Rate of Anhydride Formation in Tartrate Buffer System

Nature of Reaction Product with Aniline

By T. HIGUCHI, H. UNO*, and I. SHIMADA

Tartaric acid buffer has been shown to form a reactive intermediate in aqueous solution capable of rapidly acylating any nucleophilic compound present. With aniline as the nucleophile, the rate of formation of the intermediate, presumably an acid anhydride, has been found to proceed at roughly the same rate as that of succinic acid but slower than citric acid. Only the monoanilide was obtained in appreciable amount.

BECAUSE OF its availability, nontoxicity, and general properties, tartaric acid and its salts have had reasonably wide pharmaceutical usage. The acid and its chemical behavior, particularly in aqueous solution, are also of interest because of its biochemical origin and because of the role played by related acids in metabolism. The present communication is concerned with the measurement and study of the apparent tendency of the L (+) form of this acid (*dextro* tartaric acid) to yield an active intermediate in an aqueous solution capable of acylating nucleophilic components which may be present.

The general theory and approach basic to this study have been treated already in detail in earlier papers (1–3). The mechanism, which appears to be responsible for the observed acylation of amines introduced into such aqueous solution of acids of this type, can be written

$$\text{acid} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{active intermediate} \underset{\text{aniline}}{\overset{k_2}{\rightleftharpoons}} \underset{k_{-2}}{\text{aniline}}$$

$$\text{amide} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \text{imide} \qquad \text{(Eq. 1)}$$

It has been shown for succinic acid that the active intermediate is the corresponding acid anhydride, and that k_3 is small. Citric acid and polyalkylated succinic acid also appear to follow the same mechanism but go rather readily to the corresponding imides.

In the present study, the specific rate constant for the formation of the presumed tartaric anhydride, its pH profile and temperature coefficient, and finally the nature of the end product in the presence of aniline have been determined.

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* Present address: Sankyo and Co. Ltd., Osaka, Japan.

The experimental results suggest that any pharmaceutical formulations containing tartrate buffer should take into account the possibility of such an acylation mechanism, especially during autoclaving.

EXPERIMENTAL

Equipment

A mineral oil bath regulated to ±0.1° was used for the kinetics runs. All final determinations of concentration of the residual aniline and the reaction products were carried out spectrophotometrically on a Cary 11 MS spectrophotometer. Adjustment and determination of pH were made with a Beckman expanded scale Zeromatic pH meter.

Reagents

All reagents used throughout were of reagent grade or especially synthesized and purified. Aniline was always distilled freshly before use. Several substances, which may have been formed during the reaction studied, were prepared independently according to the method described by Casale (4) and Barrow and Atkinson (5). Only chromatographic grade silicic acid (Mallinkrodt) was used.

Tartaric Acid Phenylimide (Tartranil).—Dry solid anilinium tartrate, prepared by slowly adding 1 mole of aniline in ethanol to 1 mole of tartaric acid in ethanol, was heated in an oven to 140° for 6 hr. The product was dissolved in boiling water and immediately filtered. The imide, which precipitated on cooling, was recrystallized from glacial acetic acid, m.p. 253–254°. (Literature values vary from 254 to 262°.)

Tartaric Acid Monoanilide (Tartranilic Acid).—A calculated amount of 2 *N* NaOH aqueous solution and the imide obtained above were mixed and warmed on the steam bath for 10 min., then acidified with hydrochloric acid to yield crystals on cooling. The product was recrystallized from glacial acetic acid. The equivalent molecular weight, determined by direct titration with sodium hydroxide solution, corresponded to 222, 225 (theoretical 225). The product had a m.p. 179–180°. (Literature value 180°.)

Tartaric Acid Dianilide (Tartranilid).—Tartaric acid was heated under an air condenser with an excess of aniline at 150–170° for 5 hr. The reaction product was purified by recrystallizing from ethanol, m.p. 261–264°. (Literature values vary from 250 to 264°.)

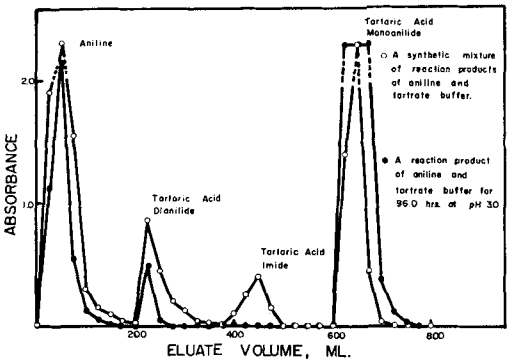

Fig. 1.—Column chromatograms of a known mixture of four substances and of a sample drawn after 96 hr. of reaction of 0.1 mole/L. aniline in 0.5 mole/L. tartrate buffer solution at pH 3.0 and 85°.

