Facilitated Reversible Formation of Amides from Carboxylic Acids in Aqueous Solutions III

Reaction of Citric Acid with Aromatic Amines

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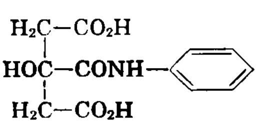
A detailed study of the rate of interaction of citrate buffers with aniline to form anilides and imides shows that the basic mechanism follows essentially that previously described. Results show in particular that the rate of anhydride formation by the polycarboxylic acid is faster than from the previously studied succinic system and probably involves both the undissociated acid and its singly charged anion. The overall equilibria and rates for the total reaction were determined and the approach to equilibria checked with an analog computer. The speed and nature of the reaction suggest that these interactions may often be responsible for loss of activity of some drugs formulated with this buffer.

IN PREVIOUS PUBLICATIONS (1, 2) it has been shown that certain dicarboxylic and polycarboxylic acids react with amines to form amides at unexpectedly fast rates. The significance of this reaction becomes apparent when one considers the wide use of some of these acids as buffers in numerous pharmaceutical systems. Because of the particular importance of citric acid in medicinal solutions, the behavior of this system has been explored in some detail with aniline as the other reactant. The reaction appears to be again mediated by the formation of cyclic anhydrides in aqueous solution which then form amido acids as described previously for the succinate system (2). The equilibria and reactions involved appear to be rather complex but may essentially be represented

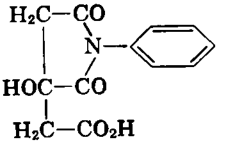
$$\left[\begin{array}{l}\text{citrate}^-\\ \text{citric acid}\end{array}\right] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{citric anhydride} \underset{\substack{-\text{aniline}\\ k_{-2}}}{\overset{\substack{k_2\\ \text{aniline}}}{\rightleftharpoons}} \begin{array}{c}\text{citric acid}\\ \text{monoanilide}\end{array} \quad \text{(Eq. 1)}$$

$$\left.\begin{array}{l}\text{citric acid monoanilide}\\ k_{-3}\ \updownarrow\ k_3\\ \text{citranilic acid}\end{array}\right\} \underset{\substack{-\text{aniline}\\ k_{-4}}}{\overset{\substack{k_4\\ \text{aniline}}}{\rightleftharpoons}} \left\{\begin{array}{l}\text{citric acid}\\ \text{dianilide}\\ k_{-5}\ \updownarrow\ k_5\\ \text{citranilic acid}\\ \text{anilide}\end{array}\right. \quad \text{(Eq. 2)}$$

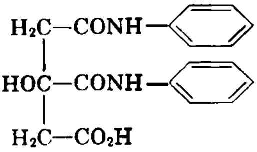
The following are the formulas of the products presumed to be formed during the reaction



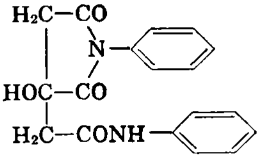
Citric acid monoanilide[1]



Citranilic acid (*IM*)



Citric acid dianilide[1]



Citranilic acid anilide

In further discussion the symbols (*CA*) for citric acid, (*AH*) for citric anhydride, (*AN*) for aniline, (*AM*) for citric acid monoanilide, and (*IM*) for citranilic acid will be used. k_n will denote apparent rate constants, with the subscript n being positive for the forward reactions and negative for the reverse.

Due to the polyfunctionality of the citric acid, other products such as the dianilide and a mixed anilide-imide are also possible. It was found from the experimental data that the latter two compounds were formed in relatively small concentrations, and Eqs. 1 and 2 represent the main reaction pathways in the concentration range studied.

RESULTS AND DISCUSSION

Identification of Reaction Products.—Sample solutions containing 0.05, 0.1, and 0.2 moles/L. of aniline were prepared in 0.5 *M* citrate buffer solutions, the pH at each concentration level being adjusted to 3.0, 3.5, 4.0, and 4.5. The solutions were heated for 100 hours at 85° and samples withdrawn at the end of 50 and 100 hours and analyzed with the *chromatographic separation* described under *Experimental*. Essentially no difference in composition could be demonstrated between the 50 and 100-hour samples, indicating the achievement of an equilibrium condition.

