

with 3.2 ml. of 1.58 *M* glutaric anhydride in dioxane solution. The reaction mixture was quenched at the end of 30 sec. with 16 ml. of a 0.33 *M* aqueous aniline solution. The pH of the resulting solution was adjusted to pH 3.13 with HCl and 30 ml. of this solution was placed on the column with 30 Gm. of silicic acid. The eluate was collected in 50-ml. fractions. The solvent was removed from each sample under reduced pressure and a mixture of chloroform-petroleum ether added to precipitate the compounds.

Characterization of Reaction Compounds.—*Glutaric Monoanilide*.—This was recrystallized from methanol-water, m.p. 129–130°. Molecular weight found by direct titration against standard NaOH was 205 (calculated 207). Known glutaric monoanilide was prepared by adding glutaric anhydride dissolved in dioxane to an aqueous solution of aniline. The sparingly soluble anilide precipitated and when recrystallized gave a m.p. 129–130°. Both known and unknown compounds gave identical infrared and ultraviolet absorption spectrums.

Citric Monoanilide.—1.—This was recrystallized from chloroform, m.p. 136–137°.

Anal.—Calcd. for C, 53.94; H, 4.87; N, 5.23. Found: C, 53.90; H, 4.94; N, 5.3.

Molecular weight determined by direct titration against NaOH was 269 (calculated 268). Infrared

and ultraviolet spectra were characteristic of an anilide. NMR spectrum strongly suggested this to be the symmetrical isomer, 2-hydroxy-2-*N*-phenylcarbamide-1,3-propanedicarboxylic acid.

Citric Monoanilide.—2.—This was recrystallized from chloroform, m.p. 127–128°. Molecular weight determined by direct titration against sodium hydroxide solution was 270 (calculated 268).

Anal.—Calcd. for C, 53.94; H, 4.87; N, 5.23. Found: C, 53.87; H, 4.85; N, 5.20.

Infrared and ultraviolet absorption spectra were characteristic of an anilide. NMR spectrum suggested this to be the unsymmetrical isomer, 2-hydroxy-1-*N*-phenylcarbamide-2,3-propanedicarboxylic acid.

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Interaction of Acetic Anhydride with Di- and Tri-carboxylic Acids in Aqueous Solution

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Earlier studies have shown that cyclic anhydrides, such as succinic, glutaric, etc., interact with citrate ions in aqueous solution to form what appears to be a citric anhydride species. These interactions have been assumed to be highly reversible through intermediate formation of a mixed anhydride. The present studies were concerned with a comparable system which was expected to be essentially irreversible. Spectrophotometric investigations have shown that acetic anhydride apparently reacts with citrate in aqueous solution to form a new species which undergoes rapid hydrolysis. The rate of the initial reaction and the rate of the subsequent step appear to depend on the citrate concentration and pH. Results of chromatographic studies on products obtained by reaction with aniline at different phases of the reaction are presented.

IN AN EARLIER report (1), experimental evidence was presented which suggested that glutaric and presumably other cyclic anhydrides formed an equilibrium system in the presence

of a large excess of citrate buffer in which the total anhydride concentration was distributed among glutaric anhydride, citric anhydride, and perhaps glutaric-citric anhydride. An attempt has been made in the present study to obtain a clearer picture of the reacting system by employing acetic anhydride to furnish the initial anhydride concentration; the noncyclic anhydride not being expected to participate effectively in any reversible process.

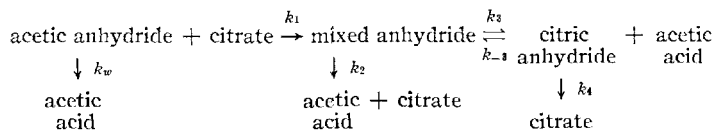
If the forward reaction pathway remains essen-

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Scheme I

tially the same as in the cyclic anhydride case, a mixed acid anhydride species would be formed by interaction of acetic anhydride and citrate ion. The loss of the acetate in this step would, however, make formation of the mixed anhydride irreversible. The net effect of this would be to drive the reaction to the right. The proposed reaction is shown in Scheme I.¹

In the present investigation the differences in the characteristics of this irreversible system from those of the previously studied reversible system have been explored.

