STUDIES ON THE CHROMOGENIC REACTION BETWEEN TERTIARY AMINES AND POLYCARBOXYLIC ACIDS

by

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DEDICATIONS

To my father, Mr. Ifan Gakem, who has endured almost in poverty to see us, his children, through school.

To the memories of my mother, Mrs. Mwuese Ifan, and of my sister, Mbawan, this would have meant the world to them. May they have eternal peace.

To my wife, Zanenge, for her ulfaltering love and support during these trying times.

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My appreciation to Professor Kenneth A. Connors for his guidance and suggestions during the course of this research work. I have benefited enormously academically from my association with him, and hope that I will forever have the benefit of counting on his friendship.

Much of my gratitude goes to Dr. Bruce R. Adams of the Chemistry Department, University of Wisconsin-Madison, who helped with obtaining the nuclear magnetic resonance spectrum of carbon suboxide. Without this spectrum, the basis for this research project probably would not have existed.

To my children, Iorwuese and Ngutor, I probably have not been the best father because of the time I spend away . But I can improve on that now. To my brothers and sisters, it has been a long wait, but thank God it has come to this point.

I wish to express my gratitude to the Faculty members, especially Professor Joseph R. Robinson, of School of Pharmacy, University of Wisconsin-Madison, for the numerous ways they have helped to mold me. I am forever indebted to all of you.

To my laboratory mate, Michael J. Jozwiakowski, you are a fine gentleman, and I wish you all the best in life.

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STUDIES ON THE CHROMOGENIC REACTION

BETWEEN TERTIARY AMINES AND POLYCARBOXYLIC ACIDS

Agber Ifan

Under the supervision of Professor Kenneth A. Connors

The color-forming reaction of polycarboxylic acids with tertiary amines in the presence of acetic anhydride, which is the subject of this study, appears to be generally applicable to the determination of tertiary amines. The limitation to its wider application lies in the almost complete lack of knowledge of the chemistry of the reaction.

Qualitative observations of many polycarboxylic acid reactants reveal that the pattern of color formation varies with the identity of the organic acid and of the tertiary amine. In this investigation, we have selected malonic acid from among the many organic acids that were considered during the preliminary stages of the investigation. The initial reaction pathway probably goes through the anhydride, which for the malonic acid system is carbon suboxide, O = C = C = C = O. We prepared carbon suboxide from malonic acid, and all subsequent work began with carbon suboxide as the reactant. The reaction solvent was diethyl ether, sometimes containing acetic anhydride.

Aromatic and aliphatic amines behave differently in this

system. Apparently, aromatic amines are not consumed in the reaction process, although the intensity of absorption is proportional to the concentration of the aromatic amine. The aromatic amines require the presence of acetic anhydride to react, and probably function as nucleophilic catalysts. The aliphatic amines do not require the presence of acetic anhydride to react. Although they may act as catalysts (probably general base), they are also consumed. The initial orders of reaction with respect to aliphatic amines, aromatic amines, and to carbon suboxide are approximately 1, 0.5 and 2, respectively.

Absorption spectrophotometric data indicate the appearance of multiple products in sequence. High performance liquid chromatography reveals the presence of possibly five products of reaction, indicating perhaps that the reaction is very complex. Products of the reaction between triethylamine and carbon suboxide have been proposed based upon mass spectral data, which indicate covalent bonding of triethylamine to product.

The analytical reaction always has an apparent featureless lag-time phase followed by an abrupt appearance of coloration and increase in absorbance. Kinetic schemes have been devised in an attempt to explain this behavior.

Approved: Kenneth A. Connors

Professor Kenneth A. Connors, Ph.D.

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I. INTRODUCTION

A. Analytical Chemistry of Tertiary Amines

A simple listing of some classes of pharmaceutical products, alkaloids, antihistamines, antimalarials, tranquilizers, hypotensives, narcotics, etc., sufficiently emphasizes the wide distribution of the tertiary amine functionality among drug compounds. Various analytical methodologies have been used to characterize these compounds, some of them employing the reactivity of the tertiary amine group as the basis of the analytical determination. But none of these methods can be considered to be generally applicable to the determination of all tertiary amines. Although a detailed discussion of the various analytical procedures is beyond the scope of this thesis, a survey of some of these analytical methodologies will be undertaken. This, it is hoped, will emphasize the variability, difficulty, and limitations of these methods.

1. Survey of Analytical Methods

Compounds containing a tertiary amine functional group have been determined in a number of ways. Many of the published

analytical methods are based upon some modification of a dye complex formation with a halogenated acid, a phenomenon first observed by LeMer and Downes (1), and later supplemented by Griffits (2). Perhaps the first analytical application of the dye complex formation principle to the determination of a drug compound was by Prudhomme (3), who utilized this principle as the basis of his method for the determination of quinine with eosin. Lehman and Aitken (4) used bromothymol blue to form their amine (meperidine) complex, which was extracted into benzene and reextracted into aqueous alkali for measurement. Higuchi and Bodin (5) have suggested that the procedure of Lehman and Aitken for the determination of meperidine is readily applicable to the determination of other bases such as quinine, codeine, aminopyrine, ephedrine, and nicotine (but not morphine) in pharmaceutical systems. Durick et al. (6) have used bromocresol purple in a procedure similar to that by Lehman and Aitken, and have also succeeded in eliminating one of the extraction steps requiring shaking of the extracted base with a buffered dye solution. It would appear that the theoretical considerations which aid in choosing a rational system for a particular determination involve a knowledge of the dissociation constants of the acid dye and the basic compound, and the pH dependence of the partition characteristics of the two substances and their addition product between aqueous and organic solvents (5). A compilation of dyes and dye pK as well as the recommended pH and solvents of extraction was

undertaken by Higuchi and Bodin. Yu et al. (7), using an adaptation of the method of Lehman and Aitken, reported that the dye-amine complex was best achieved with haloalkane solvents. They also reported that the chloroform extraction efficiencies for different dye-amine complexes were different, and were a function of pH. This prompted the authors to suggest that the different extraction efficiencies for different dye-amine complexes in a given solvent system at a particular pH, in addition to the molar absorptivities, could possibly be explored as a means to determine some tertiary amines in mixtures. It would appear though, that the capability of differentiation of tertiary amines in mixtures by such a procedure, even if desirable, would be a highly specialized analytical process, which may even be cumbersome to carry out.

The dye, methyl orange, has also been used widely (8-12) to complex tertiary amine compounds prior to extraction into an organic solvent, treatment of sample and subsequent photometric determination. Kuzel (13) developed an automated method for the analysis of tertiary amines based upon a modification of the dye complex extraction method. Modifications to the dye reagent formulation were suggested in order to enhance specificity, enabling the analysis of certain amines in the presence of others. Irving and Markham (14) used the sulfonphthalein indicator, bromocresol green, for the determination of long-chain tertiary alkylamines and quaternary ammonium salts in water and in acidic raffinate solutions. Booth (15) used the complex of bromophenol blue to

determine reserpine. Nonaqueous photometric titration of tertiary amines has also been examined with bromophenol blue in benzene as titrant (16).

Titrimetric methods based upon principles other than those employing acid dye complex formation have also been used. For example, acetylation as a means of end-point indication has been employed in the catalytic thermometric titration of tertiary amines and metal carboxylates (17). In this method, the acid-catalyzed acetylation of alcohols and phenols with acetic anhydride was used as an end-point indicator in the thermometric titration of tertiary amines and metal carboxylates using aceteous perchloric acid as titrant. Gaal et al. (18) have employed the biamperometric neutralization process, using a tin electrode pair for end-point detection, to determine tertiary amines. It was reported that not only were the results in good agreement with potentiometric determinations, but also agreed with those obtained by catalytic thermometric and photometric determinations.

Other complexometric methods for the determination of tertiary amine compounds have also been presented in the literature. Schenk et al. (19) have reported a procedure for the determination of aromatic tertiary amines by measurement of the absorbance of the colored π-complex formed between the amine and tetracyanoethylene (TCNE). Primary and secondary amines were prevented from interfering in the determination process by pretreating the tertiary amine samples with acetic anhydride. The analytical procedure

described by Schenk et al. is very elaborate and could be time consuming. Tawa et al. (20) have also studied the amine-TCNE complexation as a means to determine tertiary amines, employing a kinetic approach in their procedure. Determination of the tertiary amine functionality was based upon absorbance measurements of the π-complex formed as a function of time. Silverstein (21) developed a spectrophotometric method for the determination of primary, secondary, and tertiary fatty amines. Conditions were established for the reaction between the fatty amine and methyl orange in aqueous solutions, and for the interference to this reaction by salicylaldehyde and acetic anhydride, thus enabling differentiation between the classes of amines. Variations of the picric acid method in which the amine-picric acid precipitate is cleaved with ammonia in aqueous solution, and the base extracted with an organic solvent system have been described and applied to the photometric determination of secondary and tertiary aliphatic amines (22), and sympathomimetics in blood and urine (23). Various other methods in which colorimetric-absorbance measurements have been employed in the determination of the tertiary amine group, such as in high performance liquid chromatography, will be introduced in the ensuing paragraph of this section. However, colorimetric-absorbance measurements in which organic polycarboxylic acid-acetic anhydride combinations are involved will be presented in greater detail in the next section.

Tertiary amine compounds have also been determined by the

so-called fluorogenic reaction process, i.e. the process by which non-fluorescent compounds are converted into fluorescent derivatives via chemical methods. Kaul et al. (24), and Lehr and Kaul (25) determined chlorpromazine in extracts of biological fluids first by reacting with 9-bromomethylacridine, isolation of the product by thin-layer chromatography, and subsequent photolysis to fluorescent products. Van Buuren et al. (26) have described a fluorogenic ion-pair extraction detector for use in reversed-phased liquid chromatography. Brinkman et al. (27) have also used post column ion-pair extraction fluorometric detection in high performance liquid chromatographic determination of tertiary amine compounds. Guebitz et al. (28, 29) have used acridone-2-sulfonic acid and 2-naphthyl chloroformate to obtain fluorescent tertiary amine derivatives as a means for their determination. The dealkylation of tertiary amines to fluorescent carbamates when heated with 2-naphthyl chloroformate was applied to the precolumn derivatization of some antihistamines. Syoyama and Sano (30) have used lumogallion and calcon in ion-pair extraction fluorimetry, and reported that primary and secondary amines at concentrations lower than those of the test tertiary amines do not interfere. Hansen and Nordholm (31) have studied the N-alkylation reaction of the tertiary amine, imipramine, with chloroform, dichloromethane and 1,2-dichloroethane. The reaction products were quantified and isolated by means of high performance liquid chromatography.

Gas chromatographic methods have also been applied to the

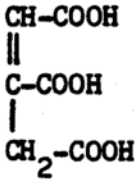
determination of tertiary amine compounds. Vessman et al. (32) reacted tertiary amines with ethylchloroformate to form a carbamate, which, upon being cleaved to a secondary amine, is transformed to heptafluorobutyramide (an electrophore) enabling determination by gas chromatography using electron capture detection. The abilities of pentafluorobenzyl carbamate, trichloroethyl chloroformate, and pentafluorobenzyl chloroformate to interact and form carbamates with tertiary amines, enabling conversion to electrophoric species capable of gas chromatographic determination, had been investigated (33-36). It was reported that pentafluorobenzyl chloroformate probably possessed the best carbamation properties owing to its high sensitivity, excellent gas chromatographic properties, and good stability (35). Sternson and Cooper (37) have, however, reportedly found limitations with the use of pentafluorobenzyl chloroformate; it failed especially for dimethylaminoalkanes which contained a pyridine nucleus. A comparison of the carbamation efficiencies of trichloroethyl chloroformate and pentafluorobenzyl chloroformate reportedly revealed the former to be 3 to 4 times more effective.

Certain phenothiazine derivatives, for example imipramine and amitriptyline, have been characterized by application of the Hofmann degradation reaction together with gas-liquid chromatography (38). The process involved alkylation of the drug molecule with an alkyl halide, and treatment of the resulting quaternary ammonium compound with moist silver oxide to yield the quaternary ammonium hydroxide.

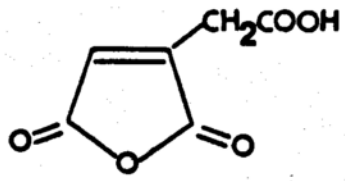
On injecting the quaternary ammonium hydroxide into the gas chromatograph, it decomposed to yield a compound with a new retention time, thus enabling its determination.

2. The Polycarboxylic Acid--Acetic Anhydride Method

Tertiary amine compounds form colored solutions when dissolved in a reagent solution consisting of an organic polycarboxylic acid, e.g. cis-aconitic acid (I), and acetic anhydride. Cromwell (39) used cis-aconitic anhydride (II) in combination with acetic



I

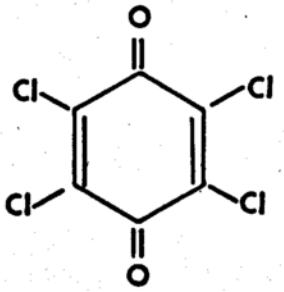


II

anhydride in the microestimation of toluene solutions of trimethylamine derived from Chenopodium Vulvaria L. The aqueous extracts of trimethylamine were carefully treated to eliminate water prior to dissolution in toluene; water had been reported in a related analytical procedure to be an inhibitor of this chromogenic reaction process (40). It was suggested (39) that the mixture of cis-aconitic anhydride and acetic anhydride not be allowed to age prior to the addition of the toluene solution of tertiary amine; aging would lead to color development in the cis-aconitic anhydride-acetic anhydride reagent solution. No reason was,

however, advanced to explain the appearance of color on aging the reagent mixture. The detection limit for the analysis was reported to be 5 µg per 5 ml.

Sass et al. (41) have reported on the colorimetric estimation of tertiary and quaternary amines using, in the one case, the reaction of amine with aconitic anhydride in the presence of acetic anhydride and, in the other case, the reaction of amine with chloranil (III) in toluene. Although the chloranil method when used



III

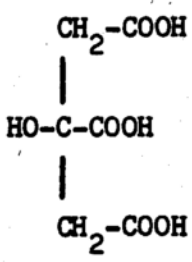
alone is not capable of distinguishing between the amine and its salts, when used in conjunction with the aconitic anhydride method, it reportedly can provide for the quantitative differentiation between tertiary amines and amine salts or quaternary amines. Unlike Cromwell, Sass et al. have encouraged that the aconitic anhydride reagent solution be aged 24 hours before using. This, in their view, led to the attainment of maximum intensity and of reproducible color. Yamamoto and Uno (42) used alcoholic solutions of cis-aconitic anhydride and of tertiary aliphatic amines, reacting them in the presence of acetic anhydride to develop a colored solution. They did not employ any preaging of the reagent

solutions, but reported that the extent of color development may be dependent on the identity of the amine. The limits of determination for the range of tertiary amine compounds considered ranged from 1 to 3 µg per 0.5 ml. Pesez and Bartos (43) have also commented on the principle of the reaction of tertiary amines with a mixture of aconitic acid and acetic anhydride. They have given an elaborate procedure for reagent preparation, which, presumably, should improve the analytical process.

Groth and Dahlen (44) studied the trans-aconitic acid-acetic anhydride system. The reagent was prepared by mixing trans-aconitic acid and acetic anhydride in the ratio 2 to 3, and aging the mixture for 1 to 3 days. Depending on the temperature used during the aging process, the reagent mixture, hitherto a suspension, reportedly becomes either a light yellow brown or sometimes a greenish homogeneous liquid. This is probably the ensuing chemical reactivity within the reagent solution that Cromwell (39) alluded to by warning that the reagent solution not be allowed to stand for extended periods of time before treatment with the toluene solution of trimethylamine. But whereas Sass et al. (41), and Groth and Dahlen (44) chose to age the reagent mixture comprising organic polycarboxylic acid and acetic anhydride, Cromwell did not find the aging process essential to his analytical procedure. Obviously, color development in the reagent solution upon aging suggests a chemical process with a potential for influencing the subsequent interaction between the reagent and the tertiary amine moiety. The

prescription of such varied reaction conditions by different authors is evidently a consequence of a lack of understanding for the chemical process(es) involved. This lack should be motivation for a careful investigation.