Based on separation of the reaction products by a partition chromatographic method with a pH 3.13 phosphate buffer as the fixed phase and mixtures of chloroform and butanol as the mobile phase, it was possible to establish the nature of the products. The identification was based on: (*a*) comparison of the elution characteristics of the products to known compounds treated in a duplicate manner; (*b*) ultraviolet absorption spectra of the unknown and known

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[1] The exact position, whether α, β, or γ of the amine groups in these structures has not yet been determined.

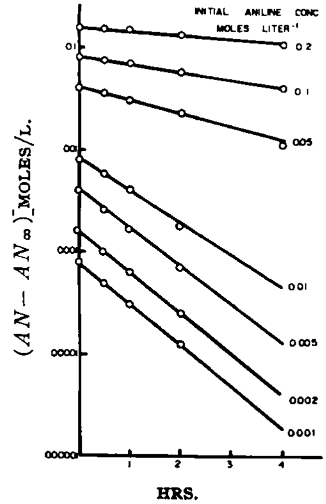


Fig. 1.—Plot showing logarithmic approach to equilibrium for aniline citrate systems. at pH 4. The several lines represent differing aniline concentration.

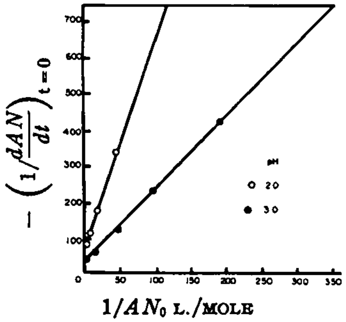


Fig. 2.—Plots of reciprocal initial rate of aniline disappearance in hours/liter/mole against reciprocal initial aniline concentration in liters/mole for aniline in presence of aqueous 0.5 *M* citrate buffer at pH 2.0 and 3.0 and at 85°. The relationship corresponds to Eq. 3.

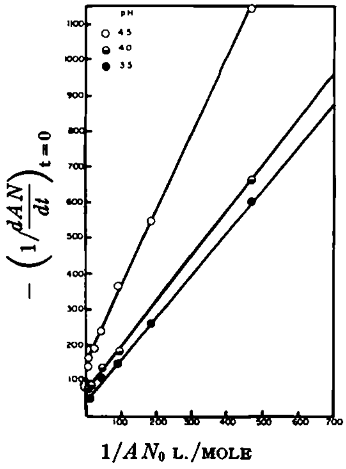


Fig. 3.—Plots of reciprocal initial rate of aniline disappearance in hours/liter/mole against reciprocal initial aniline concentration in liters/mole for aniline in presence of aqueous 0.5 *M* citrate buffer at pH 4.5, 4.0, and 3.5 and at 85°. The relationship corresponds to Eq. 3.

TABLE I.—EFFECT OF pH ON THE APPARENT CHANGE OF k_1 AND THE RATIO OF $\frac{k_2}{k_{-1}}$ AT 85°C.

pH	k_1 (hr.$^{-1}$)	$\frac{k_2}{k_{-1}}$ L./mole
2.0	4×10^{-2}	7
2.5	7.4×10^{-2}	7
3.0	7.2×10^{-2}	14
3.5	5.2×10^{-2}	33
4.0	3.1×10^{-2}	60
4.5	1.4×10^{-2}	70

compounds; and (*c*) equivalent weight and melting point comparison.

The results indicated that the main products of the reaction were the monoanilide (*AM*) and the imide (*IM*). Even under conditions of highest aniline concentration, less than 1% of the aniline was converted to the dianilide at pH 4.5. The amounts of citranilic acid anilide were higher than the dianilide at all pH values, the maximum being formed at pH 4.0. Even at this pH and highest aniline concentration, less than 3% of the aniline was present in the form of citranilic acid anilide.

The equilibrium ratios of the aniline: anilide: imide appeared to be independent of aniline concentration at a given pH and were in good agreement with the kinetically determined equilibrium values.