RESULTS AND OBSERVATIONS

Spectrophotometric Studies.—Although acetic anhydride hydrolyzes rather rapidly in cold water ($t_{1/2} \cong 5$ min. at 25°), it appears to be capable of reacting with nucleophilic species such as citrate anions. When the hydrolysis is followed spectrophotometrically at 248 $m\mu$ in citrate buffer, at pH values where there is an appreciable quantity of di-ionized citrate, a species appears to be formed which has an ultraviolet absorptivity greater than the original anhydride. The absorbance-time profile observed for a system initially containing 1.08×10^{-3} M acetic anhydride in 0.3 M citrate buffer, pH = 5 and 25°, is shown in Fig. 1. The shape of the plot suggests that the reaction involves relatively rapid formation and subsequent hydrolysis of some intermediate species.

As shown previously (1), the concentration of citric anhydride can be expressed² as:

$$[CA] = \frac{k_1[B][AA]_0}{(k_1[B] + k_w - k_4)} [e^{-k_4 t} - e^{-(k_1[B] + k_w)t}]$$

If $(k_1[B] + k_w) \gg k_4$, a semilogarithmic plot of $(A_t - A_\infty)$ against time (where A_t is the absorbance at any time t and A_∞ is the limiting absorbance) will have a terminal slope equal to $-k_4$. Extrapolation of this terminal line to zero time will give a value $(A_0' - A_\infty)$ which corresponds to the imaginary absorbance of the new species at time zero. A semilogarithmic plot of $[(A_t' - A_\infty) - (A_t - A_\infty)]$, at various times, against time will yield a linear relationship with a slope equal to $-(k_1[B] + k_w)$.

The rates of appearance $(k_1[B] + k_w)$ of the more strongly absorbing species in the system are influenced by both hydrogen-ion concentration and buffer concentration as shown in Fig. 2. As is evident from the plots, the rate of formation appears to

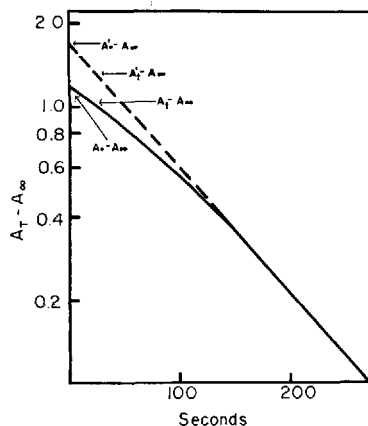


Fig. 1.—Semilogarithmic plot of absorbance change at 248 $m\mu$ for the system acetic anhydride in citrate buffer. pH = 5.0; $T = 25^\circ$.

depend upon either the di- and/or the tri-ionized form of citric acid. It should be noted here that the apparent transfer of anhydride character was observed only at a pH = 3 or greater where the concentration of di-ionized citrate was appreciable.

The observed rates of loss (k_4) of the new species, found from the terminal slopes as described above are shown in Fig. 3 for various pH values and different buffer concentrations. From the proposed reaction Scheme I, if $k_4 \gg k_3$, one would be essentially observing the rate of loss of the mixed anhydride. Similarly, if $k_3 \gg k_4$, the observed hydrolysis would be that of citric anhydride. It is felt that the latter situation probably exists and that acetic anhydride reacts with the citrate buffer to produce citric anhydride which subsequently undergoes hydrolysis.

The apparent dependency upon hydrogen-ion concentration observed in Fig. 3 can be explained on the basis that if the rate of formation of citric anhydride is slow at low pH values and low buffer concentration, extrapolations of the terminal slope does not reflect the true hydrolytic rates for the disappearance of the species. It is suggested, therefore, that only above pH = 6 is the terminal slope representative of the true hydrolytic rate of citric anhydride.

The pH dependency is similar to that observed in the glutaric anhydride-citric acid case (1). The extrapolations of the higher pH values to zero buffer concentration would be expected to yield the uncatalyzed hydrolytic rate for citric anhydride. Since in both systems citric anhydride is apparently formed, the extrapolated rate value should be the same. The value of 11.5×10^{-3} sec.^{-1} obtained in

¹ The reaction steps k_w , k_2 , and k_4 are considered irreversible, since under the conditions employed here (25°, aqueous solution) the reverse reaction would be negligible (2).

² The assumptions involved in this derivation are the same as previously reported (1). The reaction step, k_3 , is considered irreversible since at least a sixtyfold excess of buffer was employed at all times.

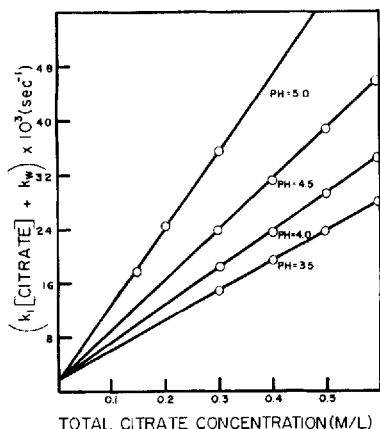


Fig. 2.—Rate of formation of the species formed from the acetic anhydride-citrate system at various pH values and different buffer concentrations.