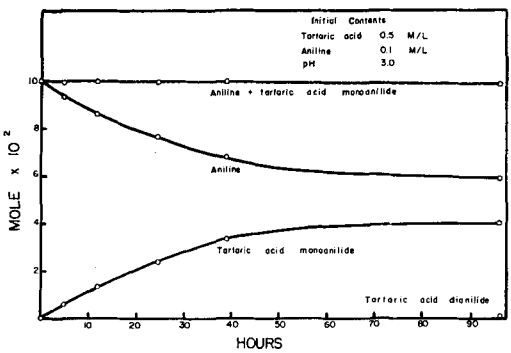

Fig. 2.—Curves obtained quantitatively by column chromatogram of a sample at various time intervals at pH 3.0 and 85°.

Chromatographic Separation

The reaction products formed during interaction of aniline with tartrate buffer were analyzed qualitatively and quantitatively by column partition chromatography. Studies first were carried out singly, then on mixtures of the three possible reaction products and aniline in a manner similar to that described in a previous paper (3). The results then were compared with those obtained on the reaction products.

The column was formed from a slurry made by adding an appropriate quantity of cyclohexane to a mixture of 10 Gm. of silicic acid and 10 ml. of a 0.5 *M* pH 5.5 citrate buffer; this was packed into a 22 mm. bore chromatography column. The sample to be analyzed was added to 2.0 ml. of a pH 5.5 citrate buffer solution and was adsorbed on 2 Gm. of silicic acid, which in turn was packed as a slurry in 10 ml. of cyclohexane onto the column. A third batch of slurry was prepared in the same beaker used for the sample using 1 Gm. of silicic acid, 1 ml. of pH 5.5 citrate buffer solution, and 5 ml. of cyclohexane to insure quantitative transfer of the sample to the column. The column was developed then with the following solvents: cyclohexane, 200 ml.; 10% butanol in cyclohexane, 200 ml.; 30% butanol in cyclohexane and pure butanol, 200 ml. Each elution solvent was saturated with the pH 5.5 citrate buffer before use. The eluate was collected in 25-ml. fractions which then were analyzed spectrophotometrically.

Kinetics Study

The rate of disappearance of aniline in 0.5 *M* tartrate buffer system was followed in a manner already described in a previous paper (3). The study was run under air rather than nitrogen since no differences were noted for this system.

OBSERVATIONS

Nature of the Reaction Product.—Column chromatograms of a sample drawn after 96 hr. of reaction of 0.1 mole/L. aniline in a 0.5 mole/L. tartrate buffer solution at pH 3.0 and 85° and of a known mixture of aniline, tartaric acid monoanilide, tartaric acid phenylimide, and tartaric acid dianilide are shown in Fig. 1. Comparison of these two chromatograms strongly suggests that only the monoanilide is produced in any significant amount under these conditions. The fraction isolated from the reaction solution, which eluted at the position corresponding to that of the monoanilide, gave on recovery from the column infrared and ultraviolet absorption spectra matching those obtained from directly synthesized monoanilide. The eluted material also exhibited the same melting point (178–180°). Equivalent weight, determined by alkalimetric titration, was found to be 233 (theoretical 225).

Figure 2 shows the amount of various presumed fractions isolated chromatographically at various time intervals from a solution of 0.1 mole/L. of aniline in 0.5 mole/L. tartrate buffer at pH 3.0 and kept at 85°. It is evident that essentially all of the aniline lost appeared as the monoanilide. There was a small amount of a component which appeared in the dianilide fraction, but this represented an insignificant fraction of the total.

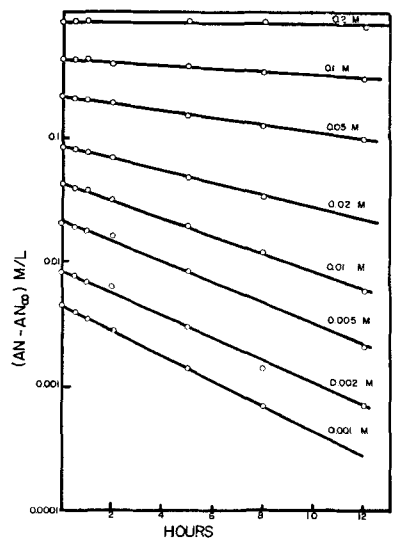

Fig. 3.—Plots showing logarithmic approach to an equilibrium for aniline tartrate system at pH 3.0. The several lines represent differing initial aniline concentration.

