intercept is $1/k_1 f_1 [\text{SH}]$ and whose ratio of intercept to its slope is $k_2 f_2 / k_{-1}$. Since f_2 can be readily evaluated from the $\text{p}K_a'$ of amine and the pH of the system, k_2 / k_{-1} can be determined from the plot.

Determination of the Rate of Formation of Anhydride and k_1 .—A plot of the above type obtained for the system citric acid and aniline is shown in Fig. 3 at two pH values and for succinic acid and aniline in Fig. 4. In both instances fairly respectable linear relationships are followed. Values of $(dA/dt)_{t=0}$ were obtained from plots such as shown in Fig. 1 and 2. According to eq. 5 the logarithmic plot should not give

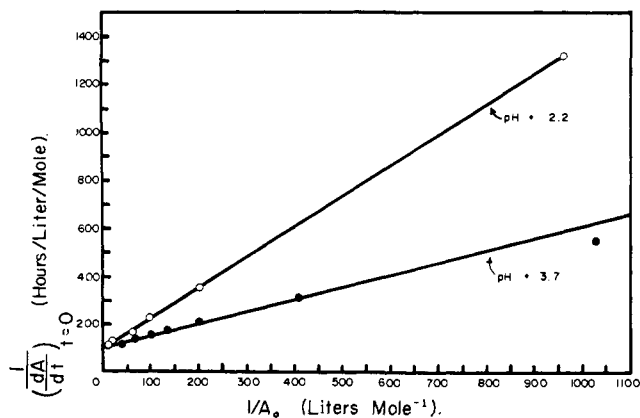


Fig. 4.—Reciprocal plots of the rate of disappearance of aniline in pH 2.2 and pH 3.7 succinate buffer (0.5 *M*) at 75° as functions of the reciprocal initial aniline concentration.

strictly linear plots, but in practice it is evident that it is difficult to see any serious deviation. Actual calculations show that this is to be expected on the basis of eq. 5. The log plots proved useful in obtaining extrapolated initial rate values for faster reactions.

It is evident from the reciprocal rate plots that at low amine concentrations these systems would behave essentially as simple second-order reactions. This would be particularly true for acidic solutions in which the free amine concentration would be relatively low as for the succinate system at pH 2.20 and at higher temperatures where k_2/k_{-1} values are smaller. The reality of the intercept values, however, appears to be beyond question. The method, therefore, seems to provide a useful tool for the determination of the specific rate of formation of acid anhydride, k_1 , in these buffers.

The rate of formation of succinic anhydride from succinic acid as calculated from such reciprocal plots at several temperatures and based on $pK_{a_1} = 4.4$ for succinic acid has been plotted in Fig. 5. Actual measurements were all made at pH 3.7 in presence of 0.5 *M* succinate buffer. The average slope of the line in the figure indicates an apparent heat of activation for this reaction of approximately 22.5 kcal./mole over the temperature range. The plot exhibits a slightly negative second derivative suggesting somewhat higher energy requirement for the reaction at lower temperatures. A crude estimation of the equilibrium concentration of free succinic anhydride in aqueous solutions of succinic acid is possible, based on k_1 values from the plot and k_{-1} values obtained directly by Koskikallio.² These calculations show that approximately 2 parts per 10^7 at 25° and 3 parts per 10^5 at 95° of free succinic acid exist in the form of the anhydrides.

The pH rate profile obtained for conversion of succinic acid to succinic anhydride is shown in Fig. 6. The f_1k_1 values obtained from the reciprocal plots are shown compared with the theoretical, normalized profile expected if it is assumed that only the free acid is capable of undergoing the reaction and $pK_{a_1} = 4.4$ and $pK_{a_2} = 4.8$. The fair fit between the two supports the assumption that only uncharged succinic acid is capable of undergoing the reaction or that some equivalent isoprotonic reaction is occurring.