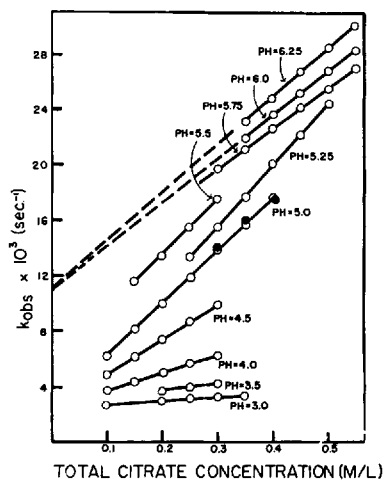


Fig. 3.—Observed rate of hydrolysis of the species formed from the interaction of acetic anhydride in citrate buffer at various pH values and different buffer concentrations. Key: ●, 1 M KCl; ○, H₂O.

this investigation is in good agreement with that reported in the earlier work (1).

Apparently the pH values, necessary to obtain a limiting maximum rate constant upon extrapolation to zero buffer at a given pH, are higher (pH = 6.0) for the present case than those found for the glutaric-citrate case (pH = 4.5). This dependence may be rationalized on the basis of steric effects. Since both glutaric and acetic anhydrides have approximately the same rate of hydrolysis in water, one cannot ascribe the observed phenomenon singly to the different sensitivities of the two species to nucleophilic attack.

As previously mentioned (1), the data presented can also be rationalized by an alternate pathway. This essentially requires that the rate-determining

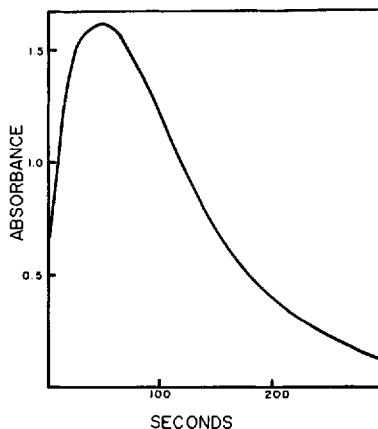


Fig. 4.—Absorbance-time profile for acetic anhydride, initial concentration $4.5 \times 10^{-3} M$, in 0.5 M phthalate buffer. The absorbance change was followed at 315 μ . pH = 5.5; T = 25°.

step corresponds to the decomposition of the mixed anhydride. A mechanism such as this requires that acetic-citric anhydride be a relatively stable species which seems unlikely. It should be pointed out that direct hydrolysis of the mixed anhydride is also possible, but the slow rate of hydrolysis observed would again require that the mixed anhydride be a relatively stable species.

The catalytic effect of citrate buffer on the hydrolytic rate of the assumed citric anhydride has been observed with other anhydrides. An example of this is the catalytic hydrolysis of acetic anhydride by acetate ions. The most acceptable theory for this catalysis is a general base effect, since nucleophilic attack would result in an identical anhydride (3). A general base effect might be a satisfactory explanation in the citrate case, but it should be pointed out that attack of citrate ions on citric anhydride would produce an intermediate citric-citric anhydride which could undergo hydrolysis at a faster rate than citric anhydride.

Supportive evidence for the formation of citric anhydride rather than the mixed anhydride was obtained by using phthalate as the buffer rather than citrate. Addition of acetic anhydride to phthalate buffer produces a species which can be followed at a wavelength where there is no interfering absorbance from the acetic anhydride. As shown in Fig. 4, a species forms and subsequently undergoes hydrolysis. Applying the same kinetic treatment as was described for the citric case allows for separation of the individual rate constants. Figure 5 shows the rate of disappearance of this species as a function of phthalate concentration and pH. The proposed mechanism for this interaction is relatively rapid formation of phthalic anhydride, followed by a somewhat slower hydrolysis. The apparent pH dependency observed here is explained on the same basis as the acetic anhydride-citrate case, *i.e.*, at low pH values and low buffer concentrations the rate of formation is slow, and, therefore, extrapolation of the terminal slope does not reflect the true hydrolytic rate. Above pH = 5.5 the limiting intercepts at zero buffer concentration value giving

an uncatalyzed velocity constant of 9.3×10^{-3} sec.⁻¹ which is in good agreement with reported work (4).