Variations of this chromogenic reaction in which organic polycarboxylic acids other than aconitic acid have been used are also available in the literature (45-51). Citric acid (IV)-acetic anhydride reagent has been used to determine alkali metal ions (40).



IV

Langley (47) has attempted to establish conditions under which a set of recommended reagents may be used, whether in aqueous or anhydrous solutions, to relate the intensity of the color developed upon reaction to the amount of chromogenic substance present. The color which was developed in the reaction process reportedly was stable, and the lower limit for detection was given to be 5 μg. Citric acid impregnated paper, in the presence of acetic anhydride, has been used as reagent in the spot testing of organic pollutants in water (50). The limit of detection was reported to be 5 to 10 μg per ml for the range of tertiary amine compounds tested.

Malonic acid (V)-acetic anhydride reagent system has also found some applications in the identification and/or determination of tertiary amines (46, 48, 49). A 2% solution (46), or a 10% solution

$$HOOC\text{-}CH_2\text{-}COOH$$

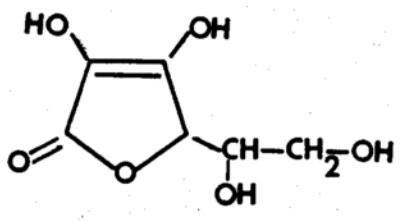
V

(48, 49) of malonic acid in acetic anhydride was used as reagent solution. In all of these cases, although there was no mention of preaging the malonic acid-acetic anhydride mixture, the mixture was nonetheless subjected to heat leading, probably, to stronger dehydrating conditions than simple prolonged exposure of the malonic acid to the acetic anhydride. In one case (46), this was intended to hasten the reaction, while in the others (48, 49), it was to facilitate dissolution of the acid in the anhydride. The dissolved malonic acid reagent solution was reported to be stable for approximately 7 hours. But when allowed to stand, i.e. to age, it developed a yellow coloration, which, reportedly (48), did not affect the fluorescence of the reaction product. It was not mentioned whether or not ultraviolet-visible spectrophotometric measurements were affected by such color development in the reagent solution (49).

Groth and Wallerberg (46) carried out thin-layer chromatography of reaction mixtures of various organic polycarboxylic acid-acetic anhydride and tertiary amine combinations. They reported the

chromatographic patterns to be dependent on the nature of the amine compound as well as of the polycarboxylic acid employed. The general mechanism for the reactions was postulated to be a base-catalyzed condensation process (46). Thomas (49), implying that malonic acid forms a mixed anhydride species when heated with acetic anhydride, reported formation of the base-catalyzed condensation product to be a function of the basicity of the tertiary amine catalyst. But it is doubtful at this point whether the catalytic role being assigned to the amine has been properly defined. If we were to cast the tertiary amine in a purely catalytic role, we would expect that it neither be consumed nor transformed in the overall reaction process. But that is not likely to lead to different product(s) using different tertiary amine compounds (46), if in fact this assertion is true; only thin layer chromatographic evidence was presented in making such a claim.

Taha et al. (51) developed a new spectrophotometric assay suitable for the determination of tertiary amine drugs. They employed a 1% solution of ascorbic acid (VI) in acetic anhydride as the reagent. They evaluated the effect of dilution of the



VI

interaction products by different solvent systems including methanol, ethanol, isopropanol, and dioxane. While the position of the wavelength of maximum absorption (λ_{max}) was reportedly not affected by the solvent system, the intensities of absorption at the λ_{max} were.

The aging phenomenon, i.e. the deliberate extended exposure of the polycarboxylic acid to acetic anhydride, alluded to several times in the preceding paragraphs probably deserves further mention. It may, to a great extent, determine the concentration of the intermediate species formed, if in fact there is any formed. For most of the studies presented in the literature, whether or not the aging phenomenon was used, it is perhaps not unrealistic to expect, given the strongly dehydrating conditions of most investigations, i.e. combination of acetic anhydride and heat, formation of an intermediate product. Color formation in the aconitic acid-acetic anhydride combination (39, 44, 52), as well as the delayed color development on aging a previously heated solution of malonic acid in acetic anhydride (48), have been documented. As a cautionary step in the analytical microestimation of trimethylamine (39), it was advised that the solution of amine be added as soon as possible to the reagent solution, which otherwise gradually developed a red coloration on standing at room temperature. Attempts have been made, using the aging process, to isolate the intermediate species in this reaction process, details of which will be presented in section C.1. The evidence presented in such attempts, although much

less than conclusive, further dramatize the apparent lack of comprehensive knowledge concerning the chemistry of this reaction. The times of aging used range from a few hours to as much as three days.

The range of compounds containing a tertiary amine functional group that have been determined using the principle of this reaction is wide. A partial listing includes chlorpheniramine, promethazine, aminopyrine, N,N-dimethylcyclohexylamine, imipramine, atropine, cocaine, pholcodeine, ergotamine, and so on.

The potential exists for the application of any of the various analytical methodologies already described to the determination of the tertiary amine functional group in pharmaceutical products. To do so in some instances, however, would require complex sample manipulation. Most of the published analytical methods for the tertiary amine group may be viewed only as so-called specialized procedures, which are at best applicable to the determination of a few compounds. Because of a lack of a replaceable hydrogen on the tertiary amine group, compounds containing this functionality cannot be subjected to a generalized derivatization procedure that would enable their determination. Even then, the complexity of most derivatization reactions is such that they are less than desirable in 'routine' applications. Most of the mentioned chromatographic methods depend on some form of derivatization reaction; the acid dye complexation methods as well as the titrimetric methods are similarly specialized procedures. The polycarboxylic acid-acetic

anhydride procedure is one that offers the hope of a method which, if properly characterized, could be applied to the determination of all tertiary amine compounds. We chose to examine the polycarboxylic acid-acetic anhydride system in greater detail.

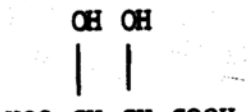
B. Background to this Study

1. Early Experiments

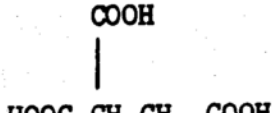
The phenomenon of the color-producing reaction of organic polycarboxylic acid-acetic anhydride reagents with basic compounds has been known for several years. Taylor (52) in 1919 used the color reaction of aconitic acid with acetic anhydride as a means for the determination of aconitic acid in sugar cane. He also used the method in an indirect way to determine citric acid. The addition of acetic anhydride to aconitic acid resulted in the appearance of a pink coloration, which rapidly turned to deep red and then to magenta. Further heating produced a bluish-green liquid, finally turning to a brown, almost opaque, solution. Analysis of the reaction solution in the intermediate stage, i.e. when the magenta liquid was obtained, indicated the solution to consist of two colored substances, a red one which was soluble in water and a blue one which was soluble in ether. Attempts at isolation of these red and blue components failed, since they were exceedingly unstable, being rapidly destroyed by water, acids, or alkalis. Although Taylor did not report using any tertiary amine or other basic compounds in the reaction process, almost all subsequent literature reports appear to have; it is not quite clear just how these basic compounds 'crept' into use in this reaction process.

Furth and Herrmann (53) described color reactions of citric, aconitic, tartaric (VII), tricarballylic (VIII), and agaricic (IX)

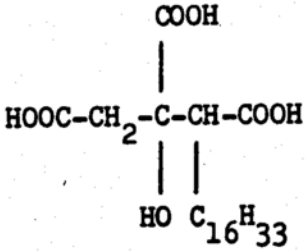
acids with a mixture of 35 parts of pyridine and 15 parts of acetic anhydride. Under the conditions of the reaction, the following



VII



VIII



IX

colors were reportedly obtained: Tartaric acid gave an emerald - green, citric acid a carmine, aconitic acid a purplish red and agaricic acid a purplish brown. No coloration was reported for tricarballylic acid. It was postulated that the colors were produced by condensation of the acids with pyridine.

In 1941, Roeder (45) reported making the unexpected observation, in the course of some other research, that alkali citrates heated with acetic anhydride produced deep red colored solutions. He attempted to substitute acetic anhydride with acetic acid but failed to observe color formation. On the basis of this

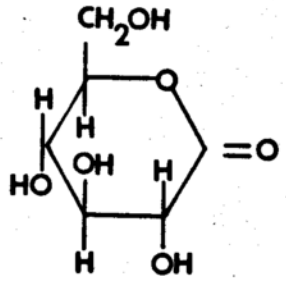
finding, he postulated that the first phase of the reaction probably is formation of alkali acetate and a mixed anhydride of acetylated citric and acetic acid. Since citric acid dissolved in hot acetic anhydride gave a colorless solution, he further inferred that the alkali acetate formed in the first phase of the reaction brought about the condensation of the mentioned mixed anhydride into the colored compound. He proceeded to test a wider range of organic polycarboxylic acids including lactones of sugar acids and reported obtaining coloration with most of them.

The conduct of the reaction usually involves heating the acetic anhydride solution of polycarboxylic acid with the basic compounds. Variations of the procedure in which alcoholic solutions of the acid and the base are prepared separately and then brought into contact in the presence of acetic anhydride also have been presented. The principle of the reaction has been used quite extensively by many analysts for the detection and/or determination of compounds containing a tertiary amine group, as described earlier. But hardly any chemistry has been studied. Connors (54) alludes to this colorimetric reaction and comments that the nature of the reaction with tertiary amines is not known. Feigl (55) has stated how the reaction can be applied in the spot testing of tertiary amines. It is perhaps surprising that, despite the acceptance of the analytical utility of the reaction, so little is known about its chemistry. Since not all organic polycarboxylic acids are capable of participating in this reaction to produce a coloration, it may be

worthwhile, as a first step, to examine what the minimal structural requirements on the acid ought to be for it to be able to take part in the reaction.

2. Structural Studies

Aconitic acid and aconitic anhydride have been used very commonly as reagents in this reaction process. But a color is also produced when these two compounds are substituted by other reagents such as malonic, tartaric, citric, or ascorbic acids. It has been reported (45), that some lactones of sugar acids, e.g. glucono-d-lactone(X), also give coloration in this reaction process.



X

Apparently, there is probably even a wider range of other organic polycarboxylic acids capable of a chromogen development under the conditions of the reaction. It may be desirable to attempt to group and classify these acids.

In the preliminary stages of this investigation, a number of

di- or tricarboxylic acids were reacted with three basic compounds, pyridine, triethylamine, and N,N-dimethylaniline. The matrix of results obtained is presented in Table I. The conditions of the various reactions were not normalized; in some instances the polycarboxylic acid in acetic anhydride treated with the basic compound was sometimes heated in order to ensure progress of the reaction, while in other instances this was not required. The preliminary findings indicated there may be a minimal structural requirement on the acid to enable it to take part in the reaction. However, the essential structural features are not obvious from these results, although some inferences may be drawn, as in section C.1.

3. Postulated Products and Reactions

Beyond the question concerning the minimal structural requirements on the acids to enable them to take part in this reaction process, there are also interesting questions concerning the nature of the colored product(s) and the reaction mechanism(s). It has been suggested (44, 45, 46, 49) that the reaction is a base-catalyzed condensation process. If this is correct, the basic compound serves only as a condensation catalyst and perhaps to form salts or some other kind of addition product (45). As for the tertiary amine compounds, they are not acylable to yield a stable neutral amide. They may therefore either persist in the acylating

Table I. Matrix of Results of Color Reactions of Some Organic Acid-Acetic Anhydride Reagents with Pyridine (PYR), Triethylamine (TEA), and N,N-Dimethylaniline (DMA).[a,b]

Organic Acid	Chemical Structure	Basic Compound		
		PYR	TEA	DMA
Oxalic	HOOC-COOH	no color	no color	no color
Malonic	$HOOC-CH_2-COOH$	yellow-orange	orange	orange
Itaconic	$HOOC-C(=CH_2)-CH_2-COOH$	yellow	red	red
Maleic	HOOC-CH=CH-COOH	no color	no color	no color
Succinic	$HOOC-(CH_2)_2-COOH$	no color	no color	no color
Malic	$HOOC-CH(OH)-CH_2-COOH$	no color	no color	no color
Glutaric	$HOOC-(CH_2)_3-COOH$	no color	no color	no color
Tartaric	$HOOC-CH(OH)-CH-COOH$	brown	yellow	yellow
Citric	$HOOC-CH_2-C(OH)-CH_2-COOH$	red	red	red
Tricarballylic	$HOOC-CH(COOH)-CH_2-COOH$	no color	no color	no color

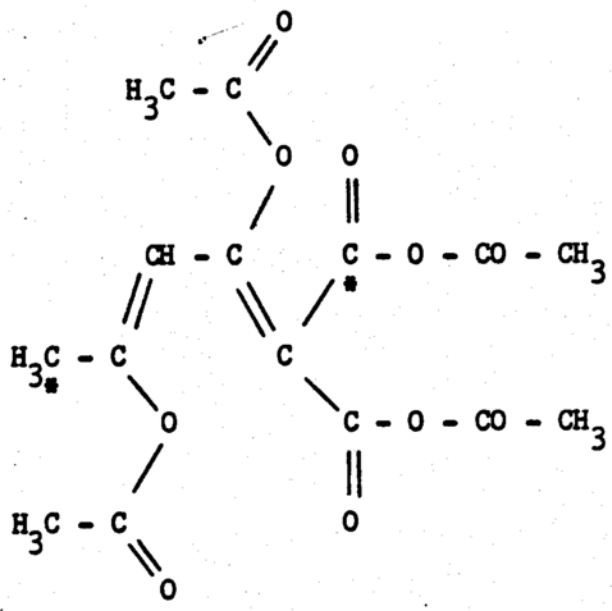
Table I (continued)

Organic Acid	Chemical Structure	Basic Compound		
		PYR	TEA	DMA
Aconitic	HOOC-CH=C(COOH)-CH_2-COOH	green	green	green

[a]For a given acid, the shade of the colored product obtained in going from pyridine through triethylamine to N,N-dimethylaniline is not necessarily a reflection of the nature of the product. It is in this case more a function of the quantity of base used; these were qualitative observations.

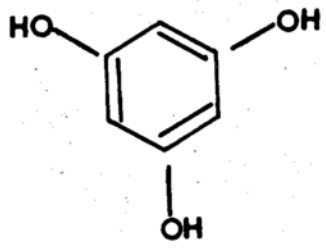
[b]In all cases, approximately 5 ml of a 20% of the acid solution in acetic anhydride were reacted with 1/2 ml to 2 ml of base. Where necessary, heat was applied using a water bath maintained at 80°C for up to 30 min.

medium, acting only as catalysts, or may be consumed in the reaction process, or both. Furth and Herrmann (53) proposed that the colors were produced by condensation of the acids with pyridine. It has been argued (45), however, that the fact that the color reactions occur with alkali acetates and with trialkylamine excludes such a proposition. Apparently, the assumption was made in these cases that the final product is the same irrespective of which basic compound was employed. Thin layer chromatographic evidence has been presented by Groth and Wallerberg which suggested that the chromatographic pattern depended on the amine and, probably not surprisingly, on the organic acid used; the intense colors, together with strong fluorescence, led to the inference (46) that the products may be aromatic. Furthermore, it was reported that elementary analysis of the product prepared from malonic acid, pyridine, and acetic anhydride (10:5:500) indicated it to be a pyridine salt of a compound produced by the condensation of 2 to 3 moles of acetic anhydride and 1 mole of malonic acid with the loss of 5 to 6 moles of water. The structure of the compound that is cyclized, if malonic acid reacted as the mixed anhydride with acetic acid, was thought to be as shown (XI); condensation would occur between the marked carbon atoms. The first aromatic compound formed probably would be a half anhydride of a completely acetylated phloroglucinal carboxylic acid (XII) and acetic acid. The different color components then had to be formed through condensations with the phloroglucinol derivative, together with deacytylation. This,



XI

according to Groth and Wallerberg (46), could explain why the amines are bonded to the colored reaction product leading possibly to the difference in the thin layer chromatographic patterns obtained. Concerning the mechanism, it was reported there can be little doubt



XII: Phloroglucinol

that it is a base catalyzed condensation. If the contention that

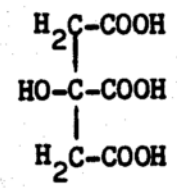
the amine may only be acting as a catalyst is true, the question then translates into what this catalytic mechanism is.