Since hydrolysis of acid anhydride is subject to proton catalysis,² it is to be expected that its formation in water would be also accelerated by acid. Apparently this effect is not significant at pH 2 but would certainly be expected under more acid conditions. Other instances of such catalysis may exist; future

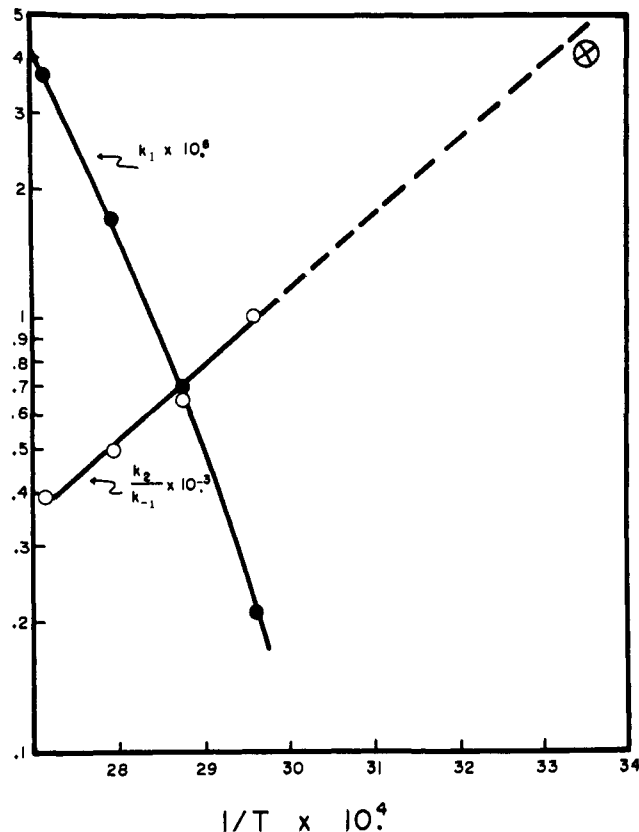


Fig. 5.—Temperature dependence of k_1 and k_2/k_{-1} . The values of k_2/k_{-1} shown were obtained from the slopes of the reciprocal plots except for the point shown as ⊗ which was determined by direct evaluation of k_2 and k_{-1} values at 25°.

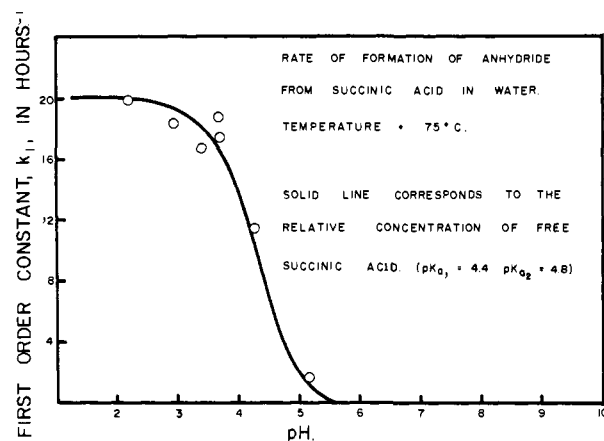


Fig. 6.—pH-rate profile of conversion rate of succinate buffer to succinic anhydride at 75° as determined from intercepts of plots similar to Fig. 2. The smooth line corresponds to the relative concentration of free succinic acid.

studies in this direction, following the more convenient reverse reaction, are planned.

Determination of k_2/k_{-1} Ratio.—An Arrhenius plot of the ratios k_2/k_{-1} obtained from the reciprocal plots and by use of eq. 4 is shown for the succinic acid-aniline interaction in Fig. 5. The values were those obtained at 95, 85, 75, and 65° at pH 3.7. It is evident that the rate of hydrolysis of succinic anhydride increases considerably faster with temperature than its reaction with aniline. It would therefore be expected as suggested above that the zero-order character of the over-all reaction would be more apparent at lower temperatures than at higher temperatures.

(2) J. Koskikallio, *Ann. Acad. Sci. Fenn. Series A-II*, **57**, 4 (1954).

