THE CHEMISTRY OF SOME CYCLIC ANHYDRIDES

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(Under the supervision of Professor Takeru Higuchi)

The polycarboxylic acids and their derivatives are biologically and pharmaceutically useful. This study concerns itself with some of the chemistry and applications of these acids and their derivatives. Specifically, the following areas were investigated: 1) The use of polycarboxylic acid derivatives in the spontaneous carbonation of aqueous systems; 2) The interactions of some polycarboxylic acids with acid anhydrides in aqueous solution; 3) The synthesis, isolation, and some of the chemistry of citric acid anhydride.

Two novel approaches for improving the process of spontaneous carbonation of aqueous solutions were investigated. The classical system used for carbonation suffers from several drawbacks among which the saline taste produced and the low levels of supersaturation of carbon dioxide obtained are perhaps the most significant. The reason for the low degree of carbonation attained is the tendency for one of the components (bicarbonate or the solid acid) to dissolve more rapidly than the other which results in the production of carbon dioxide bubbles at the surface of the undissolved particle which allows for rapid escape from the system. In order to obviate this problem,

a system employing a latentiated acidifier (glutaric anhydride) together with bicarbonate was proposed and investigated. The anhydride required time to dissolve and hydrolyze to the free acid. This lag time allowed the bicarbonate to dissolve and thus the carbon dioxide producing reaction takes place in a relatively homogeneous solution. Actual results showed that systems carbonated by this method showed superior carbonation when compared to the classical system.

Another approach to the carbonation problem is the use of a compound which hydrolyzes in water to produce carbon dioxide and acceptable solutes directly. O-Anhydrocarboxy-tartronic acid was prepared and evaluated as a model compound of this type. Although this particular compound produced copious quantities of carbon dioxide and obviously eliminated the problem of saline taste, it was found that the physical properties of the compound probably preclude its use as a potential carbonating agent.

The interaction of several anhydrides with polycarboxylic acids in aqueous solution was investigated, and it was found that the anhydrides reacted with the di-ionized polycarboxylic acids at ambient temperatures to form unstable mixed anhydrides. When both reactants were polycarboxylic, the mixed anhydride was able to either revert to the reactant species or proceed to form a new cyclic anhydride and the acid of the original anhydride. When acetic anhydride (or similar linear anhydrides) was

employed, the system was no longer reversible and the mixed anhydride went on to form the new polycarboxylic anhydride.

The stereospecificity of the reaction between d-methylsuccinic anhydride and citrate ions in aqueous solutions was also studied. The citric acid anhydride which formed was reacted with aniline and the resulting anilides were separated. The specific rotations of the unsymmetrical citric acid monoanilide and citranilic acid were determined. The optical yield of the reaction was found to be less than 0.3%, indicating that the transfer of anhydride character (in the present case at least) is not greatly influenced by the steric configuration of the diastereomeric transition states.

The final portion of this study was concerned with the preparation, isolation and some of the chemistry of citric acid anhydride, which had not previously been prepared in the crystalline form. The anhydride was synthesized by reacting acetic anhydride and citric acid under suitable conditions which decreased the production of acetylcitric anhydride which is normally the product of such a reaction. The yield of the reaction was about 40% and the crystalline compound melted at 120° to 123°. Characterization of the substance showed it to be the unsymmetrical intramolecular anhydride. It was found to react with aniline to produce the expected isomeric monoanilides. The rate of hydrolysis of citric acid anhydride, unlike the more common anhydrides, was dependent on the pH over the range investigated

(pH = 1 to 6). The half-life of the compound ranged from about 12 seconds at pH = 1 to 50 seconds at pH = 6. The hydrolysis apparently was dependent upon the dissociation of the residual carboxylic acid group in the anhydride.

APPROVED Louis A. Busse

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by

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This work is dedicated
to my wife Barbara,
my daughter Tracey,
and my parents.

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INTRODUCTION

Although polycarboxylic acids such as tartaric, citric, and malic acids are widely distributed in nature and almost as widely used in the commercial food and drug areas, apparently little of the chemistry of the interaction of such compounds and their derivatives with various reactive substances in aqueous solution has been known, let alone understood, until relatively recently. The fact that such acids exist in equilibrium with their anhydrides in aqueous solution was first demonstrated only about five or six years ago.

This thesis presents studies of the chemistry of some polycarboxylic acids, their anhydrides, and other similar derivatives which have or may have application to pharmaceutical systems. Specifically, three areas have been investigated: 1) two novel methods of producing high levels of carbonation in aqueous solutions by use of organic acid derivatives; 2) the chemistry of the interactions of polycarboxylic acids with acid anhydrides; and 3) the synthesis, isolation, and some chemistry of citric acid anhydride. Results of these studies are discussed separately in the following sections.

SECTION I

CHEMICAL CARBONATION OF AQUEOUS SYSTEMS

INTRODUCTION

The chemical carbonation of aqueous solutions has been practiced for many years without any significant changes in the systems used. The classical system usually consists of mixtures of sodium bicarbonate and one or more polycarboxylic acids such as malic, tartaric, or citric acid. Such systems suffer from several disadvantages of which the most important are its inability to achieve high levels of supersaturation of the solution with respect to carbon dioxide and the salinity caused by the sodium chloride produced.

The problem of poor supersaturation is due to the dissolution rates of the acid and the bicarbonate. One of the ingredients dissolves at a faster rate than the other and the dissolved species reacts with the undissolved at the surface of the undissolved particle which presents an excellent nucleation center for the formation of bubbles of carbon dioxide which escape from the system without supersaturating the bulk of the solution. It was felt that if the acid was introduced in a nonreactive form such as an anhydride, the time required for its dissolution and hydrolysis to the free acid would allow adequate time for the complete dissolution of the bicarbonate. Thus the reaction between the acid and the bicarbonate would for the most part take place in an homogeneous solution with

considerably less loss of carbon dioxide. When glutaric anhydride was employed together with sodium bicarbonate, the resulting carbonated aqueous solution showed a degree of carbonation which was much superior to the classical system.

Another approach to the problem is the use of a compound which when added to water dissolves and hydrolyzes rapidly to form carbon dioxide. Such a compound would require no bicarbonate for the production of carbon dioxide and therefore the problem of saline taste associated with the classical system would be obviated. If the dissolution of the compound is fast in relation to its hydrolysis the problem of nucleation would also be virtually eliminated and the resulting solution should be highly supersaturated with respect to carbon dioxide as well as being free of the saline taste. O-Anhydrocarboxytartronic acid was synthesized and isolated for use as a model of such carbonating agents. This compound hydrolyzed rapidly in water and produced copious quantities of carbon dioxide. However, the cost of preparation of O-anhydrocarboxytartronic acid, and its general physical properties such as hygroscopicity, would seem likely to severely limit the use of this particular compound as a potential carbonating agent.

PART 1

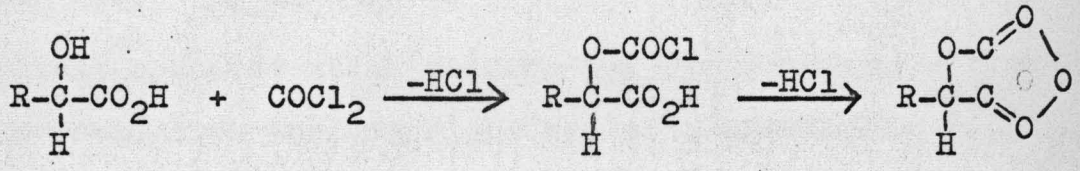
CARBONATION OF AQUEOUS SOLUTIONS WITH ACID ANHYDRIDES

PART 2

SYNTHESIS, ISOLATION, AND EVALUATION OF O-ANHYDROCARBOXYTARTRONIC ACID

INTRODUCTION

Davies (1) has described the preparation of O-anhydrocarboxy derivatives of several α-hydroxy and α-mercaptomonocarboxylic acids by the reaction of phosgene with the particular acid in dioxane. The proposed reaction mechanism for an α-hydroxy acid is shown below.



When the product is dissolved in water it is hydrolyzed to carbon dioxide and the parent acid.

The O-anhydrocarboxy acids prepared by Davies generally showed melting points ranging from 27° to 70°; which in most cases was lower than the melting point of the parent acid. This can be ascribed to a marked decrease in the intermolecular attraction of the molecules due to the alteration of the polar groups which are capable of hydrogen bonding.

It was felt that if a similar reaction was carried out using dicarboxylic acids which contained a hydroxy group the resulting compounds would be likely to have higher melting points thereby increasing their commercial utility.

Tartronic acid(2-hydroxypropanedioic acid) and α-isomalic acid(2-hydroxy-2-methylpropanedioic acid) were the simplest compounds meeting these requirements and attempts were made to synthesize their O-anhydrocarboxy derivatives. The difficulty of the procedure used by Davies arose mainly from the use of dioxane as the reaction solvent. Our work was carried out using tetrahydrofuran which shortened the reaction time from days to hours and in addition is much more volatile and thus makes it easier to isolate the products. Subsequent to the initiation of this study, a patent appeared which encompassed the entire area under investigation including the use of tetrahydrofuran as the solvent of choice (2). The reaction of α-isomalic acid and phosgene did not result in the production of significant amounts of O-anhydrocarboxy- -isomalic acid. The material obtained from the reaction was shown to produce only about 10-12% of the theoretical amount of carbon dioxide expected when dissolved in water. The O-anhydrocarboxytartronic acid was synthesized, isolated and some of its physical and chemical properties were determined.

EXPERIMENTAL

Equipment and Reagents

The infrared spectra were obtained using a Beckman model IR5A infrared spectrophotometer. A Cary model 11 recording spectrophotometer was used for uv measurements. The chromatographic columns used were 26 cm long and had an internal diameter of 2 cm. The tetrahydrofuran was refluxed over sodium hydride and distilled prior to use. Phosgene was distilled before using. All other chemicals used were of analytical or reagent grade.

Procedures

Synthesis of O-Anhydrocarboxytartronic Acid.

A solution containing 15.1 grams (0.125 moles) of tartronic acid dissolved in 110 mls of tetrahydrofuran was added dropwise over a 30 minute period to a cold (0°) stirred solution of 56 grams (0.057 moles) of phosgene and 25 mls of tetrahydrofuran. After the addition was complete, the vessel was closed and stirring was continued. The temperature of the solution was allowed to rise over a period of about two hours to 25°, and maintained at that temperature for about 6 more hours. Most of the excess phosgene was then removed by bubbling dry nitrogen gas through the solution for one to two hours. The remaining volatile components including the solvent were removed at reduced pressure and a temperature of 35 to 40° on a

rotary evaporator. After about an hour only a viscous yellow oil remained. Ten mls of absolute ether was added and the solution was cooled in a dry ice-acetone bath for about 30 minutes and then allowed to warm to 0°. As the solution began to warm, a white crystalline precipitate formed. Another 5 mls of absolute ether was added to disperse the precipitate which was removed by filtration in a low humidity environment. The precipitate was washed with 5 mls of cold ether and dried for several hours under reduced pressure. The compound was identified as O-anhydrocarboxytartronic acid by infrared spectra, and other physical measurements. The compound decomposed at 100-105°C.

Equivalent Weight

73.0 (calculated) 73.5 (found)

Carbon Dioxide Generated in Water

30.1% (calculated) 29.5% (found)

Determination of Equivalent Weight.

An appropriate weight of the O-anhydrocarboxy acid was dissolved in water and boiled to remove the carbon dioxide. The cooled solution was then titrated directly with 0.1 N aqueous sodium hydroxide using phenolphtholein as the indicator.