Nor has the role played by the acetic anhydride in this reaction process been clearly defined. One possible justification for use of the anhydride may be to ensure acylation of primary and secondary amine compounds so that they do not interfere with the determination of tertiary amines. In that case, in a situation where there is clearly no possibility of interference from primary or secondary amine compounds, it should not be necessary to use any acetic anhydride. But substituting acetic anhydride by acetic acid (45) in a reaction involving the use of malonic, tartaric, or ascorbic acids, among other polycarboxylic acids, reportedly resulted in no color production, suggesting possibly a more involved role for acetic anhydride in the reaction process.

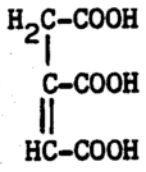
C. Definition of the Problem

1. Anhydrides as Possible Intermediates

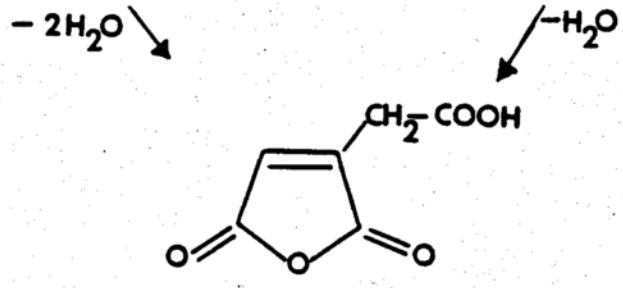
The application of strongly dehydrating conditions, i.e. combination of acetic anhydride with heat in most of these reactions, introduces the possibility that anhydride species may be formed as intermediates in the reaction process either prior to, or concomitant with, the interaction with the tertiary amine compound. A closer examination of Table I, focusing attention on citric acid and aconitic acid, which are two of the organic acids capable of generating colored solutions in this reaction process, shows that both of these acids are amenable to dehydration leading to cyclic anhydride formation as shown in Scheme I. Under the strongly



Citric acid



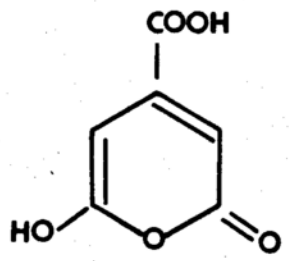
cis-Aconitic acid



cis-Aconitic Anhydride

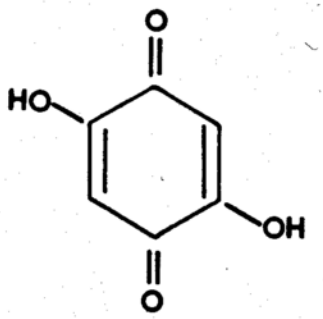
Scheme I: Dehydration of Aconitic and Citric Acids

dehydrating conditions, citric acid would lose two molecules of water, while aconitic acid would lose one molecule of water, both processes leading to formation of a cyclic anhydride, cis-aconitic anhydride. Therefore, the ability of these two compounds to give colored product(s) in this reaction process may be related through the anhydride species (40). It appears, however, that there may be a further transformation of this anhydride species into a reactive intermediate, which is as yet to be clearly determined. It had been reported (44, 52), for aconitic acid, that prolonged exposure of the acid to acetic anhydride leads to the production of a colored solution. Also, when cis-aconitic anhydride in acetic anhydride was allowed to stand for extended periods of time, a colored solution reportedly resulted (39), suggesting perhaps the further transformation of the aconitic anhydride species into some other chemical entity. Groth and Dahlen (44) have reported synthesizing such a chemical entity, or the so-called reactive intermediate. A trans-aconitic acid-acetic anhydride mixture (in a 2 to 3 ratio) was aged 1 to 3 days; the resulting freeze-dried, syrup-like material, considered to be the reactive intermediate, was shown by infrared spectroscopy to have a structure similar to the enolic form of α,γ-anhydroaconitic acid (XIII). However, the interpretation of the infrared spectral data, as well as the data themselves, are not convincing. Taylor (52) also investigated the nature of the coloration in the reaction between aconitic acid and acetic anhydride and reported the actual colored substance to be extremely



XIII

unstable. Its deep yellowish-brown color resembled that of 2,5-dihydroxybenzoquinone (XIV), but it appeared to be more unstable than that substance.



XIV

2. Malonic Acid: The System of Choice

In order to be able to study the chemistry of this analytical process, it is essential not to attempt too much, in this sense: The reactions described by Table I may represent more than one

pathway or mechanism, and selection of systems is necessary. The systems to be studied should be chemically interesting even though not general, but should bear on the ultimate analytical problem. From the matrix of results presented earlier in Table I, two interesting options are immediately obvious for possible further consideration: The first of these is the dehydration of aconitic and citric acids to give aconitic anhydride (Scheme I); the second is the curious behavior of malonic acid when compared with oxalic and succinic acids, which, taken together, are the first three members of a homologous series. This peculiar behavior of malonic acid prompted us to investigate the HOOC-$(CH_2)_n$-COOH homologous series further, and we eventually chose the malonic acid system for study.

Two additional members of the homologous series were tested and, as the results presented in Table II will indicate, malonic acid is the only member (of those members tested) that produced a colored solution. The physical properties of members of this series do not reveal anything strikingly different about malonic acid that should enable it to stand out in this manner. However, both oxalic and malonic acids offer the possibility of loss of water upon dehydration with formation of hypothetical three- and four-membered anhydride rings, respectively (Scheme II). But they do not yield any such anhydride molecules. Upon dehydration, oxalic acid yields carbon monoxide and carbon dioxide as products, whereas malonic acid affords a highly unsaturated and reactive gaseous substance known as

Table II: Physical Properties of the First Five Members of the $HOOC-(CH_2)_n-COOH$ Homologous Series (Taken from Reference 56).

Acid	n=	m.p.	Solubility g/100g	Acid Dissociation pKa_1	Acid Dissociation pKa_2
Oxalic	0	187	$10.2^{20°}$	1.46	4.40
Malonic[a]	1	135	$138^{16°}$	2.80	5.85
Succinic	2	185	$6.8^{20°}$	4.17	5.64
Glutaric	3	97.5	$63.9^{20°}$	4.33	5.57
Adipic	4	151	$1.4^{15°}$	4.43	5.52

[a]Only member of homologous series (of those tested) to have given a colored reaction solution.

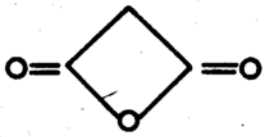
Scheme II: Anhydride Molecules of Some Members of the $HOOC(CH_2)_n\ COOH$ homologous series.

HOOC - COOH (Oxalic Acid) $\xrightarrow{-H_2O}$ ✗

HOOC - COOH (Oxalic Acid) $\downarrow -H_2O$ → $CO + CO_2$

HOOC - CH_2 - COOH (Malonic Acid) $\xrightarrow{-H_2O}$ ✗

HOOC - CH_2 - COOH (Malonic Acid) $\downarrow -2H_2O$ → O = C = C = C = O (Carbon Suboxide)



Succinic Acid and Glutaric Acid $\xrightarrow{-H_2O}$ Stable Cyclic Anhydrides

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Adipic Acid and Higher Homologs $\xrightarrow{-H_2O}$ Chain Polymers

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carbon suboxide (XV). Succinic and glutaric acids lose water to form stable cyclic anhydrides, while adipic acid and the next higher

$$O = C = C = C = O$$

XV

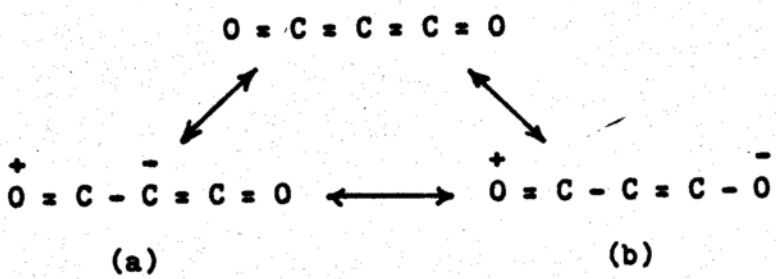
homologs are converted to a mixture of chain polymers of varying chain lengths (56). No other member of the series produces a linear anhydride molecule.

This unique behavior of malonic acid, and the remarkable structure of carbon suboxide (or malonic anhydride, as it has been called), attracted our interest, and prompted us to choose the malonic acid system for study. A reasonable hypothesis is evidently that the color-forming ability of malonic acid is a consequence of the formation of carbon suboxide, which is the actual reactant. We tested this notion with the aid of the distillation apparatus of Figure 1. In this preliminary investigation, a stream of gas was generated by heating a 20% acetic anhydride solution of malonic acid and bubbling the gas into an acetic anhydride solution of either pyridine or triethylamine. We indeed observed color development in the tertiary amine-acetic anhydride solution after less than 15 min of distillation.

3. Properties of Carbon Suboxide

Carbon suboxide is a gas under ordinary conditions (m.p.

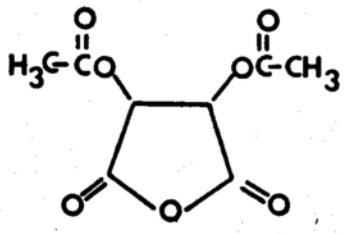
-110°C; b.p. 6-7°C). The carbon-carbon and carbon-oxygen bond distances have been determined to be approximately 1.28 and 1.16 Å, respectively. Electron diffraction data have indicated the molecule to be a linear symmetric structure but small deviations from linearity could not be discounted on the basis of such evidence (57). Scheme III shows two of the postulated mesomeric forms of the molecule; the first form, designated by the letter (a) was postulated based upon the nuclear magnetic resonance properties of the molecule (58), while form (b) is based upon quantum mechanical calculations (59).



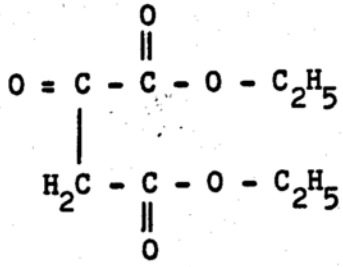
Scheme III: Mesomeric Forms of Carbon Suboxide.

Many specialized synthetic procedures have been presented in the literature for the acquisition of carbon suboxide. All of the procedures involve pyrogenic decomposition, with temperatures ranging between 140°C and 700°C. The products usually are carbon suboxide, carbon monoxide, carbon dioxide and acetic acid; these are liquified by very low temperatures (-120°C), and purified. The acetic acid is removed usually by passage through lime (CaO). Long

et al. (60) have reported a purification process that involves continuous temperature variation in a flask-to-flask distillation process. Most of the procedures have used diacetyl tartaric anhydride (XVI) as starting material (61, 62). Some (63) have used diethyloxalacetate (XVII), while others (60, 64, 65) have used malonic acid.



XVI



XVII

The solubility of carbon suboxide has been reported to be 2.5 g in 100 ml of cold anhydrous ether (63).

Carbon suboxide exhibits a range of chemical reactivity behavior, much of which is yet to be clearly elucidated. It is reported to undergo thermal polymerization leading to compounds of the polycyclic 6-membered lactone type (64). The polymerization reaction, which is reported to be a heterogeneous process, is preceded by an induction period, or a lag time, of unpredictable length, appearing to be surface-initiated and inhibited in the presence of trace amounts of adsorbed water (66). The induction

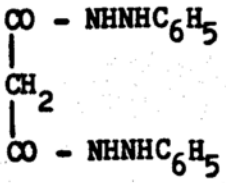
period is shortened considerably in the presence of strongly acidic sites, while reaction vessels coated with paraffin have extremely long induction periods; glass and silica appear to lie somewhere between these two extremes (64). It was found that the rate of polymerization increased with increasing temperature and monomer pressure. Furthermore, the infrared spectra of polymers prepared at various temperatures were identical, thus indicating that, regardless of the size of the polymer, the unit structure remains the same for all.

The chemical reactivity of carbon suboxide as well as its ultraviolet absorption properties have been reported to be similar to the ketenes, $R_2C = C = O$, (62, 67). The ultraviolet spectrum of carbon suboxide gas, prepared by the low temperature dehydration of malonic acid (68), shows the typical carbonyl band with maximum absorption at 265 nm (ε = 94 L/mole cm). The photolysis of a mixture of carbon suboxide and ethylene led to production of carbon monoxide and allene (67) according to the following equation:

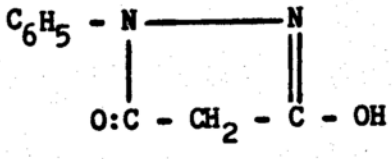
$$O = C = C = C = O + H_2C = CH_2 \xrightarrow{h\nu} 2CO + H_2C = C = CH_2$$

Gas chromatographic analysis of the reaction mixture gave the mole ratio of products CO/allene as 2.4 ± 0.3; the ratio remained constant throughout the photolysis. Evidence obtained in a separate reaction between carbon suboxide and propylene excluded the possibility that the carbon suboxide was simply exchanging its

terminal oxygen atoms for the hydrogen atoms of the ethylene in a chain reaction. The initial step in the photolysis, by analogy with ketene, probably involved cleavage of the carbon-carbon double bonds to give a C_2O radical, which might decompose to give a free carbon atom that subsequently attacks the pi electrons of the carbon-carbon double bond to form a cyclopropylcarbene intermediate (67). This intermediate then rearranges exclusively to the allene. Carbon suboxide also reacts with hydroxylamine and with β-phenylhydroxylamine giving, respectively, malonhydroxamic acid and N,N'-diphenylmalonyhydroxamic acid, much the same way as ketenes (62). But unlike ketene, which reacts with phenylhydrazine to give malonyl-bis-phenylhydrazine (XVIII), carbon suboxide reacts to give 1-phenyl-3,5-diketopyrazolidine (XIX) (69).



XVIII

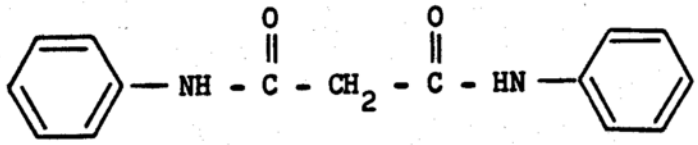


XIX

Carbon suboxide has been catalytically reduced by passing a mixture of the suboxide and hydrogen over nickel or palladium coated with silica gels (61). It was found that propylene was the principal reduction product, but acetic acid, and the polymeric form of the suboxide, brought about due to the presence of the by-product

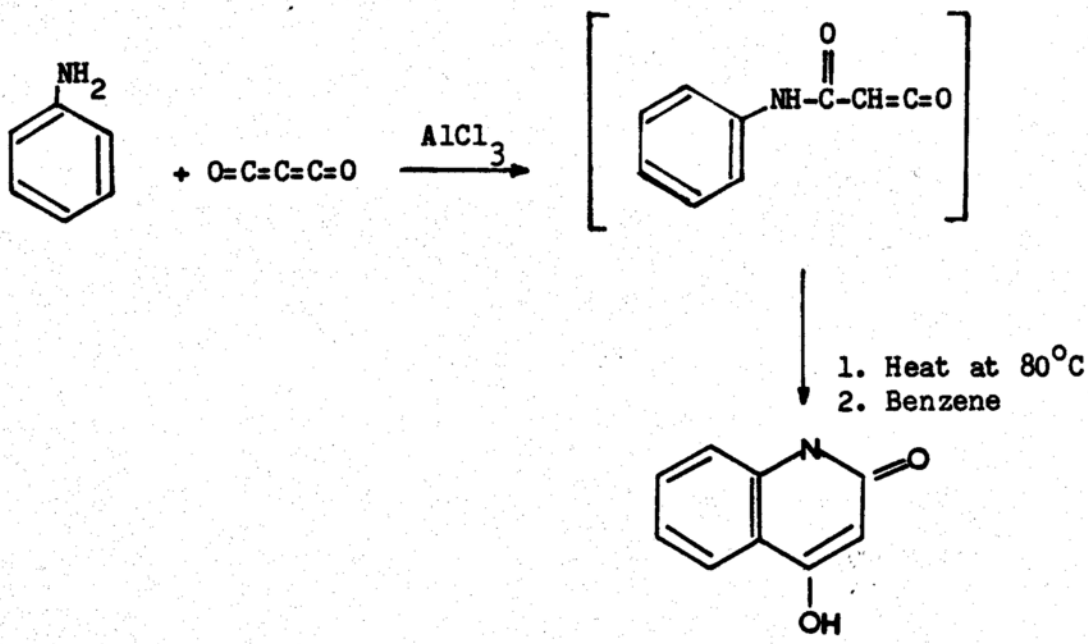
malonic acid (61, 69), were also present.