Because of the sparingly soluble nature of phthalic anhydride, it was possible to isolate it from the reaction mixture. Addition of excess acetic anhydride to the phthalate buffer gave a copious precipitate which redissolved upon standing. Isolation and characterization of this precipitate showed it to be phthalic anhydride, lending support to the hypothesis that the mixed anhydride has only a transitory existence in systems containing acetic anhydride and various carboxylic buffers.

Chromatographic Studies.—The presence of a citrate species possessing anhydride properties can be demonstrated by addition of aniline to the reaction mixture as has been previously noted (5). The anilides that are produced can be separated by chromatography as shown in Fig. 6. The typical chromatography run demonstrated in Fig. 6 was carried out using a 0.3 M, pH = 5 citrate buffer, and 8.0×10^{-2} moles of acetic anhydride; the reaction was quenched with aniline after 40 sec. The double peak corresponding to the two isomers of citric monoanilide is again in good agreement with earlier work (5). Verification that the peaks correspond to the two isomeric forms of citric monoanilide was carried out as previously described (1).

Determination of the Citric Anhydride-Time Profile by the Aniline Method.—The citric anhydride concentration in a mixture containing initially only acetic anhydride and citrate buffer can be estimated by quenching the reaction system with aniline and determining the concentration of the total citric monoanilide products. The anilides formed from samples drawn at different times during the course of the reaction were separated by column partition chromatography and the total yield of citric monoanilide determined. The results are shown in Fig. 7. The indicated concentra-

tion of citric anhydride as reflected by the total yield of citric monoanilide apparently increased initially with time. The data suggest that the hydrolysis of citric anhydride resulted in a decrease in the amount of the anilides formed in the terminal phase of the reaction. The yield of citric anilide was calculated based on a molar absorptivity for the citric monoanilide of 11,000.

It is apparent in this system that the anilide could have been formed from the mixed anhydride or from citric anhydride, although the spectrophotometric evidence presented supports citric anhydride rather than a mixed anhydride.

DISCUSSION

These studies indicate that, although the irreversible interactions of acetic anhydride with citrate

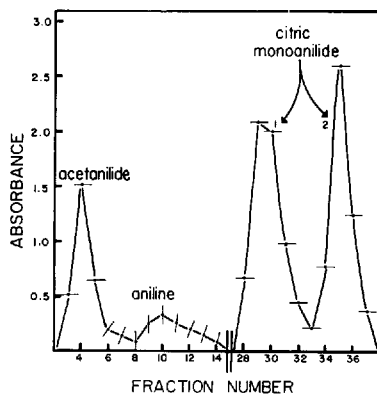


Fig. 6.—Typical chromatogram for the anilides formed by addition of aniline to the acetic anhydride-citrate system. The reaction was quenched with aniline after 40 sec. The eluate was extracted with 0.1 N NaOH and the alkaline solution analyzed spectrophotometrically at 241 $m\mu$.

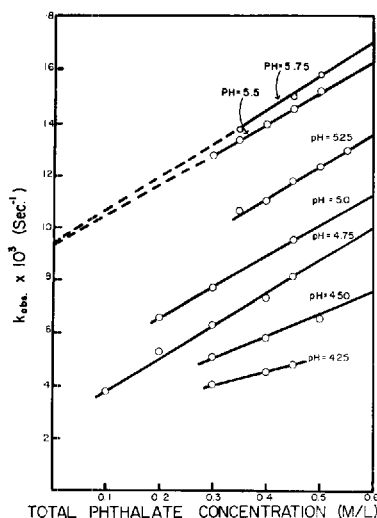


Fig. 5.—Observed rate of hydrolysis of the species formed from the interaction of acetic anhydride in phthalate buffer at various pH values and different buffer concentrations.

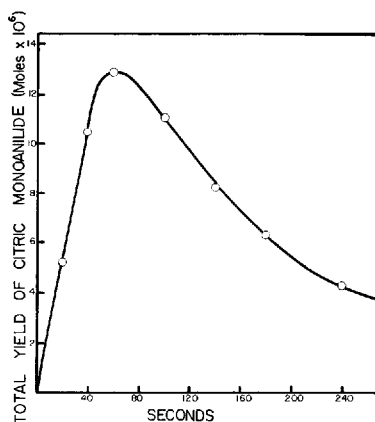


Fig. 7.—Total yield of citric monoanilide for the acetic anhydride-citrate system. The various points were obtained by withdrawing samples from the reaction mixture at various times and quenching the reaction with aniline. The total citric monoanilide present was then determined. pH = 5, 0.5 M.

differ markedly in character from the reversible reaction observed for the glutaric anhydride-citrate system, the apparent kinetic behaviors are surprisingly similar. The primary observation, aside from the irreversibility of the system, seems to be that the acetic anhydride reaction with citrate proceeds at a substantially slower second-order rate than that observed for the cyclic anhydride.