Quantitative Determination of the Carbon Dioxide Produced in Water.

The apparatus used consisted of a reaction vessel containing approximately 80 mls of 0.1 N aqueous sulfuric acid. A fritted glass gas dispersion tube was connected in such a manner that carbon dioxide-free nitrogen gas could be passed through this solution. The nitrogen was then passed through a gas dispersion bottle containing concentrated sulfuric acid. The nitrogen was subsequently subjected to further drying through a column containing magnesium perchlorate after which it passed through the detector tube containing ascarite. The entire apparatus was flushed with nitrogen for 30 minutes prior to use. The detector tube was then removed, carefully weighed, and replaced in its proper position. A sample expected to produce 80-150 mg of carbon dioxide was weighed directly into an aluminum foil container and quickly and carefully placed in the reaction vessel. The vessel was then tightly closed, the aluminum foil container was upset allowing the sample to react with the water, the nitrogen gas was again passed through the solution for a period of not less than 90 minutes. At the end of this time the detector tube was removed and weighed again. The weight difference was taken as the weight of carbon dioxide produced.

Standard samples of sodium carbonate when analyzed for carbon dioxide production in the presence of acid gave

consistant values of 99.5 to 100.5% of the theoretical. Samples of O-anhydrocarboxytartronic acid analyzed by this method were shown to contain 98% of the theoretical amount of carbon dioxide.

When different size detection tubes were used it was found that the nitrogen flow rate had to be adjusted accordingly.

Partition Column Chromatography for the Separation of the Anilides Formed by Reacting Aniline with O-Anhydrocarboxytartronic Acid.

The column consisted of 3 grams of silicic acid, 8 mls of chloroform and 3 mls of 4 N aqueous sulfuric acid which was packed on the bottom of the column and covered with a tight fitting filter paper disc. On top of this was packed a mixture of 25 grams of silicic acid, 38 mls of chloroform and 25 mls of a 0.3 M, pH = 3.13 sodium phosphate buffer.

The sample was prepared by adding 0.3 mls of 0.68 M O-anhydrocarboxytartronic acid in tetrahydrofuran to 9 mls of a 0.14 M solution of aniline in water. The pH of the solution was then adjusted to 3.13 with hydrochloric acid, and 5 mls of the resulting solution was mixed with 5 grams of silicic acid and 8 mls of chloroform and packed on the column. Elution was carried out using the following volumes of water saturated solutions:

0-100 mls	0.0%	Butyl alcohol in chloroform
100-200 mls	1.5%	Butyl alcohol in chloroform
200-300 mls	10.0%	Butyl alcohol in chloroform
300-400 mls	30.0%	Butyl alcohol in chloroform

The eluate was collected in volumes of 10 mls and the optical density was measured at 255 mμ using a corresponding butyl alcohol-chloroform solution as a reference.

Paper Chromatographic Determination of the Nonvolatile Hydrolysis Products of O-Anhydrocarboxytartronic Acid.

Two solutions, one containing 1% tartronic acid in water and the other containing 1% O-anhydrocarboxytartronic acid in water, were prepared. Several microliters of each solution were spotted on Whatman No. 1 chromatographic paper. The development of the chromatogram was achieved by using the organic layer from a 10:2:5 mixture of butyl alcohol, formic acid and water, respectively. Detection of the spots on the dried paper was done by spraying with a 0.1% bromcresol green in ethanol solution. The observed spots for both samples had identical R_f values indicating that the O-anhydrocarboxytartronic acid hydrolyzed to tartronic acid.

RESULTS AND CONCLUSIONS

The product isolated from the reaction of phosgene and tartronic acid was a white crystalline material which decomposed at 100-105°. When the infrared spectrum of the sample was taken (Figure 1) it was apparent that there were no peaks in the OH stretching frequency region indicating the removal of the alcoholic hydrogen. In addition there were three peaks in the carbonyl stretching region which is common for the cyclic anhydride structure. The overall spectrum suggested that the compound was O-anhydrocarboxy-tartronic acid.

Titration of the compound gave an equivalent weight of 73.5 which is in good agreement with the calculated value of 73.0. This evidence was not conclusive however since impurities could give rise to this equivalent weight. It was therefore felt that the carbon dioxide which was produced in water had to be determined quantitatively. Since there was no satisfactory assay for carbon dioxide produced in effervescent solutions, we devised a quantitative assay which was a variation of the procedure used for elemental analysis. The accuracy of the method was checked using sodium carbonate samples capable of producing 80-150 mg of carbon dioxide. The results showed weight changes of $100 \pm 0.5\%$ of the calculated amount.

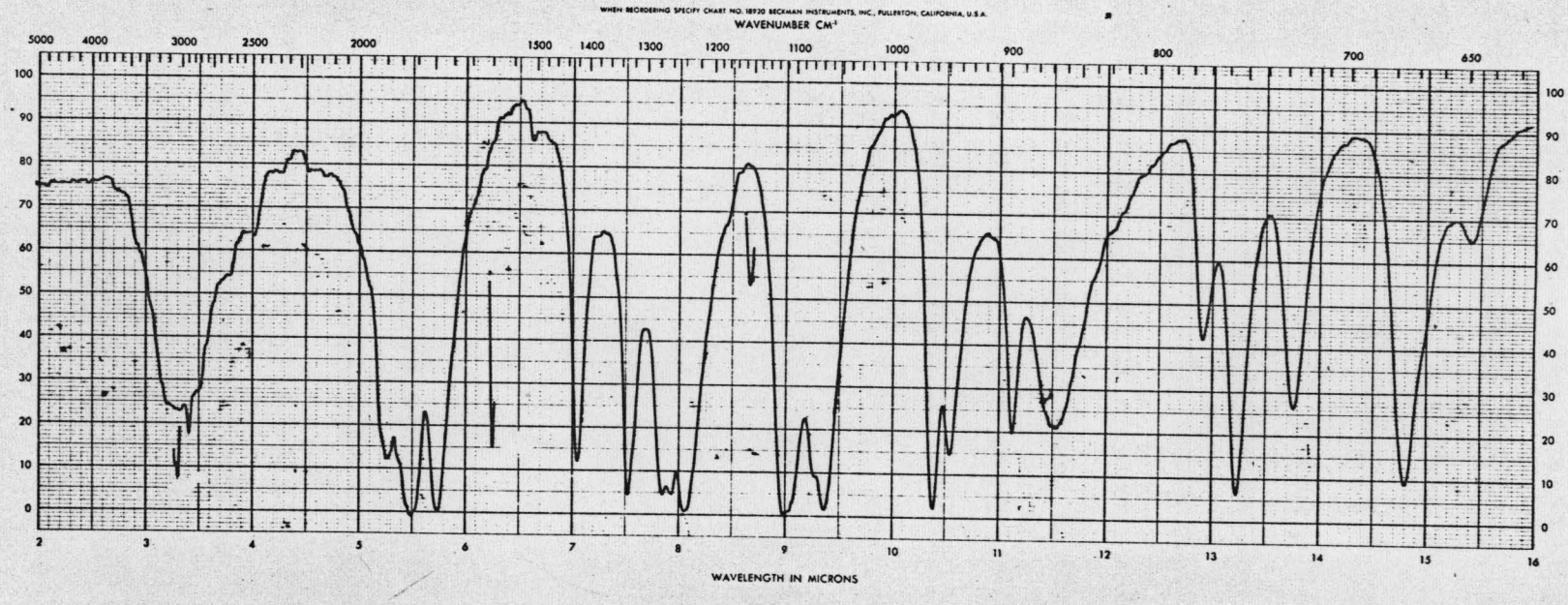
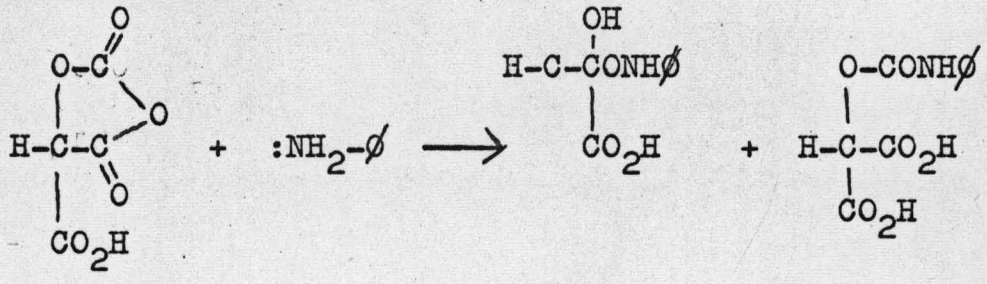


Figure 1. Infrared spectrum of O-anhydrocarboxytartronic acid. Concentration is 0.1% in potassium bromide pellet. Spectrum is calibrated at 3.302, 6.238 and 8.662 mμ.

O-Anhydrocarboxytartronic acid analyzed by this method produced 98% of the carbon dioxide which would have been expected from the pure compound, and thus further confirmed the postulated structure. In addition, the O-anhydrocarboxytartronic acid when added to water produced copious amounts of carbon dioxide and the nonvolatile product was shown by paper chromatography to be tartronic acid.

Reaction of the compound with aqueous aniline resulted in the formation of two aniline products when the reaction mixture was separated by column chromatography (see Figure 2). This seems to contradict similar work (1) in which only one aniline derivative was found. The following reaction was postulated:



The ultraviolet absorbance changes of aqueous solutions of O-anhydrocarboxytartronic acid observed at 245 mμ showed an initial decrease with a half-life of the order of 5 seconds followed by an increasing absorbance with a half-life of the order of 20 seconds. The first reaction is believed to be due to the opening of the ring structure and the second due to the release of carbon dioxide.