The reaction of carbon suboxide with some enolizable ketones in the presence of sulfuric acid to give the corresponding 1:1 pyrone or pyronopyrone derivative has been reported (70). Although the chemical equations presented in this investigation seem plausible, the experimental evidence, consisting mainly of ultraviolet absorption spectral data, leading up to such conclusions is rather weak. Carbon suboxide reacts quantitatively with aniline to give, usually, malonanilide (XX) (69). In the presence of aluminum chloride, carbon suboxide reacts with aniline in ether to produce an N-acetylated ketenic intermediate, which, in benzene under reflux conditions, cyclizes to give 4-hydroxylcarbostyril (XXI)



XX

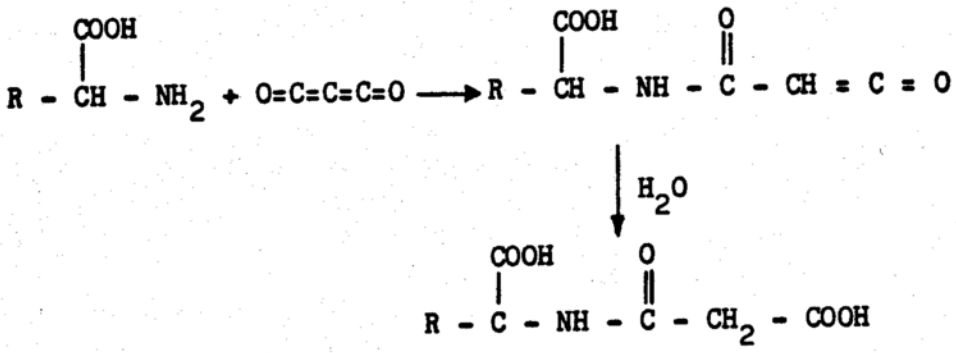
according to the following equation (71):



XXI

When 4-hydroxycarbostyril reacts with one molecule of aniline and with water, malonanilide and phenylcarbamoylacetic acid are produced (71). The influence of different electrophilic substituents in the aniline ring on the reactivity of carbon suboxide was investigated. Carboxyl, nitro, and sulfo-substituted anilines in ether did not react with carbon suboxide, whereas unsubstituted aniline reacted to give malonyl-bis-aniline (72).

α-Amino acids of the aliphatic series can react with only one ketone group of carbon suboxide, resulting first in formation of a substituted aminocarbonylcarbomethylene, which is hydrolyzed to malonyl monoamide (73, 74) according to the following equation:



It was suggested that the influence of the electrophilic carboxyl group, which acts to decrease the reactivity of the amino group, may primarily be responsible for the one-to-one reaction (72). Essentially, reactions of the suboxide with the amino and phenolic hydroxyl groups within proteins to give malonyl derivatives consist of the introduction of the radical, $-CO-CH_2-COOH$, at reactive positions (75), and also 'polymerized' forms in which the original protein molecules are coupled by $-CO-CH_2-CO-$ bridges (76).

The reaction between a series of electrophilically substituted anilines and carbon suboxide was studied in aqueous solution. Whereas electrophilically substituted anilines do not react with carbon suboxide in ether (72), it was shown that in aqueous solution, a series of substituted N,N'-malonyl-bis-anilines could be obtained (77). This is probably because the reaction rate of carbon suboxide is greater with primary amine groups than with aqueous hydroxyl, a situation which is analogous to the behavior of ketenes in their acetylation of amines in aqueous media (78). The reaction

between some β-substituted ethylamines and carbon suboxide in aqueous medium reportedly leads to formation of N,N'-malonyl-bis-β-substituted ethylamines exclusively (79).

The reactivity of carbon suboxide towards metal complexes of nickel, manganese, and rhodium decarboxylation reactions and allene polynuclear rhodium complex formation has also been studied (80).

4. Objectives of This Study

Compounds containing a tertiary amine functional group are known to produce a coloration upon treatment with a solution of cis-aconitic anhydride in acetic anhydride, and variations of this reaction have been described in which numerous polycarboxylic acids yield a color with tertiary amines in the presence of acetic anhydride. Although the reaction has been applied to the colorimetric determination of tertiary amines, it is not a widely used or widely known method.

The relative neglect of this potentially valuable analytical method is not a consequence of a lack of need for effective techniques for the analysis of compounds containing the tertiary amine functionality. A large number of pharmaceutical compounds contain a tertiary amine group. Although these types of compounds can be determined in numerous ways, for example chromatographic methods, or by the acid-dye extraction methods, there are no methods that can be regarded as both general and sensitive. This situation is in marked contrast to the wide availability of good

analytical methods for primary and secondary amines, whose possession of a replaceable hydrogen permits the application of numerous analytical derivatization reactions.

The reason for the general oversight of the organic polycarboxylic acid-acetic anhydride reaction lies in our lack of understanding of the chemistry of the method, which is yet to be established. In the present instance, not only are the reaction intermediates and product(s) still unestablished, but the structural requirements on the organic polycarboxylic acid that would permit it to take part in the reaction process, as well as the roles being played by other chemical entities used, are quite unknown. Under such circumstances, the analyst's control of the method is uncertain; the critical factors of sensitivity, reproducibility, and possible interferences are largely unknown and uncontrollable.

The chemical reactivity of the malonic acid-acetic anhydride system selected for study in this thesis project is probably not representative of the many possible pathways and mechanisms which could be followed by the various organic polycarboxylic acids capable of taking part in this reaction process. But it is a chemically interesting system to study especially from the point of view of malonic acid being probably the only member of a homologous series capable of generating a coloration in the reaction process. A more thorough characterization of this reaction system should enable its application to the detection and/or determination of the tertiary amine moiety in all compounds. The goals of this thesis

research project may be summarized as follows:

1. To undertake preliminary investigations aimed at assisting with the decision of selecting a system for study.
2. To elucidate the chemistry of the reaction process(es) involved; to attempt to identify intermediates and product(s).
3. To undertake an investigation of the role of the tertiary amine compound in the reaction process. Specifically these questions will be asked:
   a. Is there a difference in behavior between aromatic and aliphatic tertiary amines in the reaction?
   b. Is the amine consumed or is it acting purely as a catalyst as has been postulated by others?
4. To study the concentration-time behavior of reactants and reaction product(s).

II. EXPERIMENTAL

A. Chemicals and Reagents

The following chemicals were purchased from Aldrich Chemical Company, Inc., Milwaukee, WI: Triethylamine, n-tripropylamine, n-tributylamine, pyridine, 3-methylpyridine (3-picoline), 4-methylpyridine (4-picoline), 3,4-dimethylpyridine (3,4-lutidine), 2,4-dimethylpyridine (2,4-lutidine), 2,6-dimethylpyridine (2,6-lutidine), and 2,4,6-trimethylpyridine (2,4,6-collidine). Dimethylamino-2-propyne and allyldimethylamine were purchased from Pfaltz and Bauer, Inc., Waterbury, CT. Oxalic, malonic, succinic, glutaric, and adipic acids were also purchased from the Aldrich Chemical Company. Other chemicals purchased from Aldrich included cis-aconitic, itaconic, tricarballylic, tartaric, maleic, and malic acids, as well as deuterated chloroform. Acetic anhydride and anhydrous diethyl ether were from Fisher Scientific Company, Fair Lawn, NJ. Acetonitrile UV was purchased from Burdick and Jackson Laboratories, Inc., Muskegon, MI. All the chemicals were of the highest grade commercially available, and were used without further purification.

The 50% v/v acetonitrile/water mobile phase for the high performance liquid chromatography (HPLC) was prepared using triply distilled, deionized water.

B. Apparatus and Procedures

1. Production of Carbon Suboxide

Carbon suboxide was generated by heating, at approximately 150°C, a 20% solution of malonic acid in acetic anhydride and collecting the gaseous compound by passing it through water-cooled columns into 50 ml of cold anhydrous diethyl ether (see Figure 1). The heating source was a variable autotransformer (Staco Inc., Daytona, OH) set at 70% of maximum output voltage. To this was attached a 250 ml Thermowell® (Laboratory Craftsmen, Beloit, WI) heating mantle. Distillation was generally carried out for approximately 30 min. The etheral solution of carbon suboxide, which in subsequent applications may be referred to simply as carbon suboxide, was used for most analyses. Carbon suboxide gas for nuclear magnetic resonance (NMR) spectral analysis was generated similarly and distilled into 10 ml of deuterated chloroform.

2. NMR Spectrum of Carbon Suboxide

Two milliliters of the $CDCl_3$ solution of carbon suboxide were treated with 35 mg of chromium (III) acetylacetonate [$Cr(acac)_3$] (Aldrich) in a 10 mm NMR sample tube. This was immediately frozen in a dry ice-acetone bath until analyzed, which was approximately 30 min later. The NMR spectrum was obtained using a Bruker AM 500, 125 mHz instrument with the sample port maintained at $-30^{\circ} \pm 0.1^{\circ}C$. A 2-second pulse delay time was used, and a total of 2200 scans were

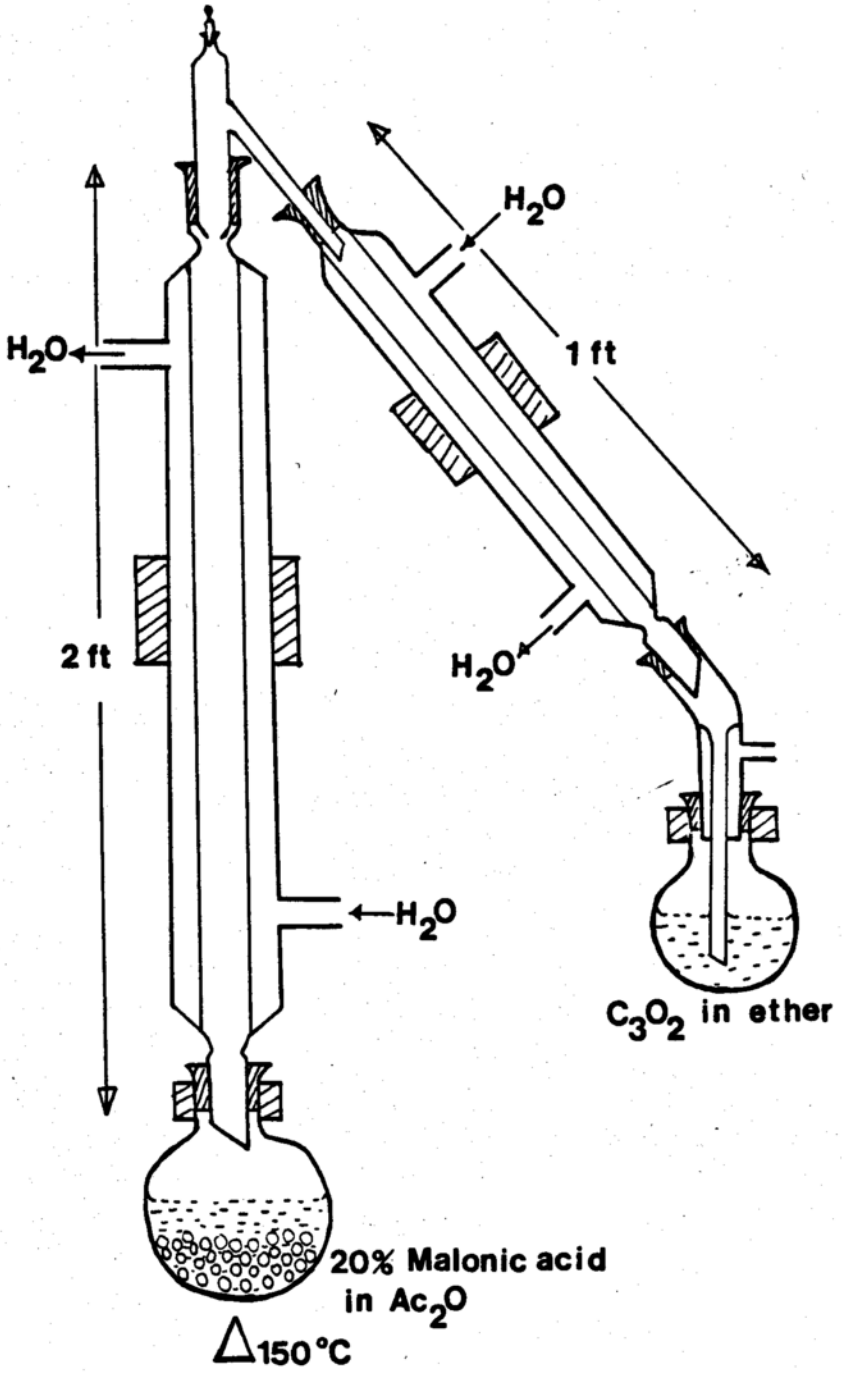


Figure 1. Distillation Set-up for the Production of Carbon Suboxide.

taken.

3. UV-VIS Spectrophotometry

Spectrophotometric measurements were made using a Varian 2200 UV-VIS double beam spectrophotometer fitted with a jacketed cell compartment maintained at 25 $\pm$ 0.1^{o}C by means of a Haake A81 constant-temperature water bath. All spectrophotometric measurements were carried out using 1 cm pathlength silica cuvettes (Spectrocell Corporation, Orland, PA) fitted with tight covers to minimize sample evaporation.

a. Absorption Spectrum of Carbon Suboxide

The absorption spectrum of carbon suboxide was obtained against an ethyl ether blank using a Varian 2200 UV-VIS spectrophotometer. The absorption spectra of a blank distillation, which was prepared using a procedure similar to the distillation of carbon suboxide except without malonic acid, of 2.00 x 10^{-2}M malonic acid, and of 3.00 x 10^{-3}M acetic anhydride in ethyl ether were also obtained.

b. Kinetic Investigations

In this section only the procedure concerning kinetic investigations performed by use of ultraviolet-visible absorption spectroscopy will be presented. Other concentration-time dependent investigative procedures performed by use of high performance liquid chromatography, or gas chromatography, will be presented in B.4.

A 10% solution of malonic acid in acetic anhydride was prepared in a 500 ml round-bottom flask. This solution was maintained at 25 $\pm$ 0.1°C by use of a water bath while being allowed to age. At timed intervals, samples were withdrawn and the absorption spectrum of the aged solution monitored against an acetic anhydride blank. Also, the reactivity of the aged solution towards a 1.60×10^{-3}M acetic anhydride solution of pyridine was monitored.

Kinetic runs were performed on reagent solutions that were preequilibrated in tightly closed containers at 25 $\pm$ 0.1°C for approximately 30 minutes. In general, kinetic runs were of two kinds: those involving reaction of acetic anhydride solutions of tertiary amine with carbon suboxide, and those involving reaction of etheral solutions of the amine with carbon suboxide. The latter reactions were performed mainly with aliphatic tertiary amines. We will explain the kinetic procedure of the former reaction first. In a typical kinetic run, 2 ml of the etheral solution of carbon suboxide was mixed with 1 ml of the amine solution prepared in acetic anhydride in a cuvette. The cuvette was shaken vigorously and absorbance measurements were initiated less than 15 seconds post mixing the reactants in the cuvette. In the second kind of kinetic run, 1.5 ml each of carbon suboxide solution and the etheral solution of the amine were brought into contact in a cuvette. Again the cuvette was shaken vigorously and absorbance measurements were initiated less than 15 seconds after mixing the reactants.

To study the influence of preaging on the lag time, or

induction time phenomenon, reagent solutions were prepared and pretreated for 20 min as outlined in Table III. Pyridine was the only tertiary amine used in this attempt to establish the influence of the preaging process on the lag time phenomenon. Triethylamine and other aliphatic tertiary amines exhibit color development upon aging with etheral solutions of carbon suboxide. The reactions were initiated by mixing the preaged pairs.

The dependence of the lag time and initial rates on the concentration of acetic anhydride was also investigated at constant pyridine and suboxide concentrations. Solutions of acetic anhydride (9.00 M, 7.00 M, 6.00 M, and 5.00 M) were constituted in ether. Two milliliters of carbon suboxide were mixed with 1 ml of the acetic anhydride solution. The reaction was initiated immediately thereafter by adding 2.40 µl of pyridine (approximately 10^{-2}M) into the cuvette and measuring the absorbance as a function of time at the appropriate wavelength.