The experimental observations again strongly suggest intermediate formation of a reactive citric anhydride species. Although the compound was not isolated in these studies, the data suggest that acetic anhydride can be employed to produce substantial concentrations of citric anhydride which, in turn, can be conveniently employed for synthesis of citric acid derivatives in aqueous solutions.

EXPERIMENTAL

Reagents and Equipment.—Commercial acetic anhydride was purified by distillation. Dioxane was purified according to Vogel (6). Aniline was purified by repeated distillation and was stored under nitrogen prior to use. All other chemicals were of analytical or reagent grade.

All pH measurements and adjustments were made with a Beckman Zeromatic pH meter with an expanded scale.

Kinetic Procedure for the Reaction of Acetic Anhydride with Various Buffers.—Fifty microliters of a 1.08 *M* solution of acetic anhydride in dioxane was introduced together with 6 ml. of the buffer into a 2-cm. photometer cell. The reaction was allowed to proceed and was followed directly on a Cary model 11- M.S. recording spectrophotometer. All solutions were equilibrated at $25 \pm 0.1^\circ$ prior to use.

The dioxane concentration employed in these runs was determined to have a negligible effect on the rate constants.

Chromatographic Separation of Reaction Mixture.—The chromatographic columns were prepared as outlined in a previous communication (5). The reaction mixture was prepared by adding 15 ml. of the appropriate citrate buffer to 300 μ l. of a 1.08 *M* acetic anhydride in dioxane solution. The reaction was allowed to proceed the requisite period of time and was quenched with 4 ml. of a 0.33 *M* aqueous aniline solution. The pH was adjusted to 3.13 with HCl and a 5-ml. sample was placed on the column with 5 Gm. of silicic acid. The eluting solutions have previously been described (1).

Blank determinations were made following the same procedure as above using (a) acetic acid, citrate buffer, and aniline solution, and (b) acetic anhydride, water, and aniline solution.

Preparative Chromatography of the Reaction Mixture.—A chromatographic column was prepared as previously reported (1). The reaction mixture was prepared by adding 100 ml. of pH = 5.0, 0.3 *M* citrate buffer to 4 ml. of a 1.08 *M* acetic anhydride in dioxane solution. At the end of 60 sec. the reaction mixture was quenched with 27 ml. of a 0.33 *M* aqueous aniline solution. The reaction mixture was placed on the column in the previously outlined manner and the eluate was collected and treated as reported (1).

Characterization of Compounds.—*Acetanilide.*—This was recrystallized from methanol, m.p. 114–115°. Commercial acetanilide was recrystallized from methanol and gave a m.p. 114–115°. A mixture of the known and unknown sample gave no depression of the melting point. In addition, both known and unknown samples gave identical infrared and ultraviolet absorption spectra.

Citric Monoanilide.—1.—*Anal.*—Calcd. for C, 53.94; H, 4.87; N, 5.23. Found: C, 54.09; H, 4.92; N, 5.22.

Molecular weight determined by direct titration against NaOH was found to be 271. (Calcd. 268.) Infrared and ultraviolet absorption spectra were characteristic of an anilide. The NMR spectrum strongly suggested this to be the symmetrical isomer, 2-hydroxy-2-*N*-phenylcarbamide-1,3-propanedicarboxylic acid.

Citric Monoanilide.—2.—This was recrystallized from chloroform, m.p. 127–128°. Molecular weight determined by direct titration against sodium hydroxide solution was 270. (Calcd. 268.)

Anal.—Calcd. for C, 53.95; H, 4.87; N, 5.23. Found: C, 53.98; H, 4.92; N, 5.19.

Infrared and ultraviolet absorption spectra were characteristic of an anilide. NMR spectrum suggested this to be the unsymmetrical isomer, 2-hydroxy-1-*N*-phenylcarbamide-2,3-propanedicarboxylic acid.

Phthalic Anhydride.—This was recrystallized from chloroform, m.p. 130–131°. Commercial phthalic anhydride was recrystallized from chloroform and a mixture of the known and unknown compounds gave no depression of the melting point. Both known and unknown samples gave identical ultraviolet absorption spectra.

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