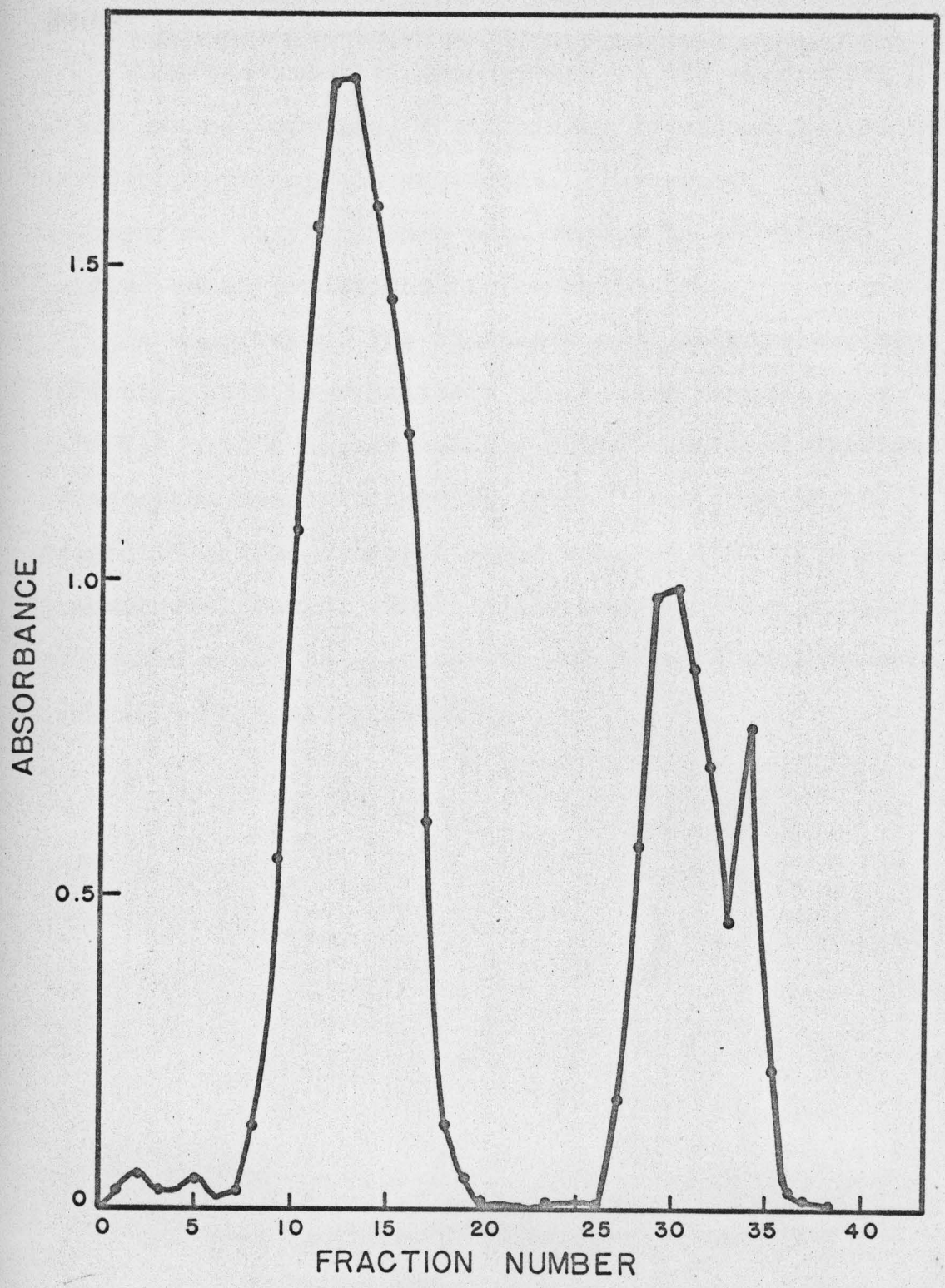


FIGURE 2. Chromatogram of the products of the reaction of 0.3 mls of 0.68 molar 0-anhydrocarboxy-tartronic acid in tetrahydrofuran with 9 mls of 0.14 molar aqueous aniline. The absorbance was determined at 255 mμ. The peak associated with fraction 34 is due to the change in eluant composition.

When the material was exposed to the atmosphere for short periods of time, a stickiness developed due to the hygroscopicity of the substance. Therefore, the O-anhydrocarboxytartronic acid should be stored and handled under conditions of low humidity.

In conclusion, the synthesis of O-anhydrocarboxy-tartronic acid is relatively simple and results in a material with a higher melting point than most previously synthesized O-anhydrocarboxy acids. But, the hygroscopicity of the compound would seem to limit its use on a commercial basis. It is felt, however, that similar compounds might be synthesized which would exhibit more desirable physical properties.

REFERENCES

1. Davies, W. H., J. Chem. Soc., 1357 (1951).

2. Feldman, J. R., and Foltz, R. L., United States Patent 3,218,338, November 16, 1965.

SECTION II

THE CHEMISTRY OF THE INTERACTIONS OF POLYCARBOXYLIC ACIDS WITH ANHYDRIDES

INTRODUCTION

The studies presented in this section are concerned with the interaction of some polycarboxylic acids of biological and pharmaceutical interest with various anhydride species in aqueous solution. The interaction apparently results in formation of a mixed anhydride which rapidly forms substantial quantities of a new anhydride of the polycarboxylic acid. The effects of pH, concentration of polycarboxylic acid, the concentration and nature of the original anhydride species, and stereochemical factors were investigated.

PART 1

INTERACTION OF DI- AND TRICARBOXYLIC ACIDS WITH GLUTARIC ANHYDRIDE IN AQUEOUS SOLUTION

PART 2

INTERACTION OF ACETIC ANHYDRIDE WITH DI- AND TRICARBOXYLIC ACIDS IN AQUEOUS SOLUTION

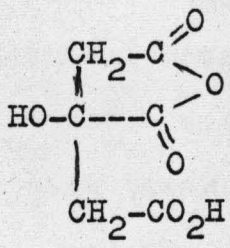
PART 3

ATTEMPTED ASYMMETRIC SYNTHESIS OF CITRIC ACID ANHYDRIDE IN AQUEOUS SOLUTION

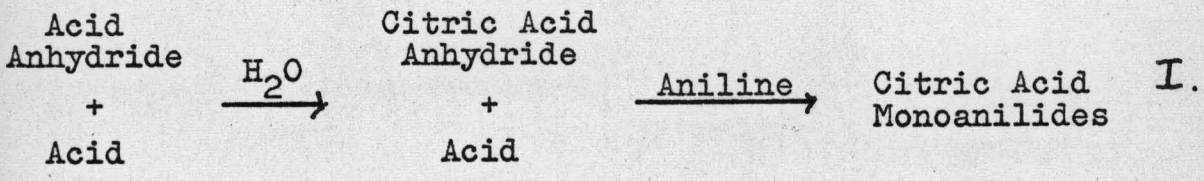
INTRODUCTION

Although the citric acid molecule has a plane of symmetry and cannot possess any asymmetric center, either of the two possible unsymmetrical citric acid anhydrides are potentially optically active. Thus, cleavage of these compounds by nucleophiles such as alcohols or amines could lead to potentially optically active derivatives if the attack occurred on the terminal carbonyl group of the anhydride. Nucleophilic attack on the center carbonyl group would lead to a symmetrical molecule with no optical activity. The present study was aimed at forming optically active citric acid anhydride in aqueous solution by reaction of an optically active anhydride and citrate ions (1-2). Specifically, the asymmetric synthesis of the unsymmetrical citric acid anhydride was attempted by reacting d-methylsuccinic anhydride with citrate ions in aqueous solution. The relative amounts of the enantiomorphs produced was monitored by addition of aniline at suitable times. Although the results of this investigation suggested that the overall optical yield of the reaction was less than 0.3%, it is felt that the general method may be made more efficient if a more complex optically active anhydride were used.

The reaction of carboxylic acid anhydrides with citrate ions in aqueous solution at ambient temperatures has already been shown to result in formation of a citric acid anhydride species (1,2) presumably of the following structure:



If the formation of this cyclic anhydride is induced with an optically active anhydride it is evident that unequal amounts of the d and l enantiomers of citric acid anhydride would be produced. Although these anhydrides cannot be isolated from aqueous solution, they can be detected and determined by quenching the reaction with aniline. The general reaction scheme may be represented as follows:



I.

The structural isomers of citric acid monoanilide result. The unsymmetrical isomer possesses an asymmetric carbon and thus may exist as an enantiomeric pair while the symmetrical isomer is incapable of exhibiting stereo-isomerism.

The citric acid anhydride formed in reaction I will be racemic if the initial anhydride is optically inactive. However, if the initial anhydride is optically active, the reaction should result in the preferential formation of one enantiomer over the other, and the proportions of the optically active unsymmetrical citric acid monoanilides formed should reflect the ratio of the enantiomers in the reaction mixture at the time the aniline is added.

PAST WORK

It was demonstrated about twenty to thirty years ago that citric acid was cycled, in the Krebs citric acid cycle, by an asymmetric pathway (3-9). The reason for this was not clearly understood until the explanation of the nature of the "meso-carbon" was made and the extreme stereospecificity of enzymes was realized. Citric acid is a symmetrical compound. It is an example of a group of compounds possessing one common feature which is the "meso-carbon" atom. This term was coined by Schwartz and Carter (10) in 1954. It refers to a carbon atom of the type Caabd where a, b, and d are symmetrical groups or atoms, but $a \neq b \neq d$ and all four groups are attached to the same carbon atom.

When one of the "a" groups of such a compound is altered by some reaction, the "meso-carbon" atom is converted to an asymmetric carbon. If the alteration is caused by reaction with some symmetrical group the result is a racemic mixture, since the transition states will be enantiomeric and thus possess the same free energy. An example of this type of reaction is the methanolysis of β-methyglutaric anhydride (11) in which case the product is racemic methyl hydrogen β-methylglutarate. If, however, the reaction of a "meso-carbon" containing compound is carried out with some optically active reagent capable of altering one of the "a" groups, the interaction

will produce diastereomeric transition states. These transition states will have different free energy levels and will cause the formation or destruction of one of the transition states at a faster rate than the other and this results in one of the stereoisomers being formed in preference to the other. This type of reaction is exemplified in the alcoholysis of β-methylglutaric anhydride by one of the enantiomers of 2-butanol. The reaction yields unequal amounts of the two dissymmetric diastereomers (11) as would be expected.

The alteration of one of the methylcarboxy groups of citric acid would also cause the formation of an asymmetric center in the molecule which if produced by interaction with an optically active compound would be expected to result in unequal amounts of the possible stereoisomers, due to the diastereomeric transition states through which the reaction must pass.

EXPERIMENTAL

Equipment and Reagents

A Cary model 60 recording polarimeter was used for all optical rotary dispersion measurements. Ultraviolet spectroscopic measurements were done on a Cary model 14 recording spectrophotometer. The Varian model A-60A nuclear magnetic resonance spectrometer was employed for obtaining the necessary nmr spectra. pH Values were determined on a Corning model 12 pH meter and read to 0.001 units. The length and internal diameter of the chromatographic columns used were 26 cm and 2 cm respectively.

Aniline was vacuum distilled from zinc dust prior to use. Tetrahydrofuran was refluxed with sodium hydride and distilled prior to use. Levo-1-*p*-nitrophenyl-2-amino-1,3-propanediol (called levo-chloramphenicol base) was provided by Parke, Davis and Company, Detroit, Michigan (batch number H700000) and used with no further purification. All other chemicals used were of analytical or reagent grades.

Procedures

Resolution of Methylsuccinic Acid.

Fifty grams (0.379 moles) of methylsuccinic acid and 160.5 grams (0.757 moles) of levo-1-*p*-nitrophenol-2-amino-1,3-propanediol (levo-chloramphenicol base) were dissolved

in 350 mls of boiling water. The yellow solution was filtered and the volume reduced to about 250 mls on a rotary evaporator under reduced pressure at a temperature of 50°. Upon cooling in an ice bath a brown oil separated and solidified. The mass was broken up, filtered off, and dissolved in 350 mls of hot water. Eight hundred mls of boiling ethanol was added and the solution was allowed to cool. Within an hour, crystals began to form and the solution was allowed to stand overnight. The crystals were subsequently removed by filtration, washed with 150 mls of cold ethanol, and dried. The dry salt (56 grams) was dissolved in a mixture consisting of 75 mls of water and 350 mls of ethanol heated to boiling. After cooling and standing overnight, the resulting crystals were removed by filtration and dried.

Dowex 50W-X8 strong acid cation exchange resin was employed to recover the acid from the salt. 100 Mls of the hydrated resin was placed in a chromatographic column and a solution containing 4 grams of the salt in 35 mls of water was poured into the column. This was washed through with 250 mls of water. The eluant was collected in bulk and evaporated to dryness on a rotary evaporator. The dry residue was recrystallized twice from the benzene-chloroform solutions. The melting point of 115° was identical to the value obtained from the literature for an optically pure enantiomer of methylsuccinic acid (12).

The optical activity of the acid showed it to be d-methylsuccinic acid and was in good agreement with previous work (13) ($[\alpha]_D^{27°}$ = 16.4° (c 1.36 in ethanol).

Preparation of d-Methylsuccinic Anhydride.