4. Chromatographic Methods

The high performance liquid chromatographic system consisted of an Altex Model 110A solvent metering pump (Altex Scientific, Inc., Berkeley, CA), an Altex Model 210 fixed volume (20 µl) injection valve, a Hitachi Model 100-10 variable wavelength detector equipped with an Altex spectrophotometer flow cell, and a Perkin-Elmer (Linear) Model 918 variable-sensitivity recorder (Linear Instrument, Irvine, CA). The analytical column was a 250 x 4.6 mm Spherisorb

Table III. Different Combinations of Pretreated Reaction Solutions.

No.	Designation[a]	Solution Composition
I	A - C + A - B	2 ml C_3O_2 + 0.5 ml Ac_2O 2.4 µl PYR + 0.5 ml Ac_2O
II	A - C + B - C	1 ml C_3O_2 + 1 ml Ac_2O 1 ml C_3O_2 + 2.4 µl PYR
III	B - C + A - B	2 ml C_3O_2 + 1.2 µl PYR 1 ml Ac_2O + 1.2 µl PYR
IV	A - C + B	2 ml C_3O_2 + 1 ml Ac_2O 2.4 µl PYR
V	B - C + A	2 ml C_3O_2 + 2.4 µl PYR 1 ml Ac_2O
VI	A - B + C	2 ml C_3O_2 1 ml Ac_2O + 2.4 µl PYR
VII	A + B + C	2 ml C_3O_2 + 1 ml Ac_2O + 2.4 µl PYR (No pretreatment)

[a]The designation A is for acetic anhydride (Ac_2O), B is for the base, pyridine (PYR); and C is for carbon suboxide (C_3O_2).

5CN, preceded by a 50 x 4.6 mm Spherisorb 5CN guard column (Phenomenex, Rancho Palos Verdes, CA). The mobile phase was 50% v/v acetonitrile in water, and the flow rate was 2 ml per min at a pressure of approximately 500 p.s.i. In general, samples of the reaction mixture were diluted 1 to 10 with portions of the mobile phase prior to injecting into the HPLC. Quantification of the eluent was by peak height.

The gas chromatographic system consisted of a Perkin-Elmer Model 3920B gas chromatograph, equipped with a flame ionization detector (FID) (Perkin-Elmer, Norwalk, CT). The nitrogen carrier gas flowed at 50 p.s.i.; oxygen and hydrogen gases flowed at 50 and 20 p.s.i., respectively. The column was packed with polyethylene glycol. Attenuation and range were set so as to maximize sensitivity towards the molecular species under consideration.

5. Mass Spectrometry

The spectrophotometer used was a Finnigan 4000 gas chromatography-mass spectrometer with a model 6000 data system. The emission current and the electron energy were maintained constant at 0.35 mAmp and 35 eV, respectively. The sample was introduced by a direct insertion probe and the spectrum obtained at 280°C. The magnetic field was scanned between 40 and 500 atomic mass units.

Equal volumes (5.0 ml) of approximately equimolar (3.50 x 10^{-2}M) solutions of carbon suboxide and triethylamine in ether were reacted for about 30 min. The supernatant was decanted and the dark

red precipitate, which was sticking on the walls of the reaction vessel, treated with 0.5 ml of methanol. The resulting precipitate was separated, allowed to air dry, and submitted for mass spectral analysis.

III. RESULTS AND DATA TREATMENT

The results of this investigation will be presented in this chapter. In addition, where appropriate, a more detailed description of the experimental procedure leading to a set of data will also be given.

A. Identification and Stability of Carbon Suboxide

A hypothesis had been advanced earlier, that the color-forming ability of malonic acid in this reaction process evidently is a consequence of the formation of carbon suboxide. A preliminary investigation in which a stream of the gas bubbled into an acetic anhydride solution of pyridine or triethylamine to give colored reaction solutions, strengthened this notion. But we are yet to provide the proof of the identity of this gaseous compound. Ultraviolet absorption and nuclear magnetic resonance spectral data will now be presented in support of carbon suboxide as the active substance.

1. UV Absorption Spectrum

The absorption spectrum of a 2.28×10^{-2} molar solution of

carbon suboxide in anhydrous ethyl ether, generated by distillation, obtained on a Varian 2200 UV-VIS spectrophotometer, is shown in Figure 2. The spectrum shows the typical carbonyl absorption at 265 nm, and is in good agreement with the reported ultraviolet absorption spectrum of the molecule (64, 68, 81).

Figure 2 also shows the absorption spectra of 2.00×10^{-3}M malonic acid, of 3.00×10^{-3}M acetic anhydride, and of a blank distillation. These results indicate that neither acetic anhydride nor malonic acid interferes with, or contributes significantly to, the carbonyl absorption peak at 265 nm, which is due to carbon suboxide. The possibility exists, however, that trace amounts of acetic anhydride, which the suboxide gas is in contact with prior to distilling into ethyl ether, may be carried over into the ether solution. From Figure 2, if the wavelength for maximum absorption (λ_{max}) for acetic anhydride in ether is taken to be 220 nm, the peak height ratio for the distillate to the 3.00×10^{-3}M acetic anhydride solution is 1:9. This represents approximately 3.00×10^{-4} moles of the anhydride in the distillate, which we consider not to be of any significance. Nor is there spectral evidence that significant amounts of malonic acid are distilling into the ether.

2. NMR Spectrum

The carbon-13 nuclear magnetic resonance spectrum of carbon suboxide was obtained at $-30^{\circ} \pm 0.1^{\circ}$C on a Bruker AM 500, 125 mHz spectrophotometer, operating in the Fourier Transform (FT) mode with

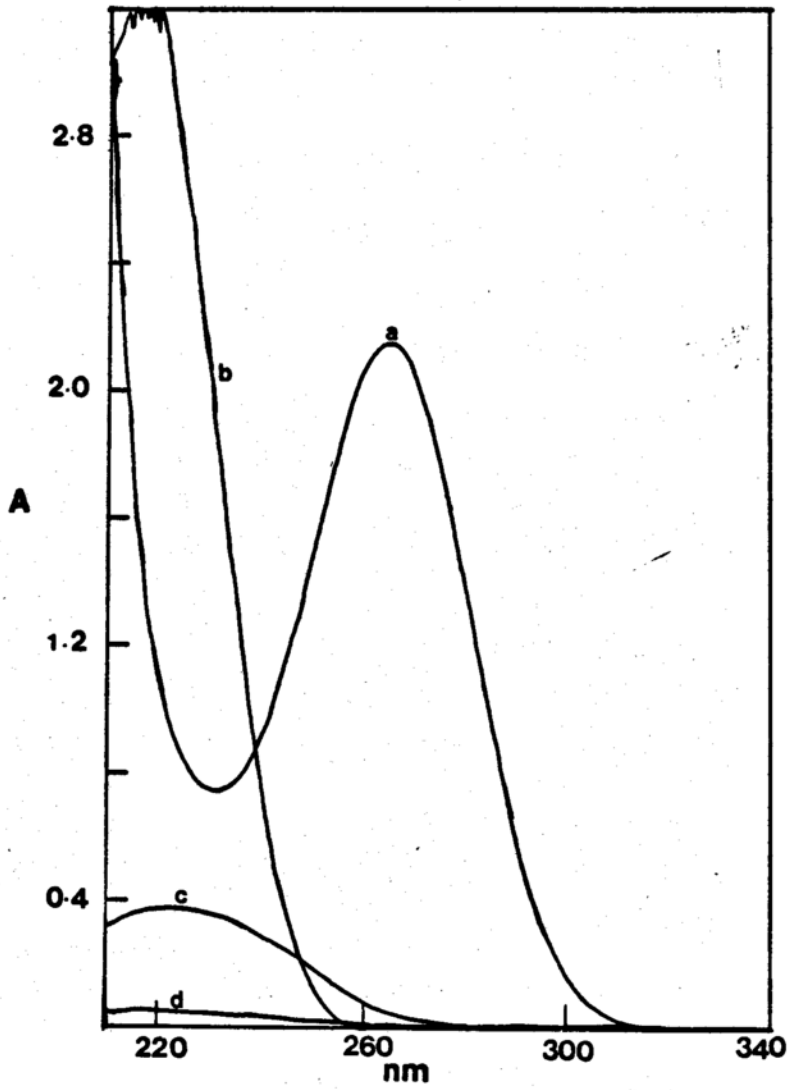


Figure 2. Ultraviolet Absorption Spectra for
a. Carbon Suboxide; b. Malonic Acid;
c. Acetic Anhydride; d. Blank Distillate

deuterated chloroform as reference standard. A paramagnetic relaxation agent, $[Cr(acac)_3]$, was added to shorten the spin-lattice relaxation times of the carbon nuclei. It has been shown (58) that the addition of the paramagnetic relaxation agent does not change carbon suboxide chemical shifts. The ^{13}C nmr spectrum of carbon suboxide is shown in Figure 3.

A remarkably high field resonance was observed for the central carbon atom (-14.93 ppm), which is in good agreement with the value reported by Williams et al. (58). A low frequency infrared bending mode of the central atom (63 cm^{-1}) had previously been observed (82). It was suggested that this arises from a low $C-C_\pi$ bond population, and from a high electron density on the central atom (83), the very high electron density on the central carbon atom, which will be expected on the basis of the mesomeric structure shown in Figure 3, should cause a high field chemical shift. Furthermore, the central carbon atom is flanked by two carbonyl groups, the so-called sp-hybridized carbon atoms, which are known to give rise to a shielding effect at adjacent carbon atoms (84). Although the relative contribution of this shielding effect is small, it nonetheless is such that will usually promote a chemical shift to high field.

The carbonyl chemical shift occurs at 129.24 ppm, close to that of carbon dioxide (124.36 ppm) which appears as an impurity in the spectrum. After this spectrum was obtained, steps were taken to decrease the amount of carbon dioxide impurity, but under our

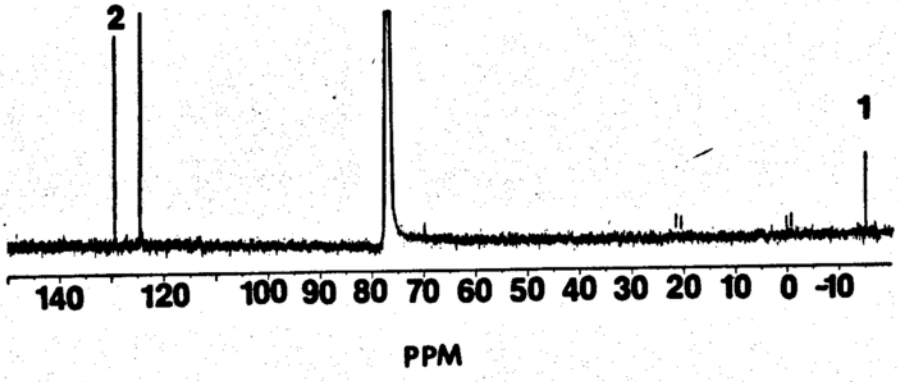


Figure 3. Carbon-13 NMR Spectrum of Carbon Suboxide in $CDCl_3$ at $-30^{\circ}C$.

conditions some carbon dioxide was always present.

3. Stability Profile

The question concerning the possibility of the instability of carbon suboxide in the anhydrous ethyl ether solvent was addressed very early in the investigation. Fifty milliliters of etheral suboxide solution was collected in a tightly covered round-bottom flask. The flask was maintained at $25^{\circ} \pm 0.1^{\circ}C$ throughout the period the stability of the suboxide was being monitored. Carbon suboxide evidently is very stable in ether.

The stability of the etheral suboxide solution was monitored by scanning its ultraviolet absorption spectrum from 340 nm to 210 nm against an ether blank. The appearance of the absorption spectrum as well as the absorbance at the wavelength of maximum absorption (265 nm), and reactivity of the suboxide solution towards pyridine were used as criteria to assess stability. Figure 4 shows the stability profile of carbon suboxide. The overall appearance of the absorption spectrum does not change with time, until after 30 days of storage. There is a gradual decrease of the absorbance at the λ_{max}, which may be a consequence of loss of the suboxide due to evaporation.

The reactivity of the aged solution of carbon suboxide towards pyridine was tested by mixing 2.0 ml of carbon suboxide with 1.0 ml of acetic anhydride, and reacting the resulting solution with 2.40 µl of pyridine (approximately 10^{-2}M final concentration).

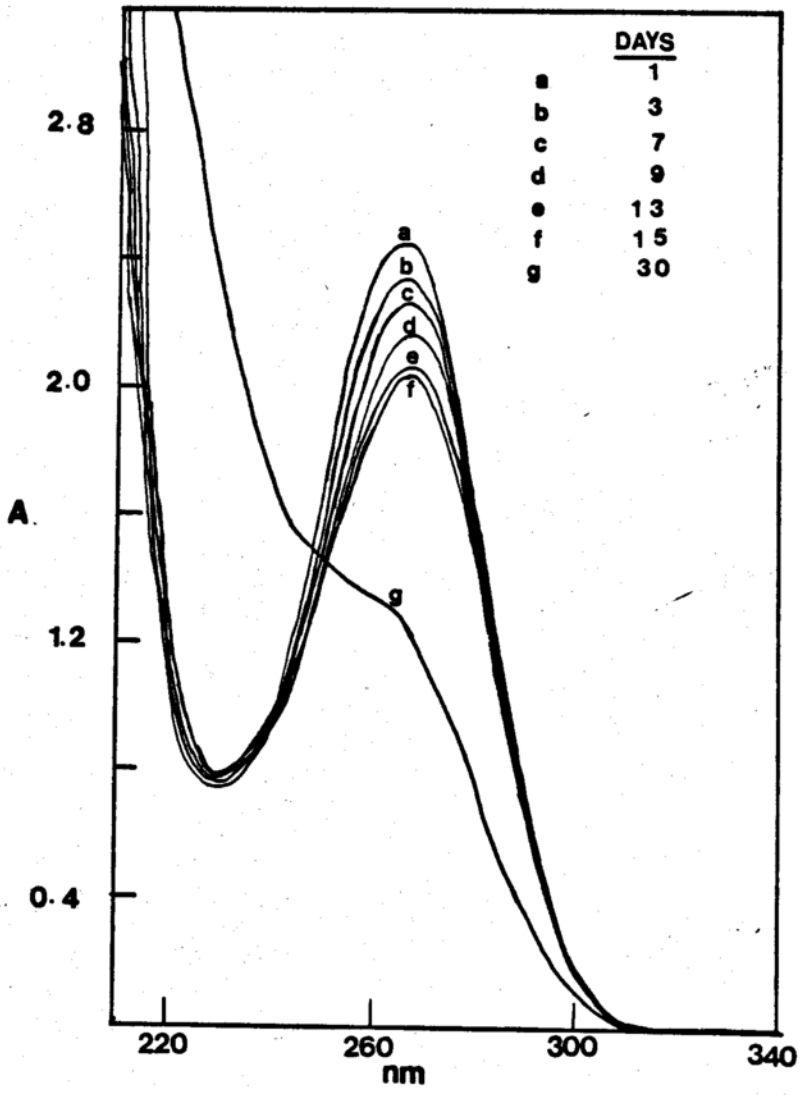


Figure 4. Stability Profile of Carbon Suboxide in Anhydrous Ethyl Ether.

The results are shown in Table IV. Although the lag times are variable, the initial rates appear to be decreasing as a consequence only of the decreasing concentration of carbon suboxide.

Table IV. Reactivity of Aged Ether Solutions of Carbon Suboxide Towards Pyridine.

	Carbon Suboxide	10^{-2}M Pyridine	
Time (Days)	A_{265}	Lag time (sec)[a]	Initial rates[b]
1	2.47	304	22.0×10^{-3}
3	2.37	384	20.4×10^{-3}
7	2.28	316	19.4×10^{-3}
9	2.18	288	19.1×10^{-3}

[a,b] The definition of the lag time and initial rates will be given in Section C.1.