Kinetics of the Forward Reaction.—The rate of disappearance of aniline from mixtures of citric acid and aniline was studied at 85° using a 0.5 *M* citrate buffer system, the aniline concentration varying from 2×10^{-1} to 1×10^{-3} moles/L. Although the reaction appeared to follow a pseudo first-order kinetic mechanism in its approach to equilibrium, the rates of the reactions were dependent on initial aniline concentration. Figure 1 depicts the typical behavior observed at pH 4.0. Analysis of these curves suggested that at low aniline concentration the reaction became first-order in respect to aniline, but at high concentrations the reaction approached a zero-order dependency on aniline. This would be in accord with the mechanism postulated by Higuchi, *et al.* (2), for the reaction of succinic acid with aniline, which apparently is mediated by the formation of cyclic anhydrides in solution. Due to the complexity of the reaction, the kinetic analysis was limited to the initial rates utilizing the following assumptions: (*a*) the initial phase of the reaction can be represented by the upper half of the total scheme given above; (*b*) in this phase k_{-2} and lower half of the scheme can be neglected as the initial amide concentration is effectively zero; and (*c*) a steady-state assumption can be made for the anhydride concentration.

These assumptions lead to the following reciprocal rate expression

$$-\frac{1}{\left(\frac{dAN}{dt}\right)_o} = \frac{1}{k_1k_2\,\frac{(AN)}{k_{-1}}\,(CA)} + \frac{1}{k_1(CA)} \qquad \text{(Eq. 3)}$$

During the initial phase we can substitute the initial aniline concentration (AN_o) and the initial citric acid concentration (CA_o) for (AN) and (CA), respectively. A plot of $[1/(dAN/dt)]_o$ *versus* $1/AN_o$ should yield a straight line with an intercept of $1/k_1(CA\text{i})$ and slope a of $\left\{1/\,\frac{k_1k_2}{k-1}\,(CA_0)\right\}$. Figures

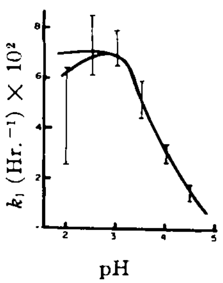


Fig. 4.—pH profile of rate of formation of citrate anhydride in citrate buffer at 85° as determined from intercepts of plots similar to Figs. 2 and 3. The lengths of the bars are approximate measures of the confidence limits of the individual values.

2 and 3 show the results of experiments conducted at pH 2.0, 3.0, 3.5, 4.0, and 4.5, treated in accord with Eq. 3. The nonzero intercept of the plots lend credence to the postulated mechanism.

From the intercept values of these plots, k_1 can be determined directly. Slope measurements were used to evaluate k_2/k_{-1} values as shown in Table I. The rate constants for the anhydride formation, k_1, measured in this fashion are shown in Table I and plotted in Fig. 4 for several pH values.

The shape of the profile strongly suggests that the species responsible for formation of the anhydride probably include both the uncharged, undissociated acid and its singly dissociated, singly charged, anionic form. If only the free acid underwent the reaction, the profile should exhibit a faster drop off at higher pH values. Unfortunately, reliable data under strongly acidic conditions were difficult to obtain because the ratio of rates of amide formation to hydrolysis of the anhydride becomes rather small as a result of protonation of the amine.

Due to the changing dependency of the reaction rate on the concentration of species, a simple pH rate profile of the total reaction at given concentrations is not fully informative regarding the expected overall rates under all conditions. A plot of the initial rate of aniline loss as a function of pH is shown in Fig. 5 at two levels of aniline. The difference in the shapes of the two curves is because at the higher aniline concentration the rate-limiting step is the formation of anhydride which is proportional to the concentration of undissociated citric acid (and, perhaps, the monocitrate anion as noted previously). Therefore, the decreasing rate at higher pH may be attributed to loss of the reactive, acidic species.