The anhydride of d-methylsuccinic acid was prepared according to the procedure of Naps and Johns (14). It was recrystallized from a benzene-petroleum ether mixture. The melting point and specific rotation were in good agreement with earlier work.

	Found	Literature Values
Melting point	66-67°C	64-65°C (14), 69.5°C (12)
$[\alpha]_D$	+31.1° at 27°C (c 1.50 in chloroform)	+31.3° at 29°C (14) (c 1.537 in chloroform)

Synthesis of Citranilic Acid.

The compound was prepared by a modification of the procedure of Nau (15). Equimolar amounts of citric acid and aniline were dissolved in warm ethanol. Upon cooling a precipitate of the monoaniline salt of citric acid separated out. The dry salt was then heated at 150°C for about two hours to effect dehydration. Boiling water was added till all of the melt had dissolved. Activated charcoal was added and boiling continued for several minutes after which the solution was filtered and allowed to stand for about 12 hours. A small amount of crystalline

material which formed was filtered off and identified as citranilic acid anilide. The filtrate was then extracted five times with chloroform. The volume of chloroform used for each extraction was equal to one-tenth of the filtrate volume. The volume of the filtrate was then reduced to half on a rotary evaporator by removal of the aqueous solvent under reduced pressure and a temperature of 50°. At this point a large amount of a white crystalline precipitate formed. The solution was filtered, the precipitate washed with water, and dried. It was identified as citranilic acid by uv and nmr spectra as well as other physical constants.

Equivalent weight	249 (theoretical)	247 (found)
Melting point	185° (literature)(15)	186-8° (found)

Resolution of Citranilic Acid.

Seventy-four grams (0.30 moles) of citranilic acid and 64.8 grams (0.30 moles) of levo-chloramphenicol base (M.W. = 212) were dissolved in 200 mls of hot ethanol. To this solution was added 600 mls of hot chloroform. The resulting solution was allowed to cool and then placed in a freezer at 20° for 48 hours. A needle-like precipitate formed and this was removed by filtration. The crystalline material was recrystallized four times from solvents containing various percentages of chloroform in ethanol as shown below. After each crystallization some of the salt was removed and a methanolic solution was prepared. The

optical activity of these solutions for each crystallization is shown below. Recrystallizations were continued till a constant specific rotation of light at 546.1 mμ was obtained.

Crystallization	% $CHCl_3$ in Ethanol	$[\alpha]^{27°C}_{546.1\ m\mu}$ (c 1% in methanol)
1	75	-26.78°
2	33	-34.22°
3	33	-35.99°
4	8	-37.11°
5	0	-37.49°

The acid portion of the salt was isolated by use of a "heavier than water" liquid-liquid continuous extraction apparatus. A solution consisting of 1.38 grams of the salt dissolved in 10 mls of 0.5 N aqueous hydrochloric acid was prepared and extracted with 90 mls of chloroform for 24 hours. The chloroform layer was separated and reduced in volume to about 45 mls on a rotary evaporator. Petroleum ether was added to the solution till it became turbid, and upon standing a crystalline precipitate formed. The precipitate was shown to be citranilic acid (mp = 123-4°C) by uv and nmr spectra as well as from its elemental analysis.

	Calculated	Found
% C	57.83	57.77
% H	4.42	4.61
% N	5.62	5.67

The specific rotation of the resolved citranilic acid was found to be:

$$[\alpha]^{27°C}_{330\ mu} = -146° \text{ (c = 1.23 in methanol)}$$

$$[\alpha]^{27°C}_{350\ mu} = -120° \text{ (c = 1.23 in methanol)}$$

The optical rotary dispersion curve of *l*-citranilic acid was a plain curve exhibiting increasing rotation with decreasing wavelength.

Column Chromatography.

Preparation of Samples To Be Chromatographed.--A reaction mixture was prepared by adding one ml of 0.455 M d-methylsuccinic anhydride to 15 ml of a 0.5 M, pH = 5.0 aqueous citric acid-sodium citrate buffer. At 50 seconds, 4.0 ml of 0.32 M aqueous aniline solution was added to quench the reaction. A blank solution was similarly prepared except that the citrate buffer was replaced by an equal volume of distilled water. An aqueous solution of a mixture of the isomeric citric acid monoanilides was prepared by dissolving 2 to 15 mg of citranilic

acid* in 10 to 15 ml of 0.1 N aqueous sodium hydroxide and allowing the resulting solution to stand for about five minutes. This caused hydrolysis of the citranilic acid to the citric acid monoanilides. All solutions were then adjusted to pH = 3.13 or 3.51 with hydrochloric acid to correspond to the internal phase of the chromatographic system used. Five ml of the desired sample solution was then mixed with 5 grams of silicic acid and 8 ml of chloroform to form a slurry which was then packed on the appropriate column.

Chromatographic System Used for the Separation of the Monoanilides of Citric and d-Methylsuccinic Acids.--Initial separations of the sample mixtures utilized a slight variation of a previously described method (16). A slurry was prepared which consisted of 3 grams of silicic acid, 5 ml of chloroform, and 3 ml of 4 N aqueous sulfuric acid. This slurry was packed on the bottom of the column and covered with a close-fitting filter paper disc.# Next a slurry composed of 30 grams of silicic acid, 45 ml of chloroform, and 30 ml of 0.5 M, pH = 3.13 aqueous sodium phosphate buffer was packed on the column. After addition of the appropriate sample, the column was eluted with the following volumes of water saturated eluants.

*In the determination of the specific rotation of the unsymmetrical citric acid monoanilide, *l*-citranilic acid was used.

#The purpose of the sulfuric acid plug is to retain the excess aniline, present in some of the samples, on the column.

0-110 ml	0%	butanol in chloroform
110-220 ml	1.5%	butanol in chloroform
220-450 ml	10.0%	butanol in chloroform
450-600 ml	30.0%	butanol in chloroform

Chromatographic System Used for the Complete Separation of the Unsymmetrical Citric Acid Monoanilide.--The column was packed with a slurry consisting of 30 grams of silicic acid, 45 ml of chloroform, and 30 ml of 0.5 M, pH = 3.51 aqueous sodium phosphate buffer. After packing on the appropriate sample, elution was accomplished using the following volumes of water saturated eluants.

0-100 ml	1.5%	butanol in chloroform
100-500 ml	12.0%	butanol in chloroform
500-650 ml	30.0%	butanol in chloroform

Qualitative Analysis of the Eluate Fractions.--The eluate fractions obtained from the columns were analyzed either by measuring the absorbance of the fraction relative to water saturated chloroform at 265 mμ in a one cm cell or by direct titration with alcoholic sodium hydroxide according to the method described in an earlier publication (17).

Preparation of Samples from the Eluate for the Determination of Optical Yield.

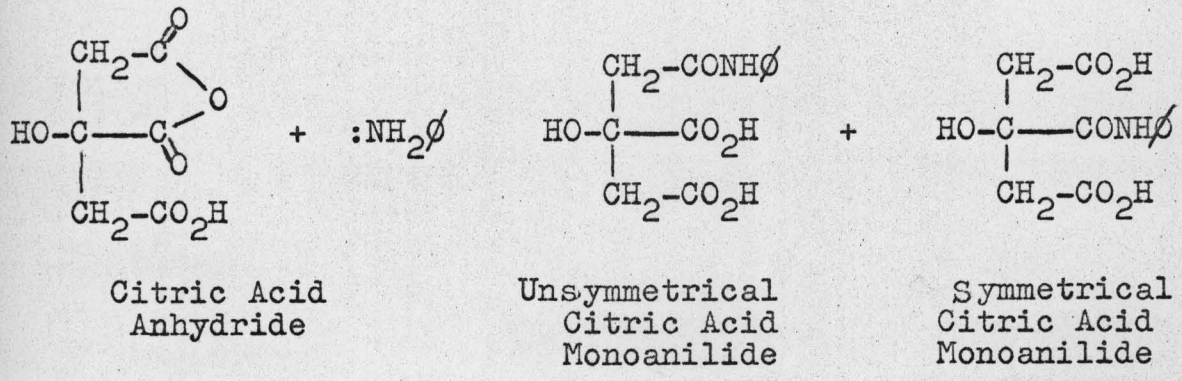
Three columns were run for the reaction mixture and for the blank solution and the fractions corresponding to those containing only the unsymmetrical citric acid monoanilide were combined and each resulting solution was extracted with 22 ml of 0.1 N aqueous sodium hydroxide solution. The aqueous solution was separated and 20 ml were placed in a 25 ml volumetric flask and brought to volume with 0.1 N sodium hydroxide to insure a homogeneous solution. The solutions were then filtered and equilibrated at 27° and the optical activity was measured at 380 mμ in a suitable 5 cm stoppered cell. The solutions were then removed, equilibrated at 25° and the uv absorption at 280 mμ was measured in a one cm cell.

Determination of the Molar Absorptivity of the Citric Acid Monoanilides Formed by Hydrolysis of Citranilic Acid.

The uv absorbance of several known concentrations of citranilic acid in 0.1 N aqueous sodium hydroxide was measured at 280 mμ in a cell having a pathlength of one cm. A plot of the absorbance of each solution versus its concentration yielded a straight line with a slope equal to the effective molar absorptivity, $a^{25^\circ}_{280\ m\mu} = 450$.

RESULTS AND DISCUSSION

The reaction of d-methylsuccinic anhydride with aqueous citrate solution apparently results in the formation of citric acid anhydride as is the case in the analogous reaction employing glutaric anhydride (1), because in both instances the addition of aniline resulted in the formation of the two isomers of citric acid monoanilide. The products presumably result from the attack of aniline on citric acid anhydride as shown below:



Citric Acid Anhydride

Unsymmetrical Citric Acid Monoanilide

Symmetrical Citric Acid Monoanilide

Separation and Identification of the Monoanilides Formed Upon Addition of Aniline to a Reaction Mixture Containing Citrate and d-Methylsuccinic Anhydride.

Figure 1 shows a typical chromatogram, obtained by uv measurements at 265 mμ, of the products obtained when aniline was added to the d-methylsuccinic anhydride-citrate system. The three major peaks showed good separation and the uv spectrum of each was characteristic of an anilide.