B. Structural Observations

1. Reactivity and Absorption Spectrum of Aged Malonic Acid Solution

This set of experiments was conducted as a preliminary investigation to assess the reactivity of an aged 10% solution of malonic acid in acetic anhydride. Furthermore, we wished to assess if there was a spectral difference between the reaction product of aliphatic and aromatic tertiary amines on the one hand, and of the aged 10% malonic acid solution and the products of reaction on the other. Since a coloration appears upon aging the acid in acetic anhydride, it may be instructive to compare these absorption spectra. Triethylamine was used as a prototype aliphatic amine, while for the aromatic amines, pyridine was used.

Figure 5 shows the ultraviolet-visible absorption spectrum of the reaction solutions for the reaction of 1.50×10^{-3} M triethylamine and 1.60×10^{-3} M pyridine with a one-day old malonic acid reagent solution. The amine solutions were prepared in acetic anhydride. One ml of the amine solution was reacted with 2 ml of the malonic acid reagent solution at room temperature (approximately 25°C). The absorption spectra were recorded 15 to 20 min after mixing the reagents, using acetic anhydride as blank. The spectral tracings indicated a difference between the aliphatic and the aromatic amines tested; the aliphatic amine compound showed a λ_{max} at 302 nm, while the aromatic amine showed a λ_{max} at 336 nm.

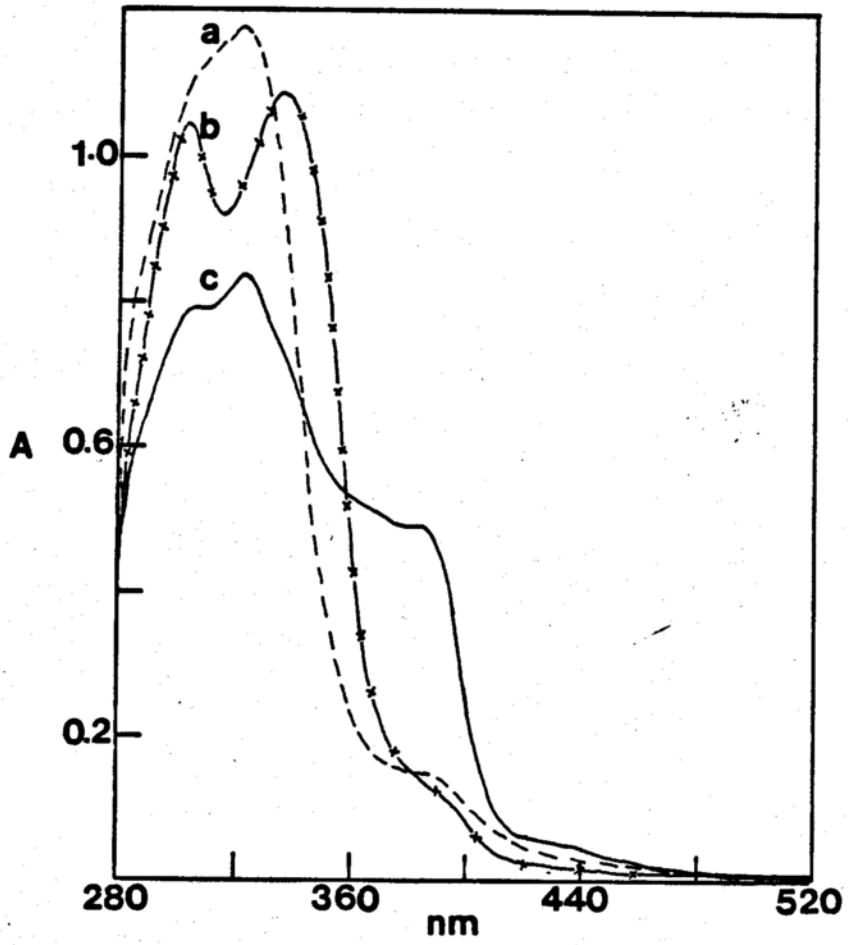


Figure 5. UV-Visible Absorption Spectra for the reaction of a. 1.5 x 10^{-3}M Triethylamine, and of b. 1.60 x 10^{-3}M Pyridine with a one-day old 10% Malonic Acid Reagent Solution. Spectrum c is for the 10% Malonic Acid Solution.

This difference could have been a consequence of the difference in base strength between pyridine, pK = 5.23 (85) and triethylamine, pK = 10.71 (86). But it may also have been due to the nature of the product(s) formed.

The spectrum of the 10% malonic acid reagent solution is also shown in Figure 5. It is different from the spectrum of the reaction product of both triethylamine and of pyridine. The absorbance of the reagent solution continued to increase with the age of the solution. During the first three days, the reagent solution showed a λ_{max} of 322 nm, but as the yellow coloration of the reagent solution intensified, a new λ_{max} appeared at 364 nm. This is perhaps an indication of formation of another intermediate species with absorption properties that are different from the one formed initially. Yet, most investigators who have used the malonic acid-acetic anhydride reagent system seem not to have taken this into account.

The reactivity of the aged malonic acid reagent solution towards 1.60×10^{-3} M acetic anhydride solution of pyridine was also tested, and the results are presented in Table V. A plot of the data from Table V is shown in Figure 6. These results show that the reactivity of the aged reagent solution towards pyridine increased as a function of time. This may, evidently, be the result of the increasing concentration of the reactive intermediate alluded to earlier. The apparent leveling of the absorbance may represent the peak concentration of this reactive intermediate species. From

Table V. Absorbance Readings of the Reaction Solution of 1.60×10^{-3}M Pyridine with Aged Malonic Acid Reagent.[a]

Time (Days)	A_{336}
1	1.08
5	1.44
7	1.84
11	1.96
16	1.92
18	2.04
20	1.84
26	1.98

[a]Two milliliters of aged reagent solution were reacted with 1 ml of the pyridine solution, freshly prepared and equilibrated at $25^{\circ} \pm 0.1^{\circ}$ C for at least 30 min.

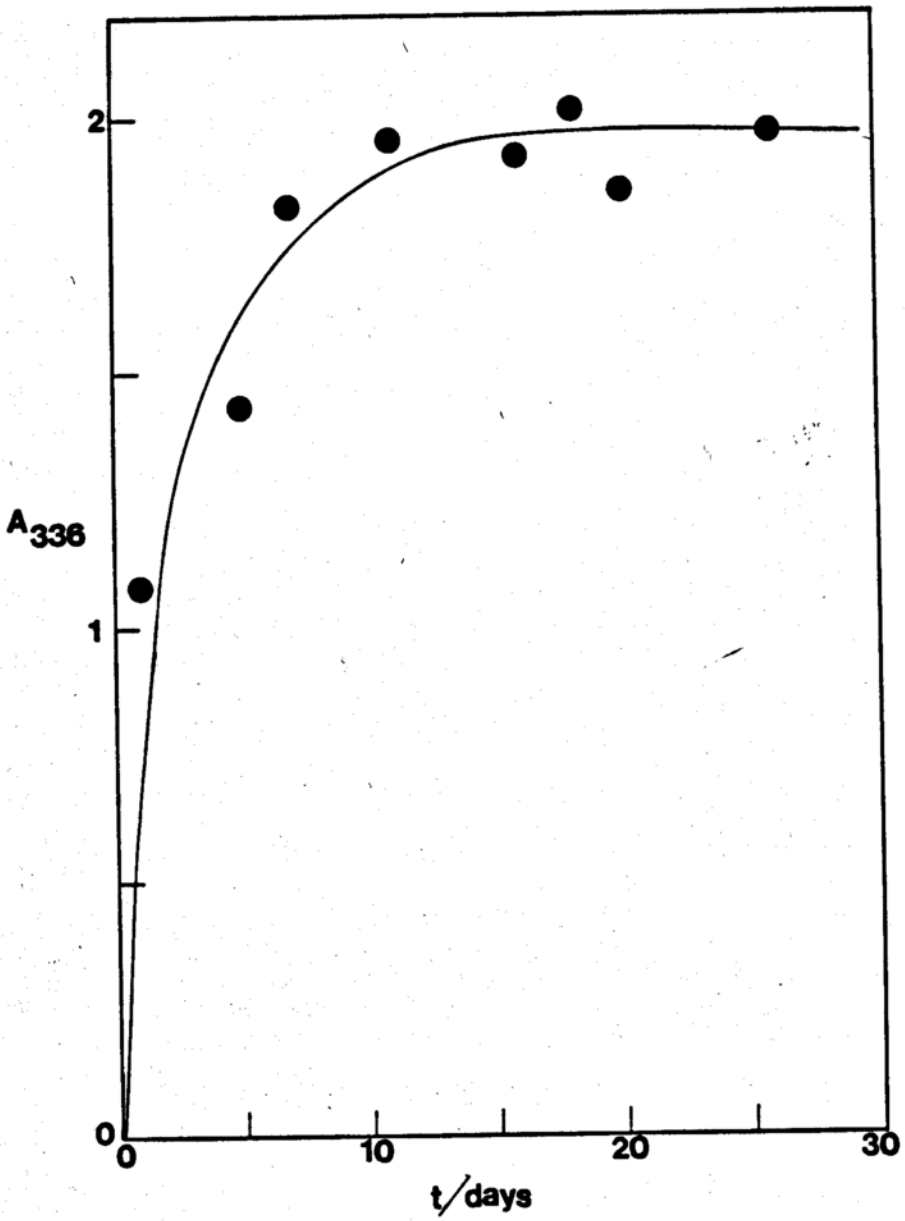


Figure 6. Absorbance versus Time Plot for the Reaction between Pyridine and Aged Malonic Acid Reagent Solution.

Figure 6, it appears that the optimal preaging time for the malonic acid-acetic anhydride reagent solution is between 10 and 15 days.

2. UV-Visible Spectra of Products

The ultraviolet-visible absorption spectra of products for the reaction between carbon suboxide solution and acetic anhydride solutions of some tertiary amine compounds are given in Figures 7 to 13. A summary of the analytical wavelengths, i.e. the wavelength of maximum absorption (λ_{max}), and the wavelengths at which shoulder peaks appear for the various amine compounds, is given in Table VI.

In all cases, 2 ml of carbon suboxide solution was reacted with 1 ml of the appropriate concentration (usually 10^{-2} to 10^{-3} M) of the acetic anhydride solution of the amine compound. The spectrum was read 10 to 20 min after mixing the reagents. Where necessary, dilutions were made in anhydrous ethyl ether prior to spectrophotometric measurements. The measurements were performed on a Varian 2200 UV-VIS spectrophotometer using ethyl ether as blank.

During the course of the investigation of the lag time phenomenon, which will be presented in section C.2 of this chapter, it was observed that ether solutions of aliphatic amine compounds interacted with carbon suboxide, in the absence of acetic anhydride, leading to formation of coloration in the reaction mixture. Depending on the concentration of aliphatic tertiary amine used, and the length of time reaction was allowed to proceed, a dark red, oily precipitate coated the walls of the reaction vessel. The

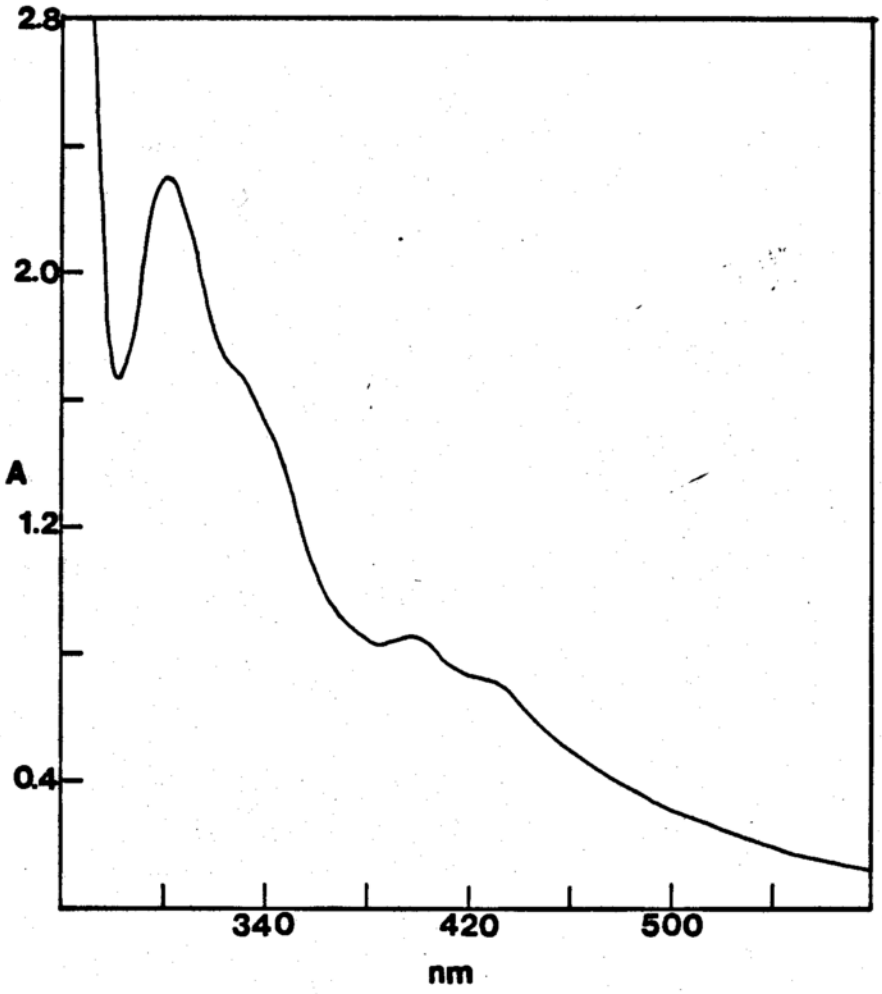


Figure 7. UV-visible Absorption Spectrum of Reaction Product Using Triethylamine.

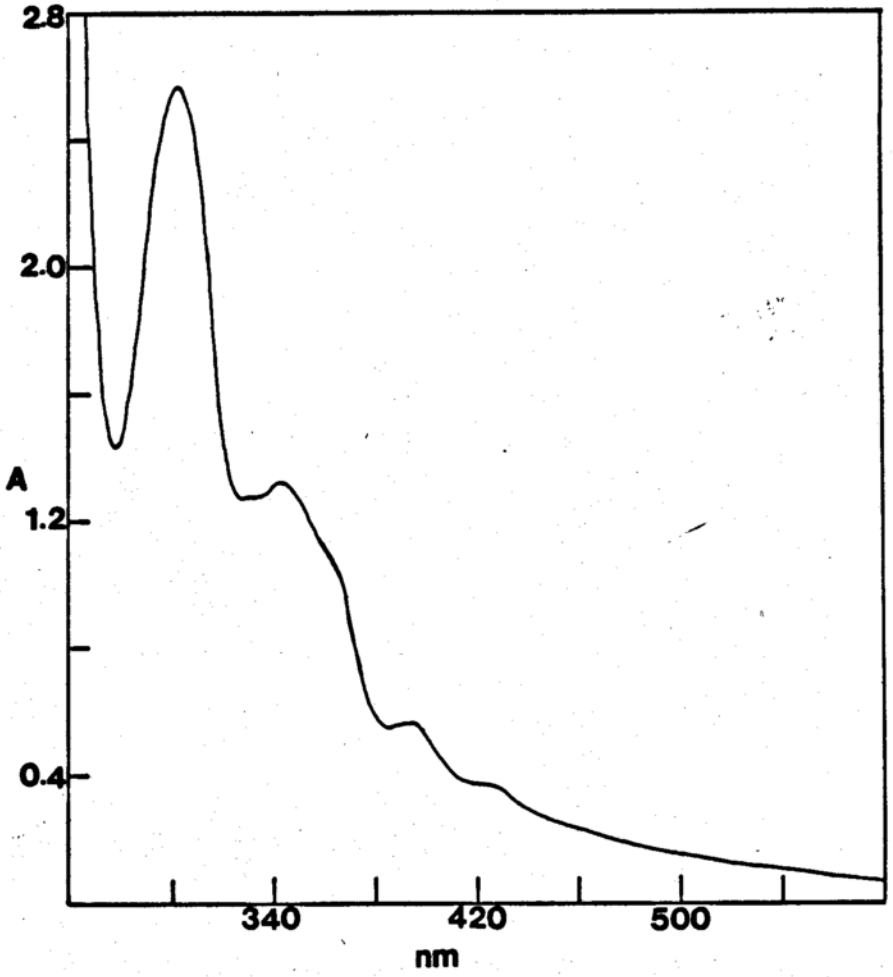


Figure 8. UV-visible Absorption Spectrum of Product Using Allyldimethylamine.