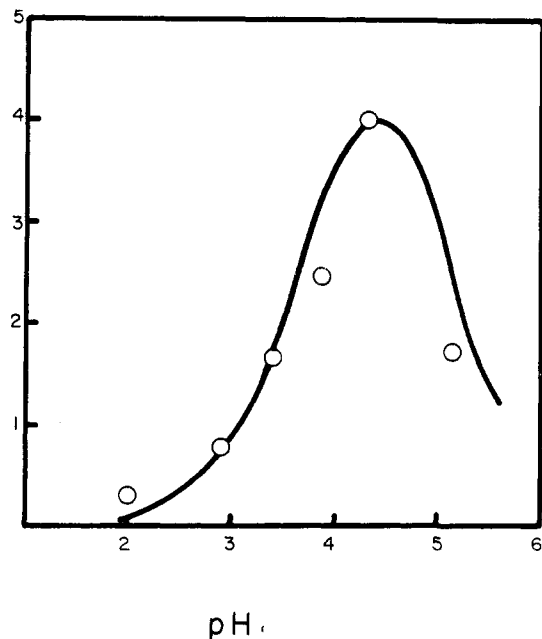


Fig. 7.—The pH profile of the apparent equilibrium constant as determined by the relationship $(A_0 - A_\infty)/[\text{SH}]A_\infty$. The solid line corresponds relatively to f_1f_2/f_{-2} .

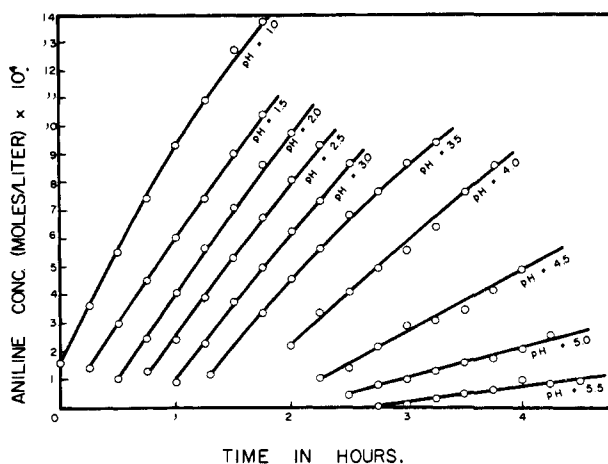


Fig. 8.—Plots showing the rate of appearance of aniline in an aqueous solution of succinanic acid in succinate buffers. The particular plots were obtained at 65°. The time axis has been displaced by a half an hour for each succeeding curve for the sake of clarity.

In further support of the postulated mechanism the individual values of k_2 and k_{-1} were determined for the succinic acid-aniline reaction. Koskikallio² obtained a value for k_{-1} , the specific rate constant for the hydrolysis of succinic anhydride, of $2.6 \times 10^{-3} \text{ sec.}^{-1}$ at 25°. He also determined k_2 for the reaction between succinic anhydride and aniline at 0°, obtaining what he called a very approximate value of $10 \text{ l. mole}^{-1} \text{ sec.}^{-1}$. These studies were repeated as a part of the present investigation and we obtained values of $k_{-1} = 2.5 \times 10^{-3} \text{ sec.}^{-1}$ at 25° for hydrolysis of succinic anhydride and $k_2 = 10.0 \pm 0.5 \text{ l. mole}^{-1} \text{ sec.}^{-1}$ also at 25° for its reaction with aniline. Thus the indicated value of k_2/k_{-1} is approximately 4000 l./mole at 25°. This is in good agreement with the extrapolated value for the same temperature as shown in Fig. 5.