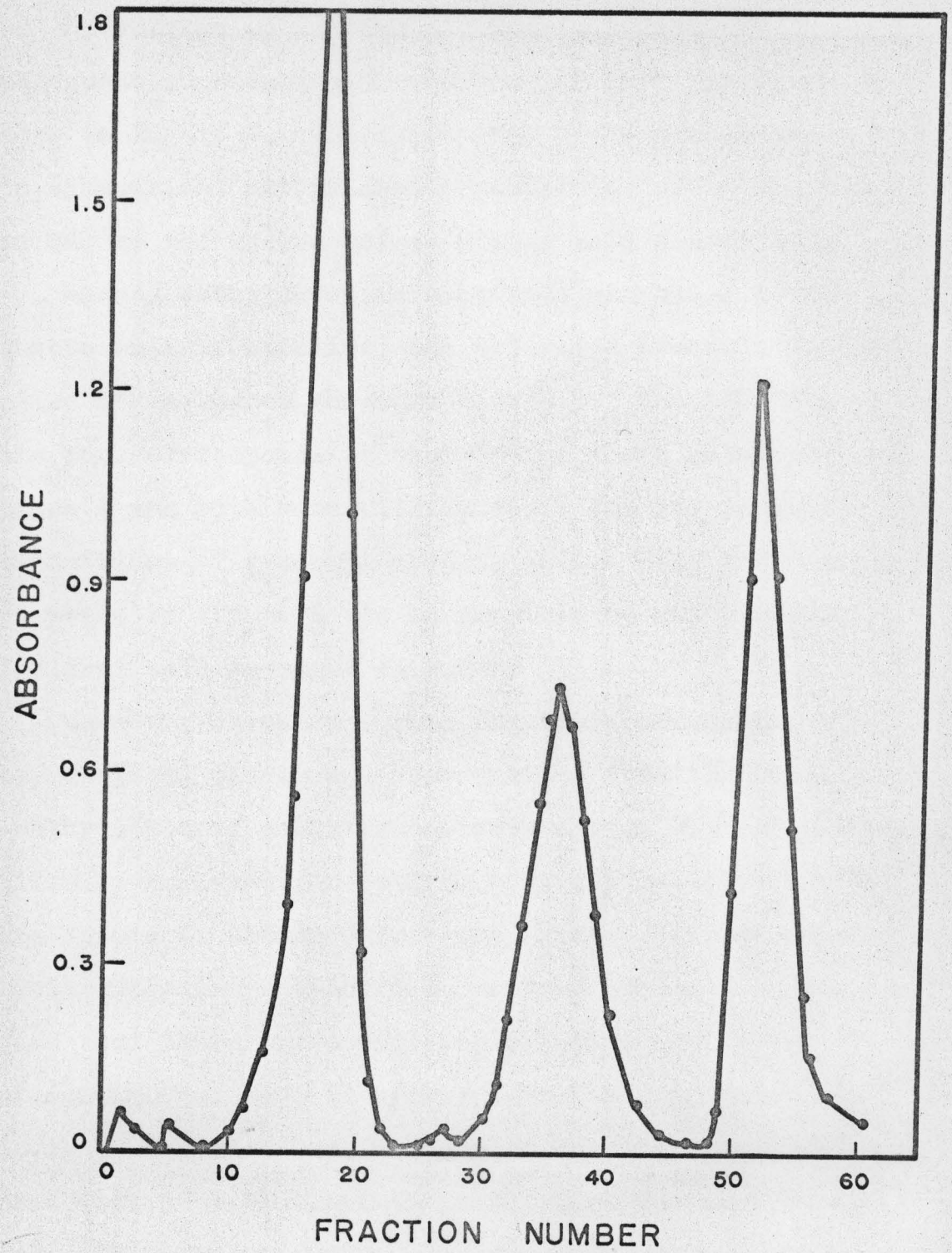


FIGURE 1. Chromatogram of the reaction mixture prepared by adding 1 ml of 0.55 M d-methylsuccinic anhydride in tetrahydrofuran to 15 mls of pH = 3.13, 0.5 M citrate buffer and quenching the reaction with 4 mls of 0.32 M aniline in water solution after 50 seconds. The absorbance measurements were done at 265 mμ.

Hydrolysis of citranilic acid in aqueous alkali and subsequent chromatographic separation gave the chromatogram shown in Figure 2. The first peak to be eluted was due to the symmetrical citric acid monoanilide, while the second was due to the unsymmetrical citric acid monoanilide.

When d-methylsuccinic anhydride was added to an aqueous aniline solution, the separated products resulted in the chromatogram shown in Figure 3. The location of this peak corresponds to that of the first major peak in Figure 1 and both were attributed to the unseparated monoanilides of d-methylsuccinic acid. Thus the remaining two peaks in Figure 1 can be ascribed to the monoanilides of citric acid as shown in Figure 2.

When the chromatographic fractions containing the unsymmetrical citric acid monoanilide from the reaction of d-methylsuccinic anhydride-citrate system with aniline were initially analyzed for optical activity relative to the same fractions obtained from the direct addition of d-methylsuccinic anhydride to aqueous aniline, it was found that there was considerable optical activity.** Subsequent work made it apparent that the unsymmetrical citric acid monoanilide containing fractions were contaminated with d-methylsuccinic acid, which was also being

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**It should be noted that d-methylsuccinic acid exhibits dextrorotation in organic solvents and levorotation in aqueous alkali.

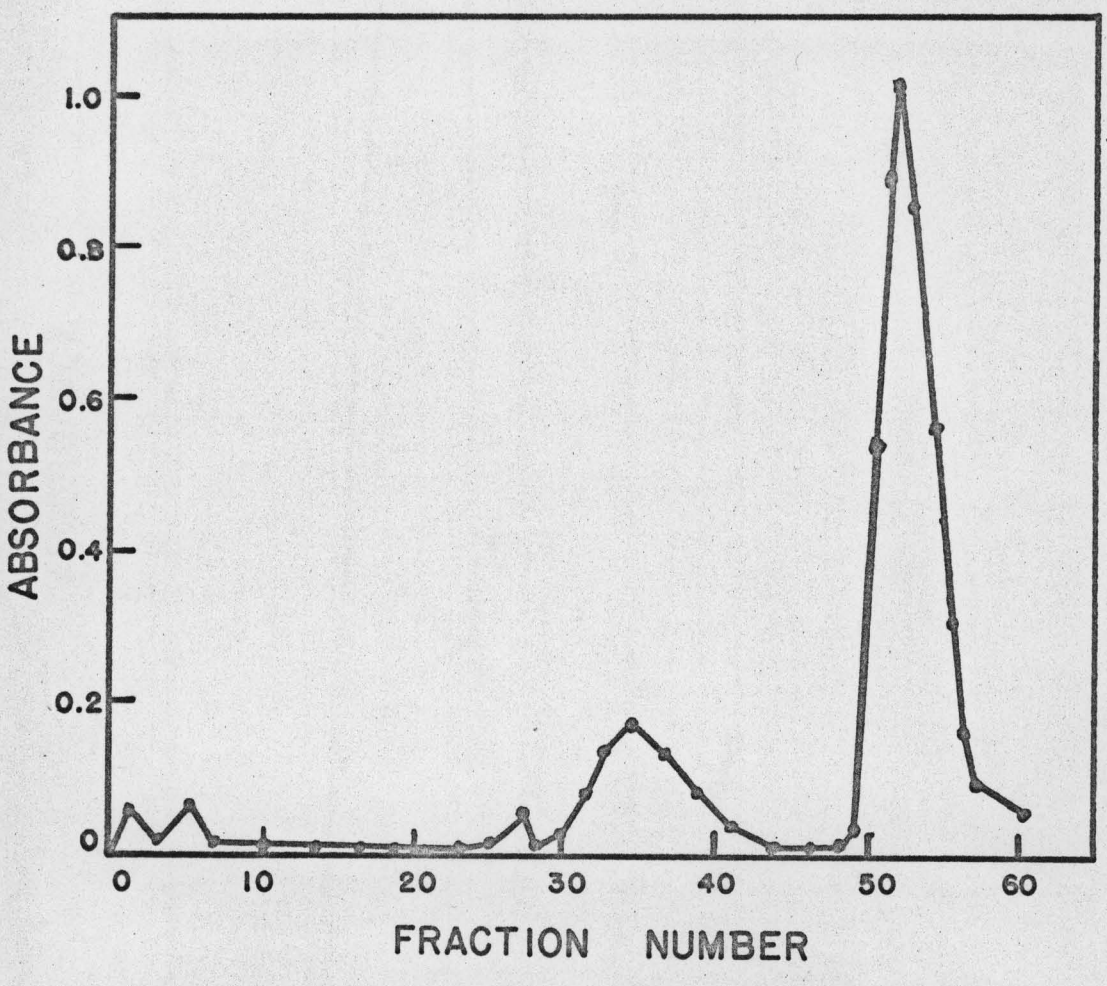


FIGURE 2. Chromatogram of the products of the hydrolysis of about 4 mgs of citranilic acid in aqueous sodium hydroxide. Absorbance was measured at 265 mμ.

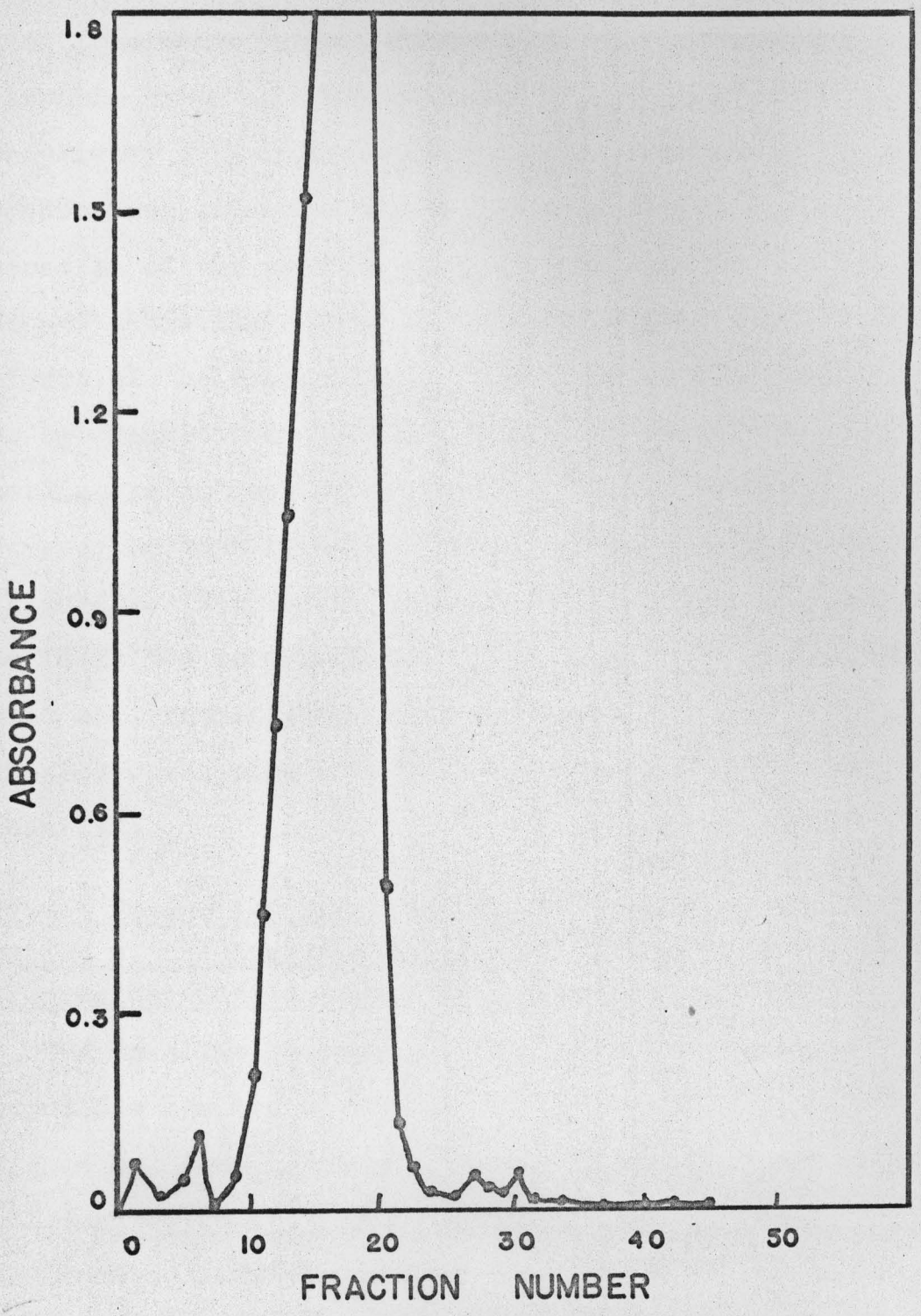


FIGURE 3. Chromatogram of a reaction mixture prepared by adding 1 ml of 0.55 M d-methylsuccinic anhydride in tetrahydrofuran to 15 mls of water and quenching the reaction at 50 seconds with 4 mls of 0.32 M aniline in aqueous solution. The absorbance measurements were done at 265 mμ.

eluted from the column. This contamination was not observable by uv spectroscopy due to the relatively low absorptivity of d-methylsuccinic acid. The chromatographic procedure was subsequently modified to obtain complete separation of the unsymmetrical citric acid monoanilide from any optically active or isomeric contaminants. The analysis of the separation obtained was made more definitive by using direct alkaline titration of the fractions according to an earlier procedure (17). A typical chromatogram showing the separation obtained is presented in Figure 4. The larger peak corresponding to fractions 8 to 16 is due to d-methylsuccinic acid and the symmetrical citric acid monoanilide. The smaller peak (in fractions 20 to 25) was assigned to the unsymmetrical citric acid monoanilide.