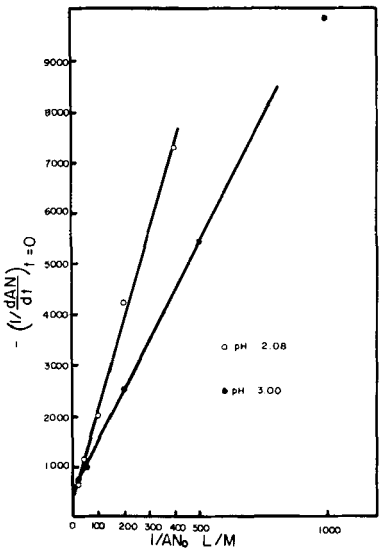

Fig. 4.—Plots of reciprocal initial rate of aniline disappearance in hours/liter/mole against reciprocal initial aniline concentration in liters/mole for aniline in presence of aqueous 0.5 *M* tartrate buffer at pH 2.08 and 3.0. The relationship corresponds to Eq. 2.

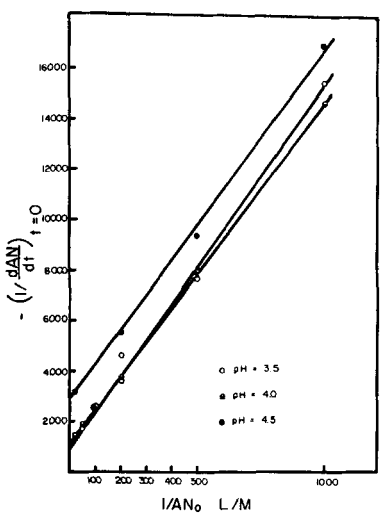

Fig. 5.—Plots of reciprocal initial rate of aniline disappearance in hours/liter/mole against reciprocal initial aniline concentration in liters/mole for aniline in presence of aqueous 0.5 *M* tartrate buffer at pH 3.5, 4.0, and 4.5. The relationship corresponds to Eq. 2.

TABLE I.—EFFECT OF pH ON THE OBSERVED CHANGE OF k_1(TH) AND RATIO OF k_2/k_{-1} AT 85°

pH	k_1(TH) (hr.$^{-1}$)	k_2/k_{-1} L./*M*
1.6	2.5×10^{-3}	15
2.08	2.5×10^{-3}	25
2.5	2.38×10^{-3}	35
3.0	1.8×10^{-3}	60
3.5	1.33×10^{-3}	71
4.0	8.3×10^{-4}	90
4.5	$\sim 2 \times 10^{-5}$	2×10^2

Rate of the Acid Anhydride Formation.—Rates of disappearance of aniline in 0.5 molar tartrate buffer system were studied at 85° at various pH values and aniline concentration varying from 2×10^{-1} to 1×10^{-3} mole/L. As would be expected from Eq. 1, the reaction approaches equilibrium, given sufficient time.

The theoretical basis of the following treatment has been reported elsewhere (2, 3); references should be made for detail to the earlier works. Typical AN-AN$_\infty$ values plotted against time are shown in Fig. 3—apparently straight lines with different slopes. As has been pointed out, a reciprocal plot of the observed initial rate of disappearance of aniline from this system can be related directly to the rate of the intermediate formation in accordance with

$$-\{1/[d(\mathrm{AN})/dt]\}_{t=0} = 1/(k_1k_2/k_{-1(\mathrm{TH})(\mathrm{AN}_0)}) + 1/[k_1(\mathrm{TH})] \quad \text{(Eq. 2)}$$

where

(AN) = concentration of aniline at time *t*,
(AN_0) = initial concentration of aniline,
(TH) = total concentration of the tartrate buffer,
k_1(TH) = over-all rate of formation of the active intermediate,
k_2 = observed specific second-order rate constant for the formation of the active intermediate, and
k_{-1} = specific rate constant for reformation of tartrate from the active intermediate.

Figures 4 and 5 show typical plots of this type for the tartrate system. The intercepts and slopes can be read from such graphs. k_1(TH) and k_2/k_{-1} values determined in this manner are shown in Table I.

In Fig. 6, the pH profile for the tartrate buffer corresponding to k_1(TH) at 85° is shown. The smooth plot corresponds to the relative concentra-

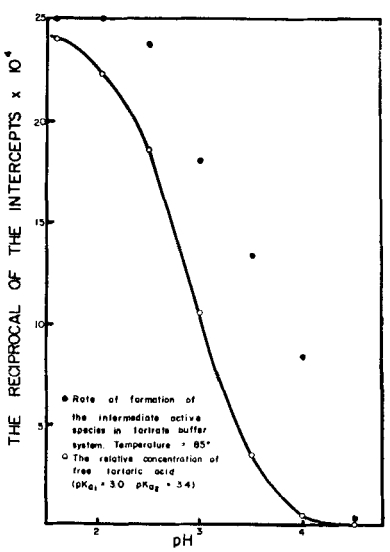

Fig. 6.—pH profile of formation of the intermediate active species in tartrate buffer at 85°, determined from intercepts of plots similar to Figs. 4 and 5, and the relative concentration of free tartaric acid.