In the lower aniline concentration the reaction would be expected to show a first-order dependency on the unprotonated form of aniline. This is in accord with the mechanism previously postulated by Higuchi and Miki (1), which stated that kinetically the mechanism could be typified as $CA + NH_2R \rightleftharpoons$ amide. Such a system would exhibit the observed bell-shaped pH dependence.

The effects of temperature on these reactions were carried out in studies at 75°, 85°, and 95° at pH = 4.0. Figure 6 shows an Arrhenius-type plot for k_1 calculated from reciprocal plots as in Fig. 3. Higher temperatures favored the formation of the anhydride as was also observed for the succinic acid system (2). The apparent activation energy for the citrate reaction—based on the plot is 26.5 Kcal. mole^{-1}. The marked positive temperature dependence is of vital importance when considering the effect of heat sterilization of amine drugs in citrate buffer systems.

Kinetics of the Intermediate Reaction.—In this phase of the work experiments were conducted to study the following equilibrium

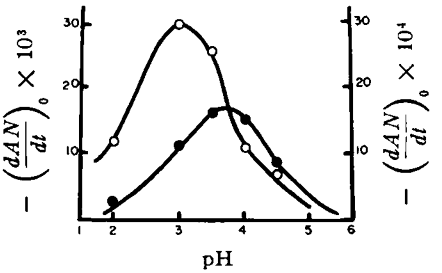


Fig. 5.—Apparent initial rate of disappearance of aniline in 0.5 M citrate buffer as a function of pH at two aniline concentrations. Key: ○, aniline = 0.2 mole/L. (left axis); ●, aniline = 0.002 mole/L. (right axis).

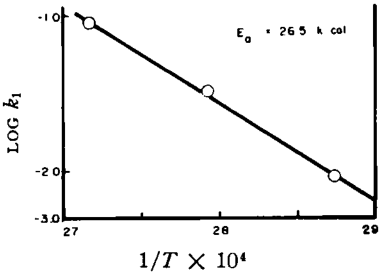


Fig. 6.—Arrhenius type plot for k_1 at pH 4.

$$\left.\begin{array}{l}\text{citrate}^-\\ \text{citric acid}\end{array}\right\} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{citric anhydride} \underset{k_{-2}\ \ -\text{aniline}}{\overset{\text{aniline}\ \ k_2'}{\rightleftharpoons}}$$

$$\text{citric monoanilide} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \text{imide}$$

where at low concentrations $k_2' = k_2\,(k_1/k_{-1})(CA)$, a constant at fixed citrate concentration and pH. Starting with 2.5–3.0 mM solutions of the monoanilide, the formation of other products was followed as a function of time. It can be assumed from previously discussed chromatographic analyses that at these low aniline concentrations the formation of products containing 2 moles of aniline can be neglected. Also because of the low aniline concentrations the reactions can be expected to follow a first-order approach to equilibrium at a given pH.

Based on these assumptions, the rate of formation of aniline and imide can be described by

$$\frac{d\,AN}{dt} = k_{-2}\,(AM) - k_2'\,(AN) \qquad \text{(Eq. 4)}$$

$$\frac{d\,IM}{dt} = k_3\,(AM) - k_{-3}\,(IM) \qquad \text{(Eq. 5)}$$

At equilibrium Eqs. 6 and 7 are valid

$$\frac{(AN)_{eq}}{(AM)_{eq}} = K_2 = \frac{k_{-2}}{k_2'} \qquad \text{(Eq. 6)}$$

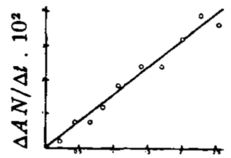
$$\frac{(IM)_{eq}}{(AM)_{eq}} = K_3 = \frac{k_3}{k_{-3}} \qquad \text{(Eq. 7)}$$

where the subscript eq refers to the equilibrium concentration. Substitution of Eqs. 6 and 7 into Eqs. 4 and 5 yields

$$\frac{d\,AN}{dt} = k_{-2}\left[(AM) - \frac{1}{K_2}(AN)\right] \quad \text{(Eq. 8)}$$