Indirect Determination of the Specific Rotation of the Enantiomers of the Unsymmetrical Citric Acid Monoanilide.

The specific rotation of the unsymmetrical citric acid monoanilide has not previously been determined. This compound has been isolated and characterized only in very small quantities (2). The difficulty of obtaining it in sufficient quantities, so that resolution can be accomplished, is further complicated by the ease with which it undergoes cyclization to yield citranilic acid upon exposure to heat.

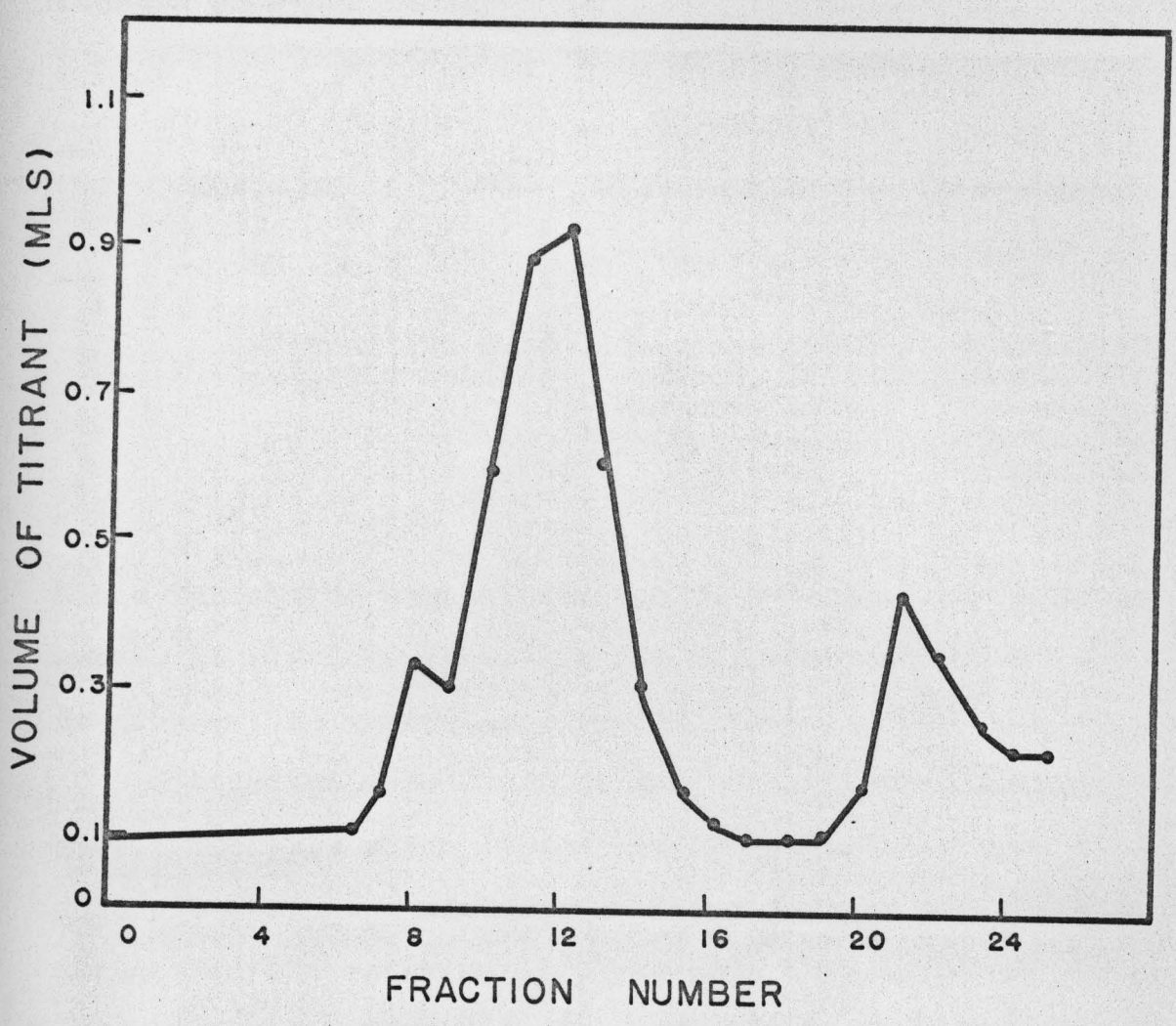
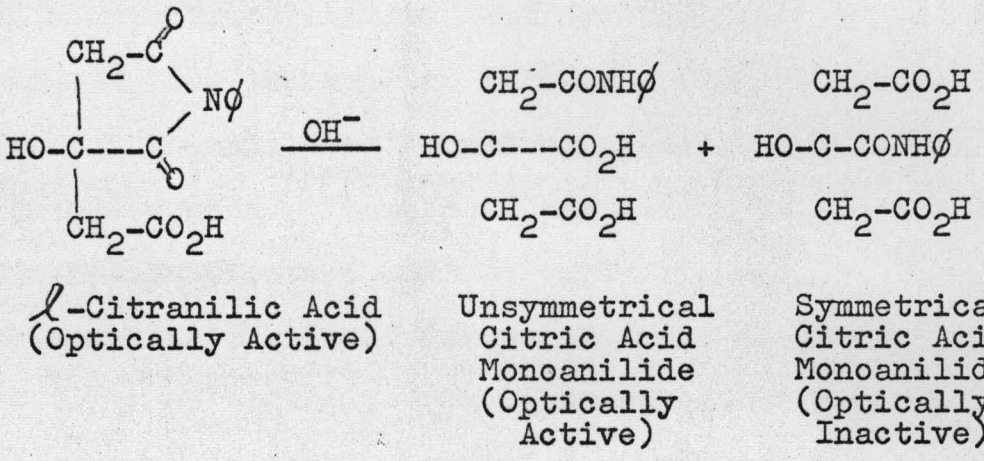


FIGURE 4. Chromatogram of a solution prepared by dissolving 2 to 3 mgs of citranilic acid and about 9 mgs of d-methylsuccinic acid in aqueous sodium hydroxide. The fractions were analyzed by direct titration with alcoholic sodium hydroxide.

It is apparent that the alkaline hydrolysis of optically pure citranilic acid results in two possible isomers as shown below:



ℓ-Citranilic Acid (Optically Active)

Unsymmetrical Citric Acid Monoanilide (Optically Active)

Symmetrical Citric Acid Monoanilide (Optically Inactive)

Since the active monoanilide can be separated as described above, it was felt that this was the most convenient way to produce the optically pure compound.

Citranilic acid was resolved using levo-chloramphenicol base. The salt which formed was recrystallized from chloroform-ethanol mixtures until no further significant changes in the rotation of plane polarized light at 546.1 mμ were observed. The acid was then reclaimed from an aqueous hydrochloric acid solution of the diastereomeric salt by continuous extraction with chloroform. The specific rotation of the crystalline enantiomer was found to be -146° (c 1.23 in methanol at 27°).

The products of the alkaline hydrolysis of l-citranilic acid were separated and the unsymmetrical citric acid monoanilide was extracted from the organic eluate with aqueous sodium hydroxide. The resulting extract was allowed

to stand for 16 hours at 25° with no change in absorbance at 280 mμ over that period of time. When the solution was chromatographed again there were no products other than the original unsymmetrical citric acid monoanilide. These data would suggest that there is no further hydrolysis or interconversion of the anilides formed by alkaline hydrolysis of l-citranilic acid. Therefore, the unsymmetrical citric acid monoanilide obtained must be optically pure.

It seems reasonable to assume that the molar absorptivities of both citric acid monoanilides will be nearly identical in aqueous alkali. Using this assumption, a Beer's law plot was constructed by measuring the absorbance of various concentrations of citranilic acid dissolved in aqueous sodium hydroxide. The effective molar absorptivity obtained at 280 mμ was 450.

An alkaline aqueous solution of the unsymmetrical citric acid monoanilide gave an absorbance of 0.790 at 280 mμ using a one cm cell at temperature of 25°. The optical rotation of the same sample solution was determined to be -0.0213° at 300 mμ in a five-cm cell and a temperature of 27°. The specific rotation of *l*-citric acid monoanilide was calculated as shown below:

$$\text{Molarity} = \text{Absorbance/pathlength} \times \text{Effective molar absorptivity}$$

$$= 0.790/1 \times 450$$

$$= 1.76 \times 10^{-3}\ \text{M}$$

$$\text{Concentration (grams/100 mls)} = [\text{Molarity} \times \text{M.W.} \times 100]/1000$$
$$= [1.76 \times 10^{-3} \times 267 \times 100]/1000$$
$$= 0.046 \text{ grams/100 mls.}$$

$$[\alpha]^{27^\circ}_{300\ m\mu} = \text{specific rotation}$$
$$= 100 / [\text{pathlength (dm.)} \times \text{concentration (grams/100 mls)}]$$
$$= 100 \times -0.0213^\circ / 0.5 \times 0.046$$
$$= -92.6^\circ.$$

Calculation of Optical Yield

When an alkaline solution of the unsymmetrical citric acid monoanilide obtained from the reaction mixture was prepared, a uv absorbance of 1.33 was found at 280 mμ at 25° in a one cm cell. The optical rotation at 300 mμ and 27° in a five cm cell was +0.0001°. It can be calculated that the rotation to be expected if the compound had been optically pure would be 0.0385°. From this information the % optical yield can be calculated as follows:

$$\%\text{ optical yield} = \frac{\text{observed rotation} \times 100}{\text{theoretical rotation}}$$
$$= +0.0001^\circ \times 100 / 0.0385^\circ$$
$$= 0.3\%.$$

Conclusions

Since the optical yield is nearly zero it can be theorized that the transition state free energies of the dissymmetric diastereomers must be nearly identical, i.e., $\Delta\Delta G^{\neq} \sim 0$. This suggests that the optical image of d-methylsuccinic anhydride has little effect on the configuration of the transition states. Perhaps the use of a more complex anhydride would have been more effective in causing the asymmetric synthesis of citric acid anhydride.