Equilibrium Constant.—For the succinic acid-aniline system the reaction appears to reach an equilibrium state as indicated in Fig. 1. If we assume this to be the case and, again, that the succinate is in large stoichiometric excess, we have from (4)

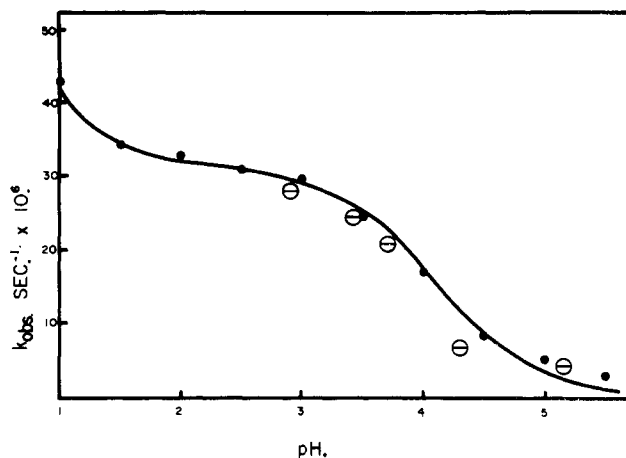


Fig. 9.—The pH profile for the rate of conversion of succinanic acid to succinic anhydride and aniline at 65° in aqueous solution. The small circles, ●, are values obtained by direct determination; ⊖ are those determined from the equilibrium constant and the remaining rate constants. The smooth curve is based on that calculated from eq. 7.

$$\frac{f_1f_2}{f_{-2}} = \frac{[1 + K_{s_1}/H^+]}{[1 + K_{s_1}/H^+ + K_{s_1}K_{s_2}/(H^+)^2][1 + H^+/K_{a_1}']} \times \frac{k_{-1}k_{-2}}{k_1k_2[\text{SH}]} \frac{(A_0 - A_\infty)}{A_\infty}$$

In Fig. 7 the experimental values found for $(A_0 - A_\infty)/A_\infty[\text{SH}]$ at 75° at 0.5 *M* succinate buffer have been plotted (open circles) against the pH of the reaction system.

The smooth line superimposed on the plot corresponds to the left-hand term in the above expression normalized to approximately the same maximum value and based on $\text{p}K_{a_1} = 4.4$, $\text{p}K_{a_2} = 4.8$, $\text{p}K_a = 4.15$, and $\text{p}K_{a_1}' = 3.8$.

It was necessary to determine the constants under conditions comparable to those existing in the reacting mixtures since the several solute species appear to mutually influence their degrees of dissociation. Succinanic acid, for example, in sodium chloride solution showed much weaker tendency to ionize than in presence of succinate, $\text{p}K_a = 4.4$ vs. 4.15 in succinate. Our value for $\text{p}K_a$ for succinic acid differs significantly from the value 5.8 reported by Pinching and Bates at this temperature.³ This can probably be attributed to a concentration effect.

Determination of k_{-2} .—The specific rate constant, k_{-2} , of the reaction corresponding to conversion of succinanic acid back to succinic anhydride and aniline can be determined by either (1) actual measurements on succinanic acid or (2) from the equilibrium constant, $k_1k_2/k_{-2}k_{-1}$ and the values for k_1 and k_2/k_{-1} determined above. Both determinations appear to give essentially the same values.

Figure 8 shows the rate of appearance of aniline from a 0.00518 *M* solution of succinanic acid in 0.25 *M* succinate buffer at 65°. The time coordinate in this plot has been displaced for successive determination to prevent overlaps of points. The initial rates obtained from the figure were used to construct Fig. 9, which shows the pH profile of the reverse reaction, the experimental pseudo-first-order constants being shown as open circles. The smooth curve, which is in substantial agreement, represents the theoretical relationship expected from eq. 1 corrected for proton attack at very high acidity

(3) G. D. Pinching and R. G. Bates, *J. Res. Natl. Bur. Std.*, **45**, 322 (1950).

$$k_{\text{obsd}} = \frac{k_{-2}}{1 + K_a/[H^+]} + k_H^+[H^+] \quad (7)$$

with k_{-2} and k_H^+ values as shown in Table I. Other isoprotonic mechanisms can be formulated but are not considered here.