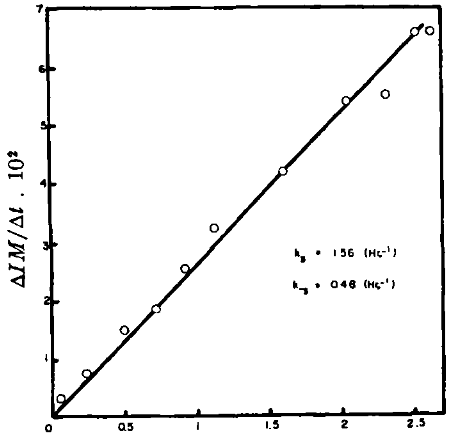
$$\frac{d\,IM}{dt} = k_3\left[(AM) - \frac{1}{K_3}(IM)\right] \quad \text{(Eq. 9)}$$

On the basis of Eqs. 8 and 9 the values of the rate constants can be estimated by plotting $\Delta AN/\Delta t$ *versus* $[(AM_{av}) - (1/K_2)\,(AN_{av})]$, where the subscript *av* refers to average concentrations during the time interval Δt. The slope of the line is equal to k_{-2}. k_3 can be estimated in a similar manner from a plot of $\Delta IM/\Delta t$ *versus* $[(AM_{av}) - (1/K_3)\,(IM_{av})]$. k_2' and k_{-3} can then be calculated by appropriate substitution into Eqs. 6 and 7. Figures 7 and 8 show the results obtained from plots of this type. The values obtained at various pH are recorded in Table II. As in the case of k_1 discussed earlier, they are apparent constants and are not corrected for species changes.



[(MONOANILIDE)$_{av}$ − 0.63(ANILINE)$_{av}$], mM/L.

Fig. 7.—Rate of aniline formation as a function of $[(AM)_{av} - 1/K_2\,(AN)_{av}]$ at pH = 3.0. k_2 = 0.96 (hours $^{-1}$); k_2' = 0.60 (hours^{-1}).



[(MONOANILIDE)$_{av}$ − 0.32 (IMIDE)$_{av}$], mM/L.

Fig. 8.—Rate of imide formation as a function of $[(AM)_{av} - 1/K_3\,(IM)_{av}]$ at pH = 3.0.

Since the reported values for the observed rate constants were not obtained by direct solution of the differential equations, their validity was checked by an analog computer. The system can be described in differential form by Eqs. 4 and 5 and the following equation for the change in amide concentration.

$$-\frac{d\,AM}{dt} = k_{-2}(AM) - k_2'(AN) + k_3(AM) - k_{-3}(IM) \quad \text{(Eq. 10)}$$

The analog computer circuit diagram representing Eqs. 4, 5, and 10 is shown in Fig. 9. When the experimentally determined constants were substituted, good agreements were found between the computer-calculated concentration-time curves for amide, imide, and aniline and the experimental values found. Figure 10 shows the computer-calculated curves and experimental values for the system at pH 3.0.

It was observed experimentally that at pH 3.0, 3.5, and 4.0 the aniline concentration passed through a maximum value. These results could not readily be anticipated by inspection of the differential equations, and consequently brought forth the speculation that some other mechanism might be active at these pH conditions. The computer-calculated concentration profile for aniline, based on the proposed reactions and rate constants, also exhibited a maximum which coincided with the experimentally observed peak, further supporting the validity of the proposed mechanism. This effect is apparently because the formation of aniline is kinetically more favored than would be expected from thermodynamic considerations.

Conclusions and Consideration of Possible Participation in Loss of Drug Activity in Citrate Buffers.—According to Table I the rate of presumed reactive intermediate (anhydride) formation is nearly two orders of magnitude greater for the cit-

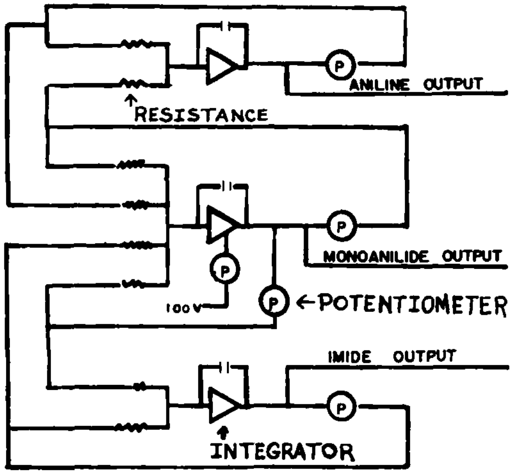


Fig. 9.—Schematic diagram of circuitry used in analog computation.