REFERENCES

1. Robinson, J., Repta, A., Higuchi, T., J. Pharm. Sci., 55, 1196 (1966).

2. Repta, A., Robinson, J., Higuchi, T., J. Pharm. Sci., 55, 1200 (1966).

3. Martius, C., Schorre, G., Ann., 570, 140 (1950).

4. Martius, C., Schorre, G., Ann., 570, 143 (1950).

5. Wilcox, P. E., Nature, 164, 757 (1949).

6. Wilcox, P. E., Heidelberger, C., Potter, V. R., J. Am. Chem. Soc., 72, 5019 (1950).

7. Werkman, C. H., Wood, H. G., Advan. Enzymol., 2, 135 (1942).

8. Krebs, H. A., Advan. Enzymol., 3, 191 (1943).

9. Evans, E. A., Jr., Slotin, L., J. Biol. Chem., 141, 439 (1941).

10. Schwartz, P., Carter, H. E., Proc. Nat. Acad. Sci. U.S., 40, 499 (1954).

11. Mislow, K., "Introduction to Stereochemistry," W. A. Benjamin, Inc., New York, N.Y., 1966, Chap. 3.

12. Berner, E., Leonardsen, R., Ann., 538, 1 (1939).

13. Freda, A., Arkiv Kemi. Mineral Geol., 15B, No. 23, 1 (1942).

14. Naps, M., Johns, I. B., J. Am. Chem. Soc., 62, 2450 (1940).

15. Nau, C. A., Brown, E. B., Bailey, J. R., J. Am. Chem. Soc., 47, 2596 (1925).

16. Higuchi, T., Eriksson, S., Uno, H., Windheuser, J., J. Pharm. Sci., 53, 280 (1964).

17. Higuchi, T., Hill, N. C., Corcoran, G. B., Anal. Chem., 24, 491 (1952).

SECTION III

SYNTHESIS, ISOLATION, AND SOME CHEMISTRY OF CITRIC ACID ANHYDRIDE

INTRODUCTION

Although cyclic anhydrides of hydroxy polycarboxylic acids such as malic, tartaric, citric, and related acids are theoretically possible, no concerted attempts seem to have been made to prepare them in significant quantities. This is probably due in large measure to their relative instability and pronounced tendency to undergo dehydration and intra- and intermolecular esterification. It has already been shown, however, that such anhydride species can form even in aqueous solution (1-4), although both cyclic malic and cyclic citric acid anhydrides hydrolize quite rapidly in these systems. The present investigation relates to the synthesis, isolation, and studies made on citric acid anhydride produced under anhydrous conditions.

With the wide use and acceptance citric acid enjoys in the food and drug industries, it would appear that the anhydride of this acid may also find similar utility. One of the most apparent uses would seem to be as a latentiated acidifier in the chemical carbonation of aqueous solutions (5).

The reaction of acetic anhydride and citric acid has been shown to produce acetylcitric anhydride when heated at about 120° for several hours (6). The product would appear to form as the result of two reactions, one of which is the acetylation of the alcohol group of citric acid while the other is the transfer of anhydride character from

acetic anhydride to citric acid. If conditions could be employed which favor the latter reaction almost exclusively, citric acid anhydride would be expected to be the major product.

EXPERIMENTAL

Equipment and Reagents

A Cary model 14 recording spectrophotometer was used for uv measurements. Nuclear magnetic resonance spectra were obtained using a Varian model A-60A spectrometer. A Lab-Con-Co dry box was used for procedures requiring a low humidity environment. The thermometer used in the cryoscopic determinations was Brooklyn Thermo Co. ASTM thermometer graduated in tenths of a degree with a total range of -8 to 32°. The length and internal diameter of the chromatographic columns used were 26 and 2 cm respectively. All pH-stat measurements were done on a Radiometer TTT11 auto-titrator used in conjunction with an SBR2c titragraph, an ABU1b auto-burette equipped with a TTA3 titration assembly, and a PHM26 pH meter.

The aniline used was distilled from zinc dust prior to use. All other chemicals used were of analytical or reagent grade and were employed without further purification.

Procedures

Synthesis and Isolation of Citric Acid Anhydride.

A heterogeneous mixture consisting of 183 grams (0.953 moles) of powdered anhydrous citric acid, 90.3 grams (1.50 moles) of glacial acetic acid and 194.4 grams (1.90 moles) of acetic anhydride was heated at 36-38°,

with good stirring, in a closed vessel until all of the citric acid had dissolved (19 to 20 hours). The solution was then filtered through glass wool. The reaction vessel was rinsed with 500 mls of hot chloroform which was then added to the filtered solution. The resulting solution was stirred and allowed to cool, and within half an hour a viscous oil began to separate. Continued stirring resulted in conversion of the oil to a white crystalline solid which was removed by filtration in a low humidity atmosphere. The precipitate was washed with 700 mls of warm chloroform and dried for 24 hours over potassium hydroxide pellets at a pressure of less than one mm of mercury and a temperature of about 45°. The white crystalline solid melted at 120 to 123° and was shown by nmr spectra and other physical measurements to be citric acid anhydride. The yield of the reaction was about 40%.

Equivalent weight found by direct titration with aqueous sodium hydroxide was 58.3 (calculated = 58.0). The molecular weight obtained from cryoscopic measurements in dioxane was 184 (calculated = 174). Elemental analysis showed 41.09% C and 3.54% H which was in good agreement with the calculated values of 41.39% C and 3.47% H.

Chromatographic Separation of the Products of the Reaction of Aniline and Citric Acid Anhydride.

The column was prepared by packing on the bottom of the column a slurry of 4 grams of silicic acid, 6 ml of

chloroform, and 4 ml of 4 N aqueous sulfuric acid (the purpose of this acid plug was to retain any excess aniline present in the sample). On top of the acid plug was packed another slurry consisting of 30 grams of silicic acid, 45 ml of chloroform, and 30 ml of 0.5 molar pH = 3.13 aqueous sodium phosphate buffer. The sample solution was prepared by mixing 100 microliters of a 1.0 M solution of citric acid anhydride in tetrahydrofuran with 10 ml of 0.16 M aqueous aniline. The resulting solution was acidified to pH = 3.13 and 5 ml of the solution was mixed with 5 grams of silicic acid and 8 ml of chloroform. The resulting slurry was then packed on the column and eluted with the following volumes of water saturated eluants:

0-110 ml	0.0%	butanol in chloroform
110-220 ml	1.5%	butanol in chloroform
220-390 ml	10.0%	butanol in chloroform
390-550 ml	30.0%	butanol in chloroform

The eluate was collected in 15 ml fractions and the absorbance of each was determined at 265 mμ using water saturated chloroform as the reference solution.

Cryoscopic Determination of Molecular Weight.

The cryoscopic measurements were carried out according to a routine procedure (7). Dioxane was the solvent chosen since it does not react with anhydrides and the freezing

point and molal freezing point depression factor were suitable. A solution containing 1.50 grams of citric acid anhydride in 8.36 grams of dioxane exhibited a freezing point depression of 4.78%. Appropriate calculations based on the molal freezing point depression constant of 4.9% (8) gave an apparent molecular weight of 183 (calculated = 174).

Kinetic Measurements of the Hydrolysis of Citric Acid Anhydride in Aqueous Solutions.

Spectrophotometric Measurements.--One hundred microliters of 0.57 M citric acid anhydride in tetrahydrofuran was added to 15 ml of the desired aqueous solution (previously equilibrated at 25±0.1°) in a spectrophotometer cell fitted with a stopper. Mixing was accomplished by rapid inversion of the closed cell, taking care not to disperse air bubbles throughout the sample. The sample was then quickly placed in the cell compartment and the absorbance changes were followed at wavelength of 240 to 250 mμ depending on the optical density of the aqueous solution used.

pH-Stat Measurements.--Kinetic measurements were carried out at pH = 5.0 and pH = 6.0 using 0.1 N aqueous sodium hydroxide. A typical sample consisted of 25 mls of water and 2 mls of 0.1 N sodium hydroxide contained in a vessel thermostatted at 25±0.1° to which was added 200 microliters of a 0.57 M citric acid anhydride in

tetrahydrofuran solution. The excess alkali in the initial reaction mixture was necessary to enable the pH-stat assembly to accommodate the large initial acidity produced by the free carboxylic acid groups of the citric acid anhydride when the sample was added.

RESULTS AND DISCUSSION

Synthesis and Isolation of Citric Acid Anhydride.

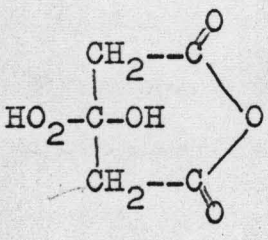
Initial synthesis and isolation of citric acid anhydride was attempted by mixing equimolar amounts of citric acid and acetic anhydride and heating the mixture at 60 to 80°C until an homogeneous solution was formed. Nmr spectra of the resulting solution diluted with deuteroacetone indicated the presence of a large amount of acetylcitric anhydride and lesser amounts of citric acid and another species suspected to be citric acid anhydride. When temperatures of 35-48°C were used total disolution of the citric acid failed to occur over a period of several days.

Since the appearance of any new anhydride species in such a system would be subject to second order kinetics, the concentration of acetic anhydride was progressively increased until a 2:1 molar ratio of acetic anhydride to citric acid was reached. It was apparent from nmr spectra of the reaction mixture at various times that initially the citric acid anhydride species was being formed but with the continued heating necessary to effect total dissolution of the citric acid, acetylation of the anhydride was occurring and resulting in appreciable quantities of acetylcitric anhydride. The initial reaction thus appeared to be dissolution controlled and in an attempt to increase the

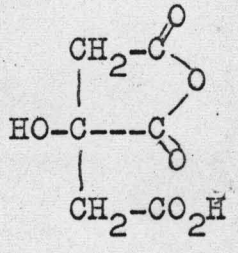
solubility of the citric acid, various amounts of glacial acetic acid were added to the reaction mixture. It was found that an approximately 2:1:1 molar ratio of acetic anhydride, citric acid, and glacial acetic acid, respectively, when mixed together and heated at 35 to 40°C with stirring in a closed container until dissolution had occurred resulted in the production of mostly citric anhydride. Precipitation of the anhydride using chloroform, washing the filtered material with chloroform and drying resulted in the isolation of citric anhydride with apparently little contamination.

Characterization of Citric Acid Anhydride.

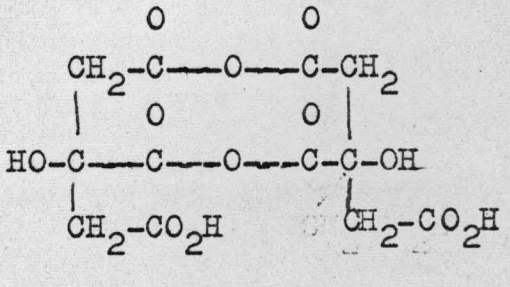
Although elemental analysis, equivalent weight obtained by direct titration of aqueous solutions of the anhydride, and equivalent weight determined by titration of solutions in which the compound had been added to aqueous aniline (9), gave mutually agreeable results, it was not possible to distinguish between the following possible anhydride structures.



Symmetrical anhydride



Unsymmetrical anhydride



Intermolecular anhydride

In order to determine whether the compound was the intermolecular or the intramolecular anhydride the freezing point depression of the compound was determined in dioxane solution. The molecular weight obtained by calculations based on these measurements was found to be about 184 which agrees quite well with the molecular weight of 174 which would be expected for the intramolecular anhydrides.

When the anhydride was reacted with aniline and the products separated by chromatography (see Figure 1), two anilides were found and they were apparently the symmetrical and the unsymmetrical citric acid monoanilides. The ratio of the total absorbances of the unsymmetrical isomer to the symmetrical was found to be 0.85 which is in good agreement with earlier results obtained by addition of aniline to aqueous solutions in which citric acid anhydride was produced by interaction of acetic (3) or glutaric (2) anhydride and citrate ions. Since both monoanilides were produced in similar amounts, it appears that the symmetrical anhydride must be the predominant species.

Nuclear magnetic resonance studies also support this structural assignment. A typical nmr spectrum is shown in Figure 2. Inspection of the spectrum and its integration curve lead to the conclusion that the anhydride structure is the unsymmetrical one. The coupling constant for the doublet at σ = 3.68 and 3.38 was calculated to be 19 cps which is in good agreement with similar calculations by Ericksen for malic anhydride (10).