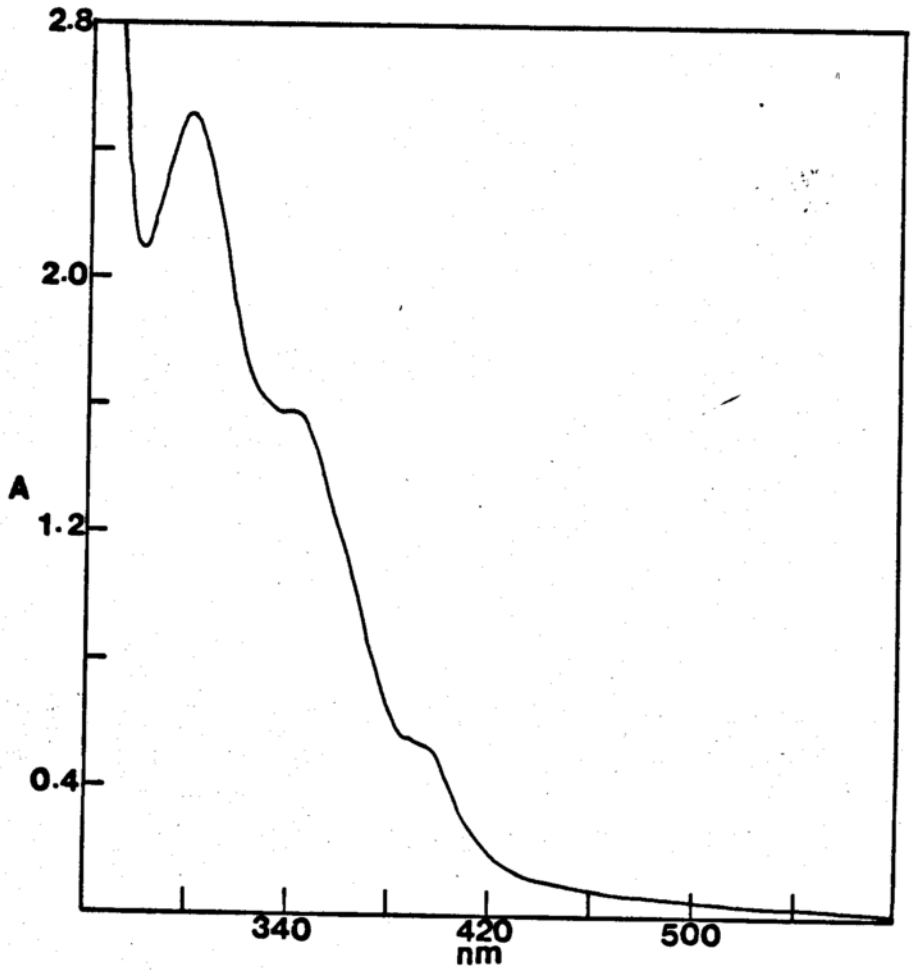


Figure 9. UV-visible Absorption Spectrum of Reaction Product Using Pyridine.

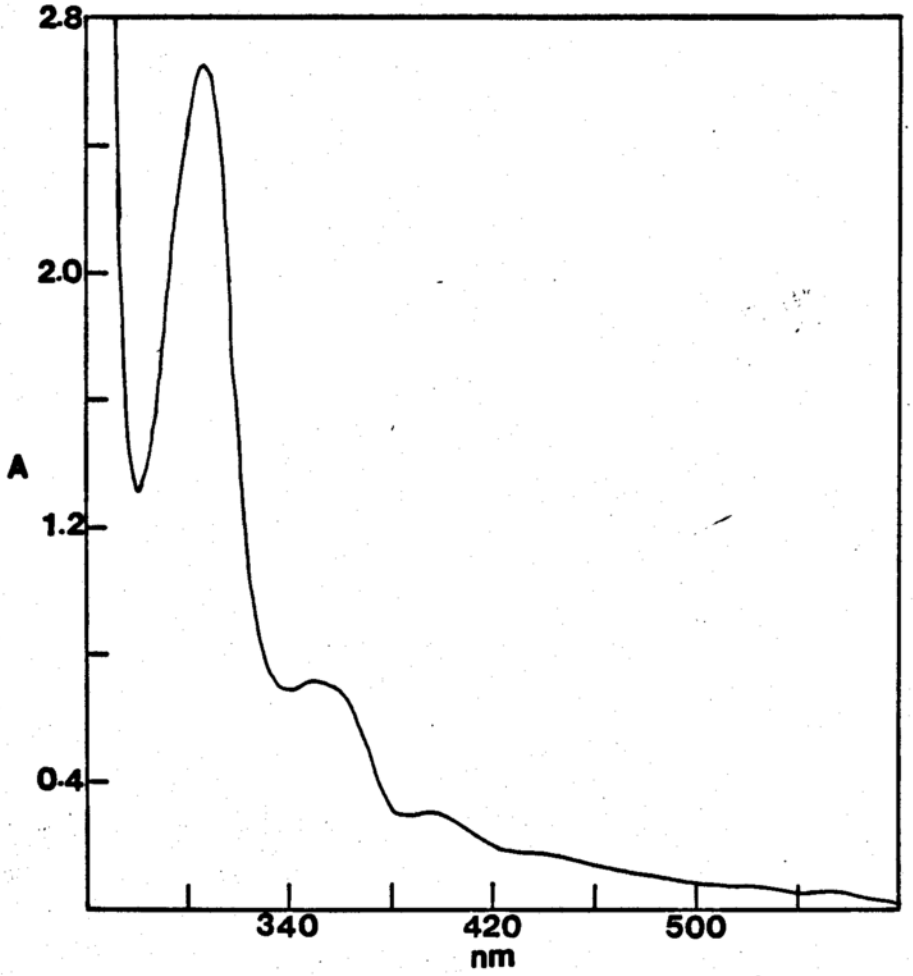


Figure 10. UV-visible Absorption Spectrum of Reaction Product Using 3,4-Lutidine.

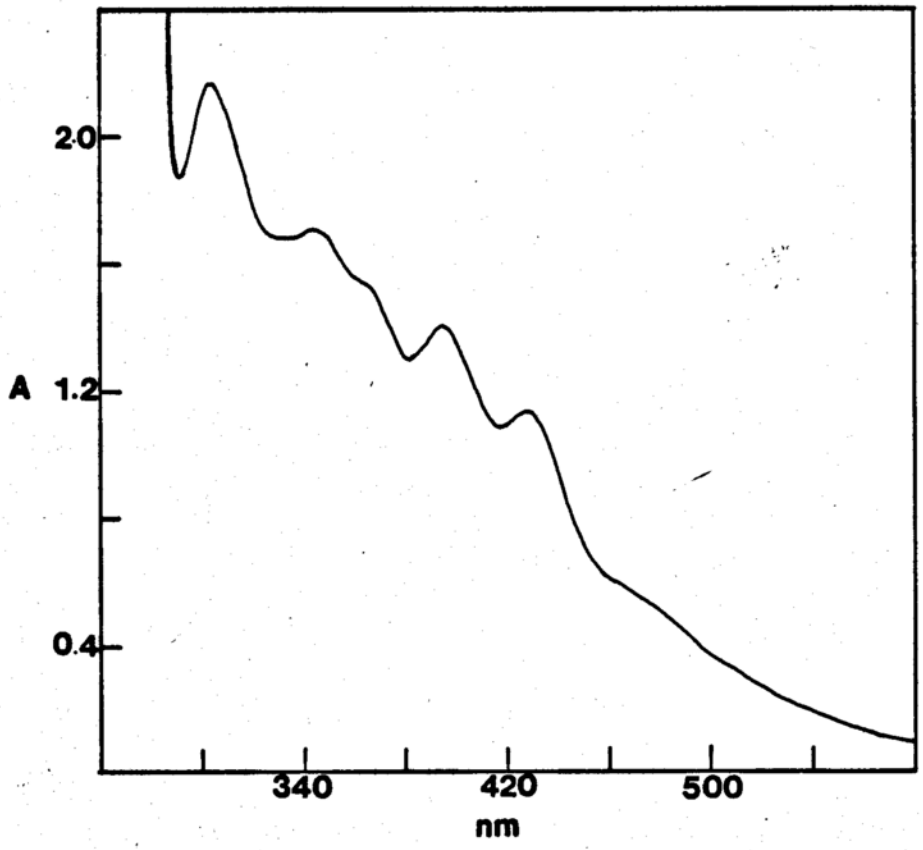


Figure 11. UV-visible Absorption Spectrum of Reaction Product Using 2,4,6-Collidine. Color development in this reaction system took approximately 60 min to become visually perceptible.

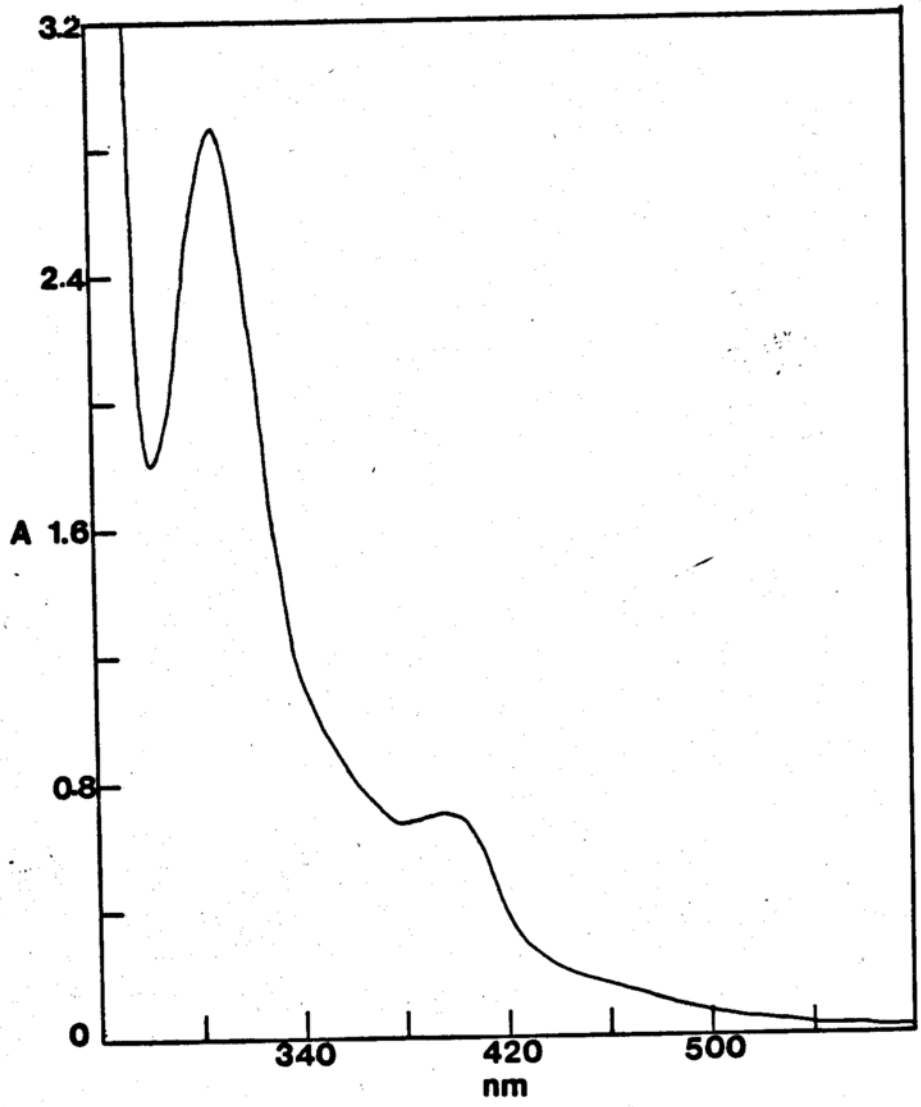


Figure 12. UV-visible Absorption Spectrum of Reaction Product Using 4-dimethylaminopyridine.

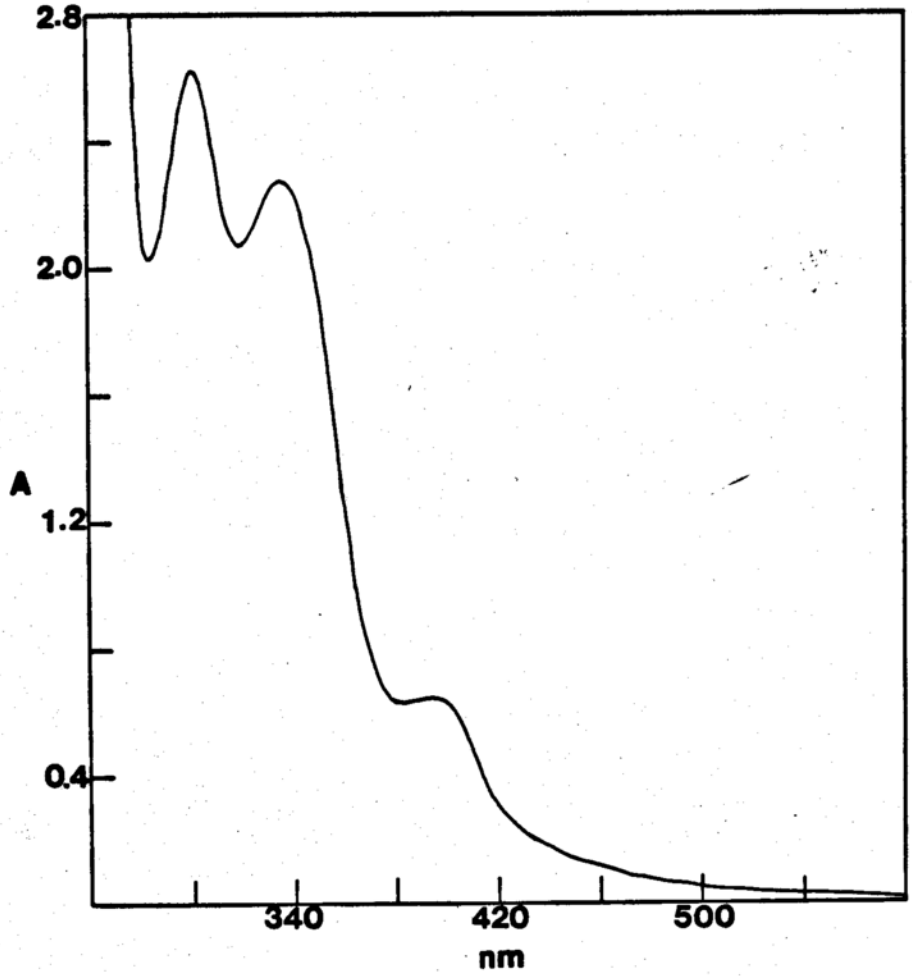


Figure 13. UV-visible Absorption Spectrum of Reaction Product Using N-methylimidazole.

Table VI. Summary of Analytical Wavelengths (λ_{max}) and Shoulder Peaks of Reaction Products

Compound	λ_{max} (nm)	Shoulder Peaks (nm)
Triethylamine	305	328, 396, 428
n-Tripropylamine	303	334, 392
n-Tributylamine	306	338, 394
Allyldimethylamine	304	345, 395, 425
1-Dimethylamino-2-propyne	301	338, 390, 430
Pyridine	300	340, 392
3-Picoline	300	344, 388
4-Picoline	303	344, 392
2,4-Lutidine	300	338, 384, 428
2,6-Lutidine	300	336, 392
3,4-Lutidine	306	348, 392
2,4,6-Collidine	303	344, 396, 428
N,N-Dimethylaniline	299	
4-Dimethylaminopyridine	307	396
N-Methylimidazole	301	336, 396

ultraviolet-visible spectra of reaction products obtained using triethylamine, n-tripropylamine, and n-tributylamine showed λ_{max} at approximately 305 nm with a shoulder at about 345 nm. The etheral supernatant of these reaction solutions exhibited similar ultraviolet-visible spectral properties to the reaction solution. Figure 14 shows the uv-visible spectra of the supernatants from reactions of triethylamine (TEA), n-tripropylamine (TPA), and n-tributylamine (TBA) with carbon suboxide. Five ml aliquots of a 2.50×10^{-2}M carbon suboxide solution were treated with sufficient quantity of an amine to give a 10^{-2}M final solution in the amine in the reaction vessel. The reaction was allowed to progress at approximately 25°C for 30 min. The supernatants were decanted and diluted 1 ml to 25 ml with anhydrous ethyl ether, and the spectra scanned against an ether blank.