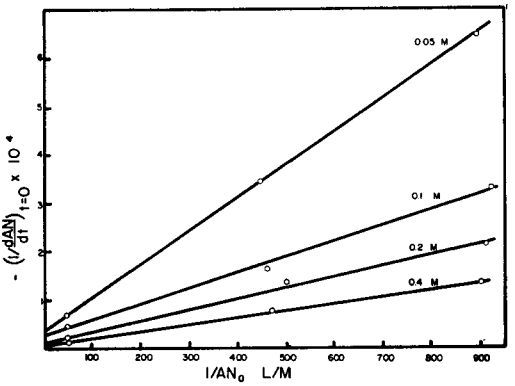

Fig. 7.—Rate dependency on tartaric acid concentration varying from 0.05 to 0.4 mole/L. at pH 3.0 and 85°. The concentration of tartaric acid is expressed in moles/liter.

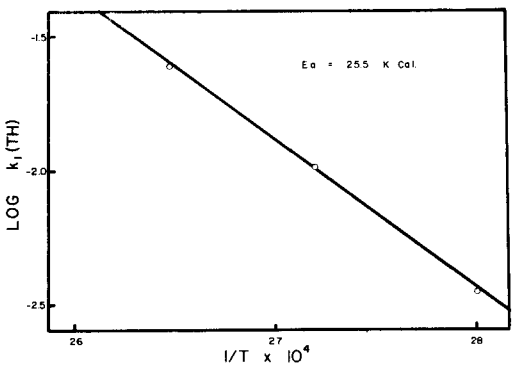

Fig. 8.—Arrhenius-type plot for k_1 (TH) at pH 2.08.

tion of the unionized acid [assumed $pKa_1 = 3.0$, $pKa_2 = 3.4$ (6)] in solution normalized for best fit. There is little doubt that the free acid is not the only participant in this reaction. The general behavior suggests the possibility that, although anhydride formation may still be involved, the reaction in such a case must be catalyzed by other acid-base species similar to that observed for succinate and citrate systems (2, 3). The rate of formation of intermediate active species in aqueous solution at 85° for tartaric acid (rate = 5×10^{-3} hr.$^{-1}$) is roughly the same as that of succinic acid (rate = 6.3×10^{-3} hr.$^{-1}$) but slower than that of monoionized citric acid (> 0.02 hr.$^{-1}$).

There are other possible intermediate active species which can form in the system. Formation of a lactone can also explain the observed kinetics relationship. Bruice and Marquardt (7) have shown that γ-lactone formation facilitates the hydrolysis of γ-hydroxybutyramide. Although this is a possibility, the similarity in behavior with the succinate system would seem to suggest that the tartrate reaction follows the same mechanism.

Another aspect of the present study which cannot be answered at the moment is the apparent lack of dependence of k_2/k_{-1} on the pH of the reacting system. If only the free amine reacted with the active species, the k_2/k_{-1} values should decrease inversely with the hydrogen ion concentration. Table I shows that this does not seem to be the case, except at the highest pH range.

Influence of Tartrate Concentration.—Rate dependency on the acid concentration was determined at 85° using various concentrations of tartaric acid from 0.05 mole/L. to 0.4 mole/L. Results are shown in Fig. 7. The intercepts (the reciprocal of rate of formation of the intermediate active species) appear to be proportional to the concentration of tartaric acid, as predicted by the theory. It is evident from these measurements that the reaction is first order with respect to tartaric acid.

Influence of Temperature.—The temperature dependency of the reaction rate was determined from measurements made at 75°, 95°, and 105°. The runs were limited to pH 2.08, where the observed rate of the reaction was relatively fast. An Arrhenius-type plot for k_1(TH) against the reciprocal of absolute temperature is shown in Fig. 8, corresponding to an apparent activation energy for the tartrate buffer system of 25.3 kcal./mole.

GENERAL DISCUSSION

Although the total mechanism accounting for the behavior of the tartrate system cannot be offered at the present time, the data presented strongly suggest that an active acylating intermediate is produced in tartrate buffers, particularly under acidic conditions and higher temperature. The introduction of the two hydroxyl groups does not appear to confer on the tartaric acid molecule a behavior much different from that of succinic acid. This is in marked contrast to the effect produced by introduction of alkyl substituents.

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