Table II.—Effect of Varying pH on the Apparent Pseudo First-Order Rate Constants and on the Ratios of k_{-2}/k_2' and k_3/k_{-3}

pH	k_{-2} (Hr.$^{-1}$)	k_2' (Hr.$^{-1}$)	k_{-2}/k_2'	k_3 (Hr.$^{-1}$)	k_{-3} (Hr.$^{-1}$)	k_3/k_{-3}
2.0	1.3	0.23	5.5	1.3	0.24	5.5
3.0	0.96	0.60	1.6	1.6	0.48	3.1
3.5	0.81	0.94	0.9	1.6	0.55	2.9
4.0	0.55	0.88	0.6	1.1	0.59	1.9
4.5	0.29	0.43	0.7	0.84	0.70	1.2
5.0	0.12	0.13	0.9	0.44	0.77	0.6

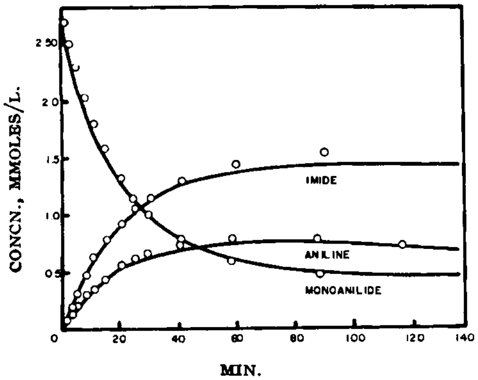


Fig. 10.—Curves obtained by computor analysis showing the concentrations of aniline, imide, and monoanilide at pH = 3 as a function of time. The points shown are experimental values. The computer curves were based on: $k_2' = 0.60$ (hours^{-1}); $k_{-2} = 0.96$ (hours^{-1}); $k_3 = 1.56$ (hours^{-1}); $k_{-3} = 0.48$ (hours^{-1}).

rate than for the succinate system (2). At 85° approximately 0.03 to 0.04 mole/liter of the suggested anhydride species would be expected to be formed per hour in a 0.5 *M* citrate buffer in the pH range 2.5 to 4.0. It would appear that this would be a significant factor during any autoclaving process. The effect of the buffer on shelf lives at room temperature would depend to a great extent on the concentration of the susceptible drug species and the value of k_2. Although k_1 may be markedly lower (approximately 1×10^{-6} hr.$^{-1}$ at 25° based on heat of activation of 26.5 Kcal./mole), the rate will still be expected to be significant for many drugs. In particular, any drug amine which may be readily acylated, including aromatic amines, hydrazine derivatives, etc., is suspect.

EXPERIMENTAL

Reagents

All reagents used were of analytical grade, except those synthesized and described below.

Citranilic Acid (N-Phenylcitric Acid Imide.)—This substance was prepared with some modifications according to a method originally described by Pebal (3) and later modified by Nau, Brown, and Bailey (4) and Leulier, Cier, and Drevon (5). The monoaniline salt, prepared by evaporating the ethanol from an equimolar solution of aniline and citric acid in ethanol, was heated in an open crucible to 150° for about 2 hours. The product was washed with ether and finally crystallized from chloroform. The equivalent weight was determined both by direct titration with sodium hydroxide solution and by adding sodium hydroxide solution in excess and back titration with hydrochloric acid. When using the latter method, the substance was hydrolyzed to the corresponding anilide. Both methods gave the molecular weight 255 (calculated 249), m.p. 186–187° (literature values vary from 185 to 189°).