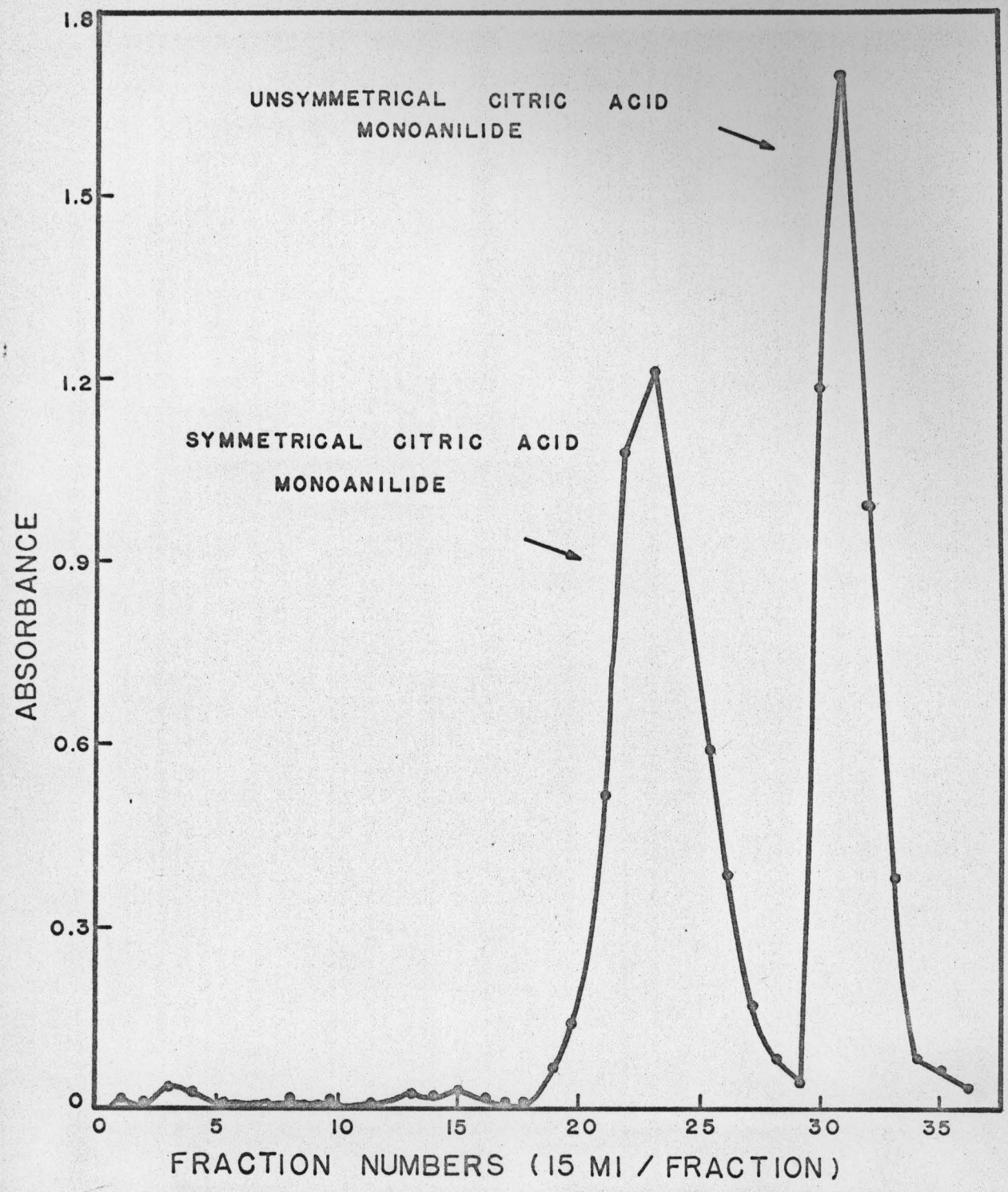


FIGURE 1. Chromatogram of the anilides formed by adding 100 microliters of 1.0 molar citric acid anhydride to a solution containing 5 mls of water and 5 mls of 0.32 molar aqueous aniline.

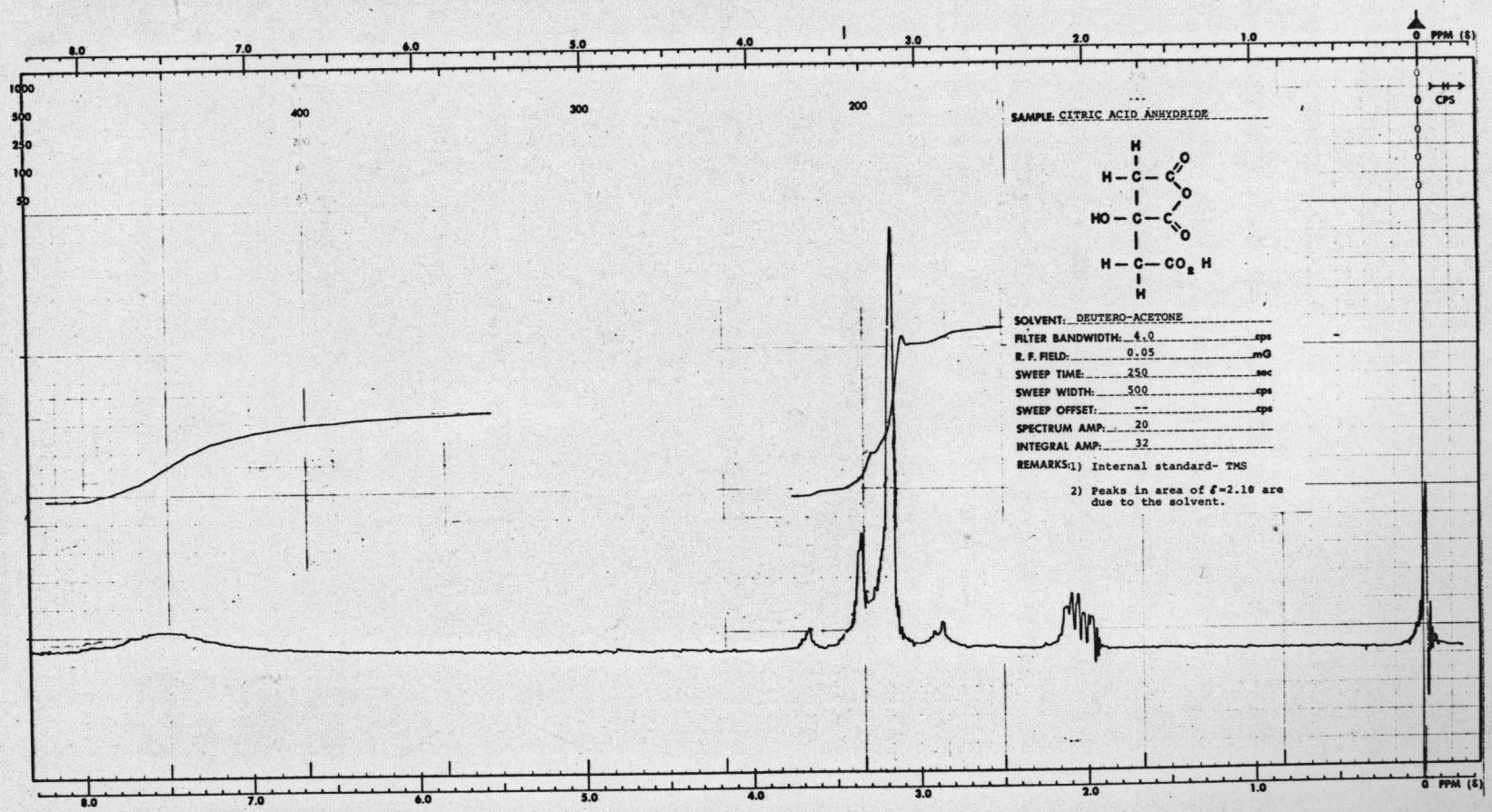


Figure 2. Nuclear magnetic resonance spectrum of citric acid anhydride in deutero-acetone.

Chemical Behavior in Aqueous Solutions.

The reaction between citric acid anhydride and water was shown to yield citric acid as determined by isolation and characterization of the nonvolatile material, as well as the nmr spectra of solutions of citric acid and citric acid anhydride in D_2O solution.

Determination of the hydrolytic rate constant of the anhydride under various conditions were obtained using uv spectrophotometry and pH-stat measurements. The results are shown in Table I, and inspection of this data indicates that the hydrolytic rate decreases as the pH is increased from 2 to 5. This behavior is quite different from that of most anhydrides whose hydrolysis over this pH range is independent of the hydrogen ion concentration. The decrease in hydrolytic rate is apparently due to the ionization of the free carboxylic acid group which would be expected to decrease the susceptibility of the molecule to nucleophilic attack by water. Similar effects have been noted in the hydrolysis of tricarballylic acid anhydride (11), and presumably such behavior could be expected of any anhydride species possessing an ionizable group. The data obtained when potassium chloride was added to the water also shows a decrease in the rate of hydrolysis and this can once again be ascribed to increased dissociation of the carboxylic acid group.

When the absorbance of the reaction of the anhydride versus time was followed at 250 mμ in aqueous acetate or

TABLE I. Kinetic data for the hydrolysis of citric acid anhydride in various aqueous solutions

Solvent composition	pH	Method of measurement	$k^{\#}$ x $10^3 sec^{-1}$	$t_{1/2}$ sec
water	-	uv	57.7	12
1.5 molar HCl	-	uv	40.9	17
0.1 molar aq. HCl	1.0	uv	63.0	11
0.01 molar aq. HCl	2.0	uv	63.0	11
aq. citrate buffer	3.08	uv	36.9*	19
water	5.0	pH-stat	13.9	50
water	6.0	pH-stat	13.9	50

#Observed pseudo-first order rate constant.

*Rate was obtained by extrapolation to "zero" buffer concentration.

citrate buffers at pH values of 4.52 and 4.47 respectively, both systems showed an initial rapid decrease in absorption followed by a slower and less pronounced increase. The magnitude of the changes seemed to be related to the concentration of the buffer species, andthe effect of acetate was markedly greater than that of citrate. The cause and nature of these changes was not determined and the behavior of the system cannot be determined by using the pH-stat since its usefulness is limited to pH values where the reacting species have no buffer capacity.

REFERENCES

1. Higuchi, T., Eriksson, S., Uno, H., Windheuser, J., J. Pharm. Sci., 53, 280 (1964).

2. Robinson, J., Repta, A., Higuchi, T., J. Pharm. Sci., 55, 1196 (1966).

3. Repta, A., Robinson, J., Higuchi, T., J. Pharm. Sci., 55, 1200 (1966).

4. This thesis, Section II, Part 3.

5. Higuchi, T., Perrin, J., Robinson, J., Repta, A., J. Pharm. Sci., 54, 1273 (1965).

6. Fuchs, C. F., United States Patent 2,520,139, August 29, 1950.

7. Pharmacopoea Nordica, Ed. Norv., Vol. I, Oslo, 1963, p.92.

8. S. H. Maron, C. F. Prutton, "Principles of Physical Chemistry," 4th ed., The Macmillan Company, New York, N.Y., 1967, p. 324.

9. Bunton, C. A., Fuller, N. A., Perry, S. G., Pitman, I. H., J. Chem. Soc., 4478 (1962).

10. Erickson, L., J. Am. Chem. Soc., 87, 1867 (1967).

11. Repta, A., Unpublished data.