Except for possibly a slight deviation at higher pH values, hydrolysis of succinamic acid thus appears to be largely mediated by formation of succinic anhydride. The formation of the anhydride is not totally unexpected in view of the work by Bender⁴ on phthalamic acid. This mechanism is probably responsible in part for the observed bifunctional intramolecular catalysis reported by Morawetz and Shafer for the hydrolysis of N-(2-carboxyphenyl)-phthalamic acid.⁵ An analogous mechanism has been shown to play a part in the hydrolysis of corresponding half esters of dicarboxylic acids which can conveniently form cyclic acid anhydrides.⁶

The large circles, \ominus , shown in Fig. 9 are rate values calculated from the over-all equilibrium constants as described above. These would be expected, of course, to be considerably less accurate since their computation involves the over-all equilibrium constant, the ratio k_2/k_{-1} , and the rate constant for the anhydride formation, k_1 , each of which being more difficultly determinable than k_{-2} by the direct method. The fair agreement between the two methods, however, lends additional support to the proposed mechanisms.

The k_{-2} and k_H^+ values obtained directly from data such as shown in Fig. 8 from hydrolysis of succinamic acid are shown in Table I for several temperatures.

TABLE I
RATE OF HYDROLYSIS OF SUCCINAMIC ACID DETERMINED BY DIRECT MEASUREMENT

Temp., °C.	k_{-2} , sec. ⁻¹	k_H^+ , l. mole ⁻¹ sec. ⁻¹
45	0.495×10^{-5}	1.37×10^{-5}
55	1.33	3.81
60	2.08	6.55
65	3.12	10.8

The apparent heats of activation for the two processes were found to be 19.5 and 21.7 kcal./mole, respectively.

General Discussion

Interaction of succinic acid with aniline apparently leads to reversible formation of the anilic acid resulting eventually in an equilibrium state. The data presented above, based on the differential eq. 3, strongly support the mechanism based on reversible intermediate participation of the cyclic anhydride, a pathway which may be much more general for the hydrolytic reactions than has hitherto been suspected. The observed behavior is fundamentally inconsistent with the assumption that the acid reacts directly with the amine.

Recently Morawetz and Otaki⁷ reported that the reaction of ammonia or methylamine is not strikingly higher with succinic acid than with monobasic acids. This is not in disagreement with the present findings in that these studies represented runs made in highly alkaline solution in which the concentration of the un-ionized form of the dibasic acid would be essentially negligible. In view of the presently reported results the rate of anhydride formation at pH of 10–11 would be inconsequential compared to the direct reaction between the carboxylate group and the free amine.

(4) M. L. Bender, U.-L. Chow, and F. Chloupek, *J. Am. Chem. Soc.*, **80**, 5380 (1958).

(5) H. Morawetz and J. Shafer, *ibid.*, **84**, 3784 (1962).

(6) U. K. Pandit and T. C. Bruice, *ibid.*, **82**, 5858 (1960).

(7) H. Morawetz and P. S. Otaki, *ibid.*, **85**, 463 (1963).

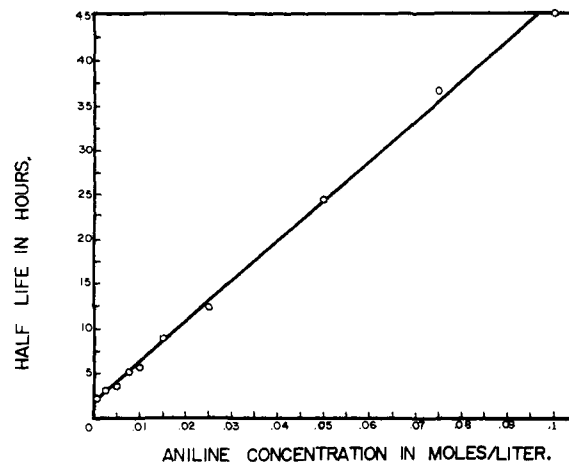


Fig. 10.—A plot of apparent half-lives as determined from plots similar to Fig. 2 shown against the initial aniline concentrations according to eq. 8. The intercept is approximately equal to $0.693/f_{-2}k_{-2}(A_0/A_\infty)$.