Citric Acid Anilide.—This substance has not been isolated, but solutions of it have been prepared by adding an excess of sodium hydroxide solution to a solution of citranilic acid.

Citranilic Acid Anilide.—After boiling an aqueous solution containing 0.5 moles/L. aniline and 0.25 moles/L. citric acid for approximately 24 hours, citranilic acid anilide precipitated upon cooling the solution. The melting point was 181–182° after recrystallization from chloroform. Although the substance was prepared in a manner different from that reported by Bertram (6) and by Leulier, Cier, and Drevon (5), the observed melting point is in good agreement with that found by these investigators.

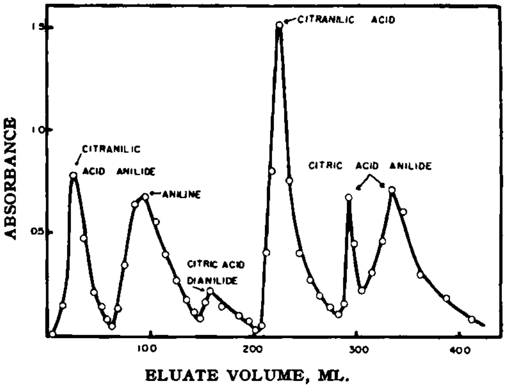


Fig. 11.—Elution chromatogram of a synthetic mixture of reaction products of aniline and citrate buffer readings in 1-cm. cells at 287 mμ for aniline and 241 mμ for the other fractions after extraction with their equal volume 0.1 *M* NaOH.

Citric Acid Dianilide.—This was prepared by adding an excess of sodium hydroxide to a solution of citranilic acid anilide which yielded the dianilide upon hydrolysis. Upon acidifying the solution with hydrochloric acid, the very sparingly soluble dianilide precipitated. The substance was also synthesized using a modification of the method originally described by Pebal (3). One mole of citric acid and 2 moles of aniline were heated at 170° for 1 hour in a test tube equipped with an air condenser. The dianilide product was extracted with sodium hydroxide solution and precipitated by acidifying with hydrochloric acid. The dissolution with alkali and precipitation with acid was repeated; finally the substance was recrystallized from ethanol. Both these methods of synthesis gave a substance with a melting point of 176° and an equivalent weight of 345 determined by titration (theoretical 342). The reported melting points in the literature vary from 180–184 (3, 6–8). The differences in the reported melting points might be attributed to the possibility of obtaining two different dianilides. Leulier, Cier, and Drevon stated that their compound with a melting point of 182° was the unsymmetrical dianilide.

Chromatographic Separation

The study of the reactions between aniline and citric acid required an assay method which permitted the determination of aniline and the four synthesized substances which might be formed. This was accomplished by the use of a partition chromatographic separation. Chromatographic columns with 18-mm. inner diameter were used. Twenty-five grams of silicic acid (Mallinckrodt, chromatographic grade) was used as supporting media, and 25 ml. of a pH 3.13 buffer of the following composition was used as the stationary phase:

Sodium phosphate monobasic, 75.90 Gm. (=0.55 mole)
Hydrochloric acid, 1 *M*, 30.00 ml.
Distilled water, *q.s.* 1000 ml.

The column was packed in the usual way using chloroform to make a slurry (9). A 2.00-ml. quantity of an aqueous solution containing approximately 0.05 mmole of aniline and its reaction products and with pH adjusted to 3.1 was adsorbed on 2 Gm. of silicic acid in a 30-ml. beaker. A slurry was made by adding 10 ml. of chloroform and then transferred to the column. To insure quantitative transfer of the sample to the column, another slurry was made in the same beaker using 1 Gm. silicic acid, 1 ml. stationary phase, and 5 ml. of chloroform. The second slurry was also added to the top of the column and packed tightly. The separation was carried out in the normal manner of gradient elution, collecting the fractions and changing solvent composition as follows.

The eluting solutions were chloroform and mixtures of chloroform and butanol according to the following compositions: eluant—0–100, 100–190, 190–270, and 270–425 ml.; butanol in chloroform—0, 1.5, 10, and 30%.

Figure 11 shows an elution chromatogram of a mixture of the five substances. Eluate was collected usually in fractions of 10 ml., but in some cases 5-ml. fractions were used. The final fractions used were 25 ml. each. The separated components are shown as separate peaks on the plot.

All the fractions were analyzed by U.V. spectrophotometry. Aniline was determined directly in the chloroform solution at 287 mμ ($\epsilon = 1760$). The other fractions were extracted with 0.1 *M* sodium hydroxide solution and determined at 241 mμ. Citranilic acid anilide was determined as the citric acid dianilide ($\epsilon = 23{,}000$), and the citranilic acid as the citric acid anilide ($\epsilon = 11{,}000$).

As Fig. 11 shows, a very small amount of the citric acid dianilide was used because this substance is sparingly soluble in both water and chloroform. Figure 11 corresponds to 1.9 mg. of aniline and about 0.9 mg. of citranilic acid and citric acid monoanilide and 0.2 mg. of citranilic acid anilide. The citric acid monoanilide always gave two peaks which suggests that both of the two possible monoanilides were formed. No complete separation of the two monoanilides has been attempted.

Kinetics and Analytical Procedures Used in the Investigation of the Interaction of Citric Acid with Aniline

The test solutions were prepared by dissolving concentrations of aniline varying from 0.2 to 0.001 moles/L. in 0.5 *M* citric acid solution which had previously been adjusted to the desired pH with the addition of sodium hydroxide or hydrochloric acid. The solution was subdivided into 5-ml. ampuls and sealed. The samples were placed in a constant temperature bath maintained at 85 ± 0.10° and withdrawn at various time intervals. The reaction was quenched by chilling in an acetone–dry ice freezing mixture.

The progress of the reaction was determined by assaying the samples for residual free aniline. A suitable aliquot was transferred to a separator containing 25 ml. of chloroform and 5 ml. of 1 *N* NaOH solution. The aniline was extracted into the chloroform layer which was separated and placed into a 100-ml. volumetric flask. The extraction was repeated twice again using 20-ml. volumes of chloroform. The chloroformic extracts were combined, and the resultant solution brought to 100.0 ml. with chloroform. The aniline was determined spectrophotometrically at 287 mμ using a Cary model 11 spectrophotometer.

Procedure for Determining Rates of Formation of Aniline and Citranilic Acid from Citric Acid Monoanilide

A 175-ml. quantity of a 0.5 *M* citrate buffer in a 250-ml. volumetric flask was preheated to 85.0°, then placed in a constant temperature bath at 85.0°. Citranilic acid (0.5 to 0.6 mmole) was dissolved in 23 ml. of 0.1 *M* sodium hydroxide solution, preheated to 85°, and placed in the constant temperature bath at 85.0°. The imide is rapidly hydrolyzed to the monoanilide in the alkaline solution. The monoanilide solution was poured quickly into the buffer solution; 10-ml. samples were withdrawn at appropriate time intervals.

These samples were pipeted directly into 50-ml. volumetric flasks containing cold water and stored in an ice water mixture for later analysis. After appropriate dilution with water the absorbance (A) of this solution was determined at 241 mμ in 1-cm. cells. A concentrated solution of sodium hydroxide was then added to make the solution substantially alkaline, and aniline was extracted with chloroform. The volume of the chloroform extracts was adjusted to 50 ml. and the absorbance read at 287 mμ in 10-cm. or 1-cm. cells. The concentration of aniline was calculated from the absorbance.

For these systems a plot of log ($A - A_{eq}$) against time always exhibited a straight line relationship. The concentration of the monoanilide at zero time for each run was obtained from these plots. From this information together with molar absorptivity values of aniline, at the pH of the particular run and of the monoanilide and the imide all at 241 mμ, the concentrations of the latter two were calculated from the A values. Experiments showed that the absorptivities of the monoanilide and the imide were not influenced by pH over the range studied.

Computer Analysis

An Applied Dynamics, Inc., model AD-2-32PB analog computer was utilized for this portion of the work.

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