Another rather interesting and separate test of the proposed mechanism is based on the integrated eq. 5. As stated before, although the approach to the equilibrium state is not strictly first order with respect to $(A - A_\infty)$, the first term on the left side does not produce a significantly observable apparent deviation from the usual linear relationship as shown in Fig. 2. If this is the case and we ignore the non-logarithmic left-hand term, the apparent half-life of approach to equilibrium readable from Fig. 2 is approximately related as follows to the constants of the system

$$\frac{0.693}{t_{1/2}} \cong \frac{f_{-2}k_{-2}(A_0/A_\infty)}{(A_0/A_\infty) + A_0 f_2(k_2/k_{-1})}$$

If we were to look at this relationship with A_0 , the initial amine concentration, as the independent variable, it is apparent since (A_0/A_∞) is independent of A_0 that

$$t_{1/2} \cong K \left(\frac{A_0}{A_\infty} + \frac{f_2 k_2}{k_{-1}} A_0 \right) \quad \text{where } K = \frac{0.693}{f_{-2} k_{-2} (A_0/A_\infty)^2} \quad (8)$$

is an equation of a straight line. A plot of this type for the succinic acid–aniline system at pH 3.4 and 75°, as shown in Fig. 10, yields a respectively straight line with a definite intercept on the time axis. The intercept value, 1.8 hr., is approximately equal to KA_0/A_∞ or $0.693/f_{-2}k_{-2}(A_0/A_\infty)$. Since the term ignored in the initial approximation is equal to zero for the extrapolated condition, the intercept should yield the true answer. The values of, for example, $f_{-2}k_{-2}$ calculated in this fashion using the observed A_0/A_∞ value of 1.83 come out to be 5.8×10^{-5} sec.⁻¹ in agreement with value 6×10^{-5} sec.⁻¹ obtained from constants calculated from the differential data. It is evident that other constants such as k_2/k_{-1} and k_1 can be estimated from the slope value. Although these results appear to be in fair agreement generally with those predicted by previous calculation, the extent of approximation involved is too great to permit accurate evaluation of the remaining constants by this method.

For certain related dibasic acids other data seem to indicate that the reaction may often proceed further to the imide state and that anilic acid may be present in only an extremely low concentration. In particular, alkyl substituted succinic acids appear to go readily to their corresponding imides. These are presently under investigation. Citric acid, for example, yields a multitude of products including two imide species.

Experimental

Equipment and Reagents.—Mineral oil baths regulated to $\pm 0.05^\circ$ were used for the kinetics runs. All final determinations were carried out spectrophotometrically on a Cary Model 11 MS spectrophotometer. The adjustment and determinations of pH were made with a Beckman Zeromatic pH meter with an expanded scale.

Only reagent or analytical grade chemicals were used throughout. Aniline was freshly distilled and kept under nitrogen.

Procedure for Kinetic Runs on Succinic Acid–Aniline Reaction.—Reaction solutions containing varying amounts of aniline were prepared by mixing appropriate quantities of 2% aniline in 0.5 *M* succinate or citrate buffer with additional quantities of the buffer. Each solution was adjusted to the desired pH value, prior to mixing, with powdered sodium hydroxide and the mixtures were checked after mixing. They were then placed in thoroughly washed, dry neutral glass ampoules and sealed under nitrogen.

The ampoules were preheated to approximately the final temperature by vigorously shaking in a water bath for 2 min. and then quickly transferred to the oil bath. The zero hour sample was taken at this time and subsequent ampoules were normally removed at 0.5, 1, 2, 4, 8, 12, 24, 50, and 75 hr. These were immediately chilled in acetone–Dry Ice mixtures.

The residual aniline content of the ampoules was determined by spectrophotometric measurements at 287 $m\mu$ on a chloroform extract of the neutralized solutions (pH \cong 7). Although a fairly adequate assay could be based on direct ultraviolet measurements on the reacted solutions, the method would be subject to varying errors if side reactions occurred.

The residual aniline concentration reported as A_∞ were largely based on runs made at low initial aniline concentration. Since these rapidly reached equilibrium states, determination of the amine concentration at effectively infinite time presented no problem.

Procedure for Determination of k_{-1} and k_2 on Succinic Anhydride.—These were determined in a conventional fashion in a thermostated Cary Model 11 spectrophotometer. The reactions were permitted to occur directly in the photometer cell. For the aniline reaction varying amounts of an alcoholic solution of the anhydride were first introduced into the cell followed by a buffered solution of aniline. The resulting rate constants were then extrapolated to zero alcohol concentration.

Procedure for Determination of the Dissociation Constants.—Dissociation constants were determined on systems comparable to that used for the rate studies except for aniline. The values for succinic acid were obtained, for example, from potentiometric titration of a 0.5 *M* solution, that for succinilic acid by partition coefficient determination on the acid from succinate buffer solutions at several pH values. The pK_a' values for aniline were measured potentiometrically in 0.5 *M* sodium chloride.

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[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY, UNIVERSITY OF ATHENS, GREECE]

New Methods in Peptide Synthesis. I. Tritylsulfonyl and *o*-Nitrophenylsulfonyl Groups as N-Protecting Groups^{1,2}

By LEONIDAS ZERVAS, DIMITRIOS BOROVS, AND EUTHYMOS GAZIS

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N-Tritylamino acid *p*-nitrophenyl esters, with the exception of the glycine derivative, do not couple with amino acid esters. On the other hand, *N*-tritylsulfonyl (TRS) or *N*-*o*-nitrophenylsulfonyl (NPS) amino acid *p*-nitrophenyl esters show no steric hindrance and have been used to synthesize *N*-protected dipeptide esters. The *N*-*o*-nitrophenylsulfonyl derivatives of L-amino acids themselves have also been prepared and isolated in the pure state as their dicyclohexylammonium (DCHA) salts. These salts have been used directly for peptide synthesis. The TRS or NPS group can be easily removed from *N*-protected peptides by means of two equivalents of hydrogen chloride in methanol or nonpolar solvents, with the generation of TRS or NPS chloride. The use of the *N*-sulfonyl protecting groups is advantageous for the lengthening of a peptide chain, especially when the peptide includes amino acids bearing protected functional groups in their side chain.

Introduction

The very progress of the synthesis of peptides depends upon finding suitable methods for (a) the protection of the α -amino group, the carboxyl group, and the side-chain functional groups; and (b) the coupling reaction.³ This communication deals with the problem of the *N*-protection of the amino acids.

Thirty years after its introduction, the carbobenzyoxy group⁴ is still most commonly used for the *N*-protection of amino acids, especially since this method of *N*-protection^{3,1} has been adapted to the peculiarities of some amino acids, such as lysine,⁵ arginine,⁶ and cysteine–cystine.^{7–9}

The great usefulness of this group may be attributed to the fact that the coupling of carbobenzyoxyamino acids with other amino acids or peptides proceeds without racemization whatever method of coupling is employed; moreover, the removal of this *N*-protecting group can be easily brought about by treatment with hydrogen bromide–acetic acid,¹⁰ by trifluoroacetic acid,¹¹ or under very mild conditions, *i.e.*, by catalytic hydrogenolysis.⁴

In spite of the wide application of the carbobenzyoxy group, other groups have proved useful in some cases, for instance the toluenesulfonyl,¹² the phthaloyl,¹³ the trifluoroacetyl,¹⁴ the butyloxycarbonyl,¹⁵ the di-

(1) A preliminary communication on this work was presented at the 5th European Peptide Symposium, Oxford, Eng., September, 1962; E. Gazis, B. Bezas, G. C. Stelakatos, and L. Zervas, "Proceedings of the 5th European Peptide Symposium," Pergamon Press, London, 1963, p. 17.

(2) This investigation was supported by the Royal Hellenic Research Foundation, to which we are greatly indebted.

(3) A detailed description of the methods for peptide synthesis can be found in J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York, N. Y., 1961.

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