It may be instructive to compare the absorption intensities obtained from the supernatant of these three aliphatic amines. The intensity of coloration and, therefore, of absorbance of the supernatant, increased in the order TBA > TPA > TEA. This may have a qualitative bearing on the solubility, or the hydrophobic properties of the reaction products. Since the aliphatic tertiary amines used in these reactions, and especially in such low concentrations, do not themselves elicit a uv-visible spectrum, we attribute these spectra to the reaction products. It appears then that the apparent increase in hydrophobicity of the products possibly arises as a result of incorporation of the amine compound

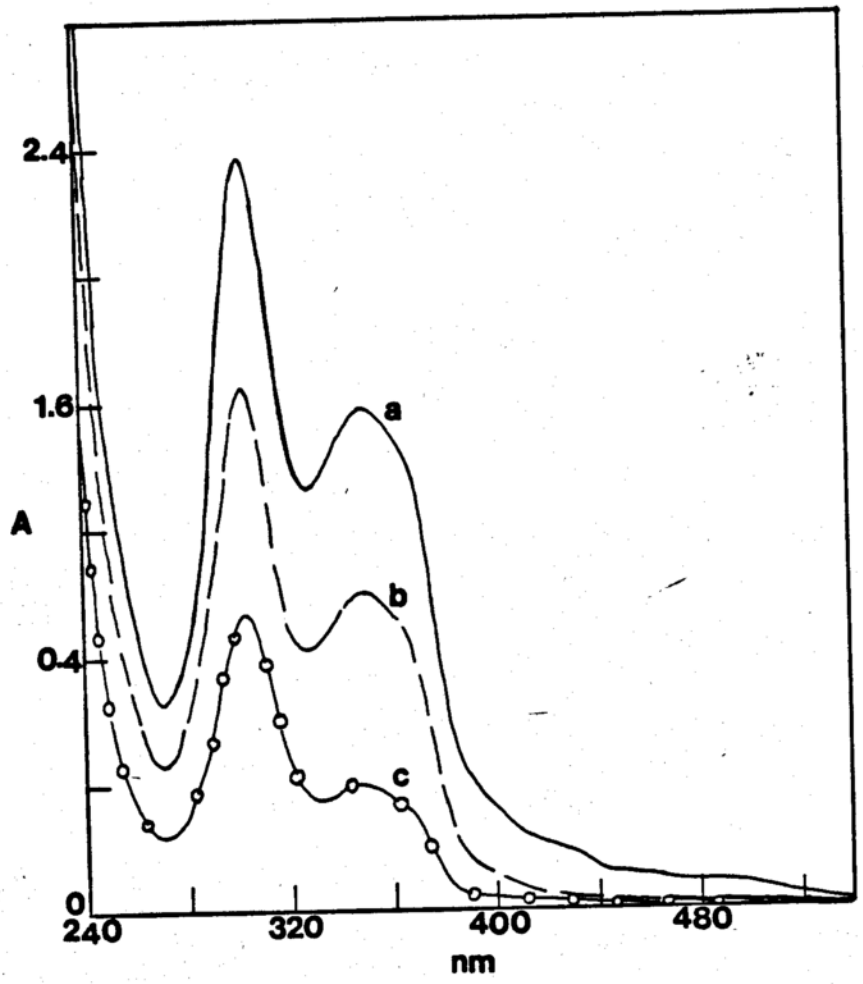


Figure 14. Ultraviolet-visible Absorption of Ether Supernatants of Reaction between Carbon Suboxide and a. n-tributylamine; b. n-tripropylamine; c. triethylamine.

into the final product. The increasing chain length of the aliphatic tertiary amine may be responsible for the enhanced ether solubility.

The elimination of acetic anhydride from the reaction mixture also provided an excellent opportunity for us to monitor, by means of absorption spectroscopy, the disappearance rate of carbon suboxide. Figure 15 depicts the spectral changes associated with the reaction of 1.50 ml of a 2.00×10^{-3}M etheral triethylamine with 1.50 ml of a 2.59×10^{-2}M carbon suboxide solution. There is a gradual decrease of the peak at 265 nm, which corresponds to the carbonyl absorption of carbon suboxide. This is followed by the appearance of coloration in the reaction mixture, and an increase in absorbance. During this period of increasing absorbance, there is an initial strong absorbance at about 260 nm, which is later surpassed by a stronger absorbance at approximately 305 nm. It was observed that as the absorbance increased further there was a slight hypsochromic shift of the peaks at 260 and 305 nm. A similar spectral behavior is observed when n-tripropylamine is used in place of triethylamine; see Figure 16. The strong absorbance at 260 nm, which is later surpassed by an even stronger absorbance at 305 nm, may be the result of the sequential appearance of two reaction products.

In light of these findings, we investigated the interaction of ether solutions of pyridine (10^{-3}M) with carbon suboxide (2.59×10^{-2}M). No spectral changes such as were observed with

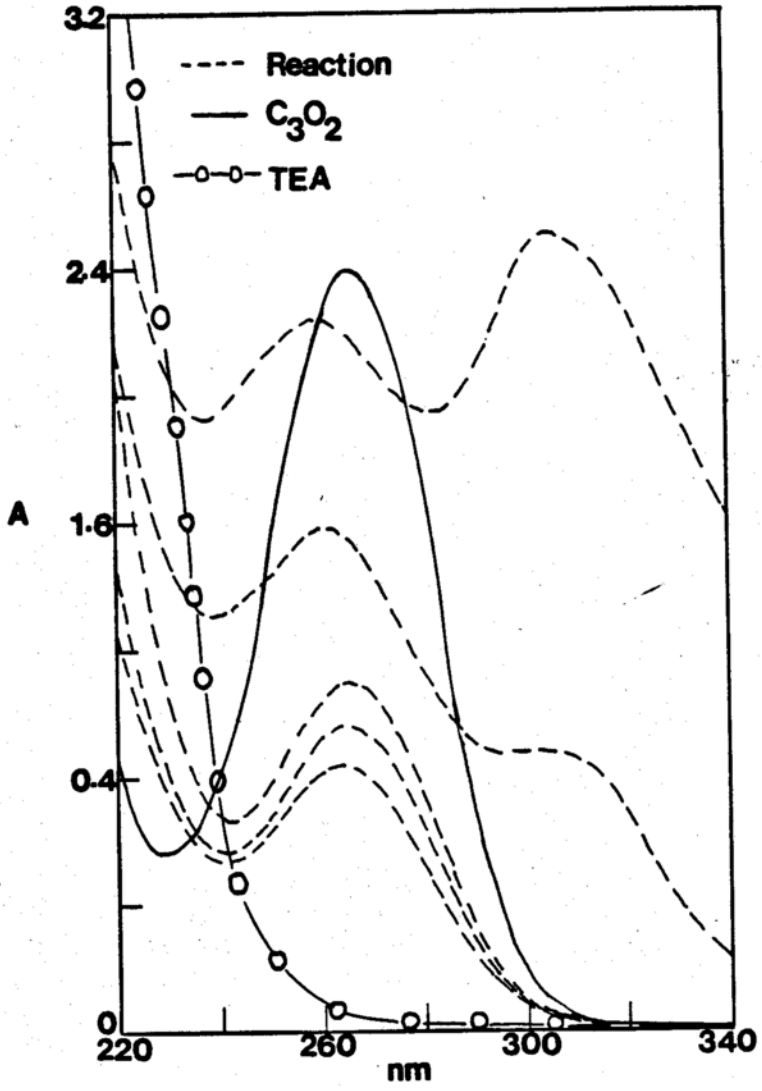


Figure 15. Time-dependence Spectral Behavior of Reaction between Carbon Suboxide and Triethylamine.

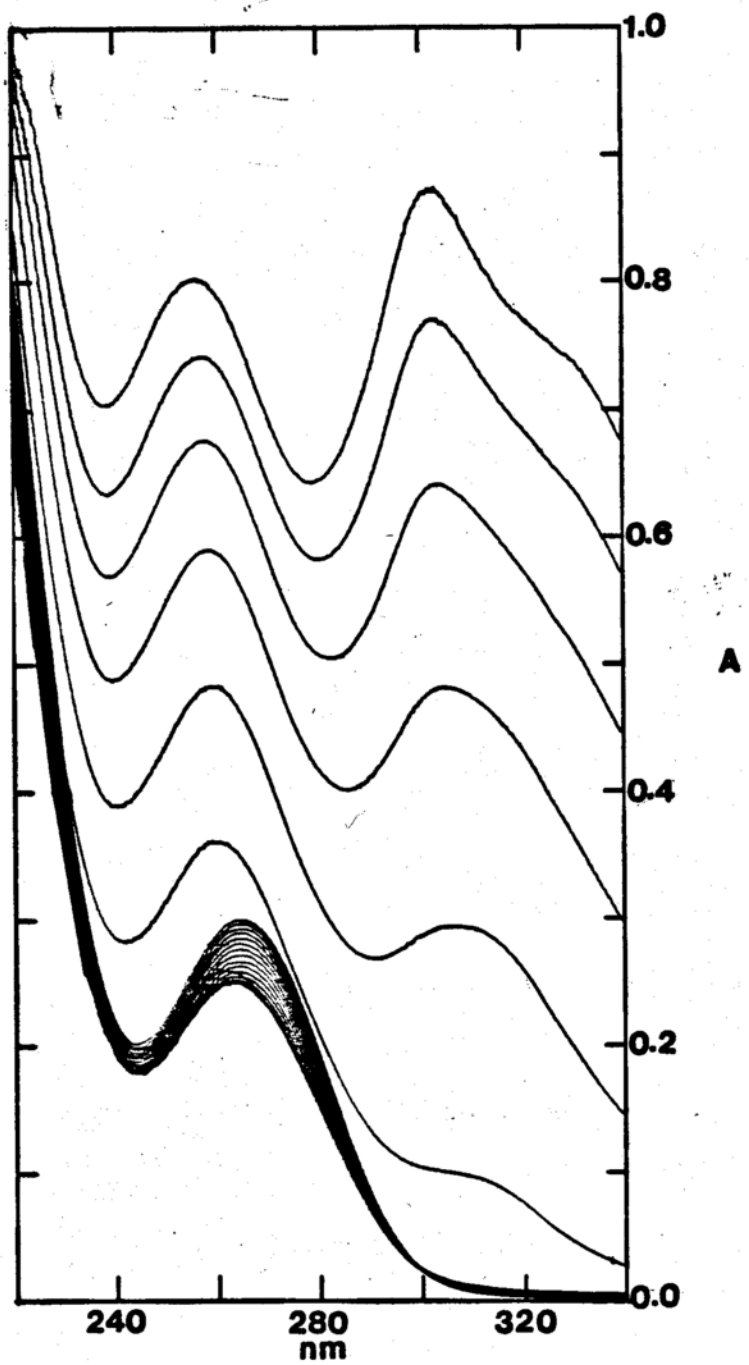


Figure 16. Time-dependence Spectral Behavior of Reaction between Carbon Suboxide and n-tripropylamine.

triethylamine-, or n-tripropylamine-carbon suboxide were seen. Results of the attempted investigation of the difference spectrum of the interaction between pyridine and carbon suboxide were inconclusive.

Figure 17 shows the spectral behavior of the carbon suboxide-triethylamine-acetic anhydride reaction system. Triethylamine and acetic anhydride were constituted in anhydrous ethyl ether such that their final concentrations were 2.00×10^{-3}M and 3.00×10^{-2}M, respectively. The ultraviolet absorption spectral behavior of the reaction mixture, consisting of 1.5 ml each of ether solutions of carbon suboxide, and of triethylamine-acetic anhydride, was monitored as a function of time. Again, the spectral behavior shows an initial decrease of absorption followed by a strong absorption. The actual shapes of the absorption spectra at wavelengths below 260 nm are obscured by the absorption of acetic anhydride, which has a uv-cut-off at approximately 260 nm. But the peak at 305 nm is still present. The amount of acetic anhydride used here is approximately 1/300th the usual concentration of acetic anhydride for reaction systems employing acetic anhydride.

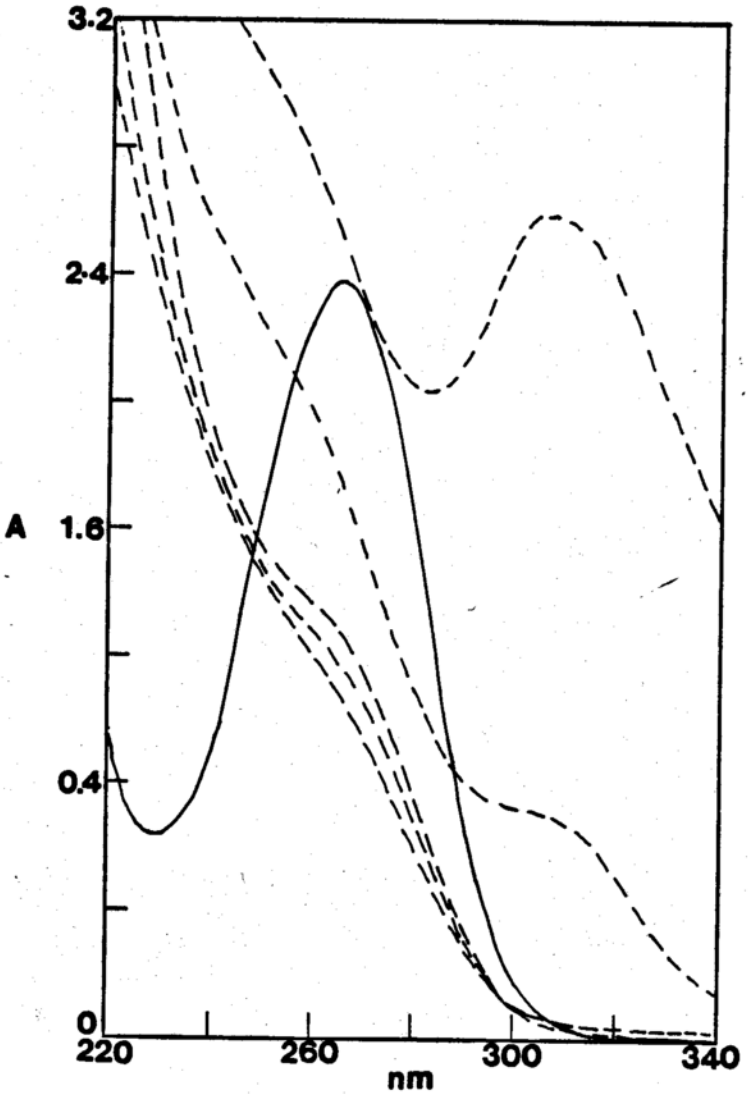


Figure 17. Time-dependence Spectral Behavior of Reaction between Carbon Suboxide and an Ether Solution of Triethylamine-acetic Anhydride.

3. Extractability and HPLC of Products

The absorption spectral properties of the aqueous extract of reaction solutions for some of the systems studied were investigated. Figure 18 shows the spectra of the reaction solutions prior to aqueous extraction, while Figure 19 shows the absorption spectra of the aqueous extracts.

One ml solutions of the amine compound in either acetic anhydride or anhydrous diethyl ether were reacted with 2 ml aliquots of a 2.45×10^{-2}M carbon suboxide solution. The reaction was allowed to progress at approximately 25°C for about 90 min in tightly closed 10 ml volumetric flasks. Triply distilled, deionized water was then added to bring the volume to 10 ml and the contents thoroughly mixed. When the two phases settled, much of the coloration had partitioned into the aqueous phase, which was separated and diluted 1 to 20 ml before recording the absorption spectrum.

In all the systems studied, there was a bathochromic shift of the aqueous spectra; this was gradual and appeared to reach an apparent steady state after approximately 30 min. The overall shapes of the spectra did not appear to change during the spectral shift. If the shapes were to have changed, that could have been linked to either degradation or other transformation of the product(s) in the aqueous medium. In the pyridine-carbon suboxide-acetic anhydride system, a peak appeared in the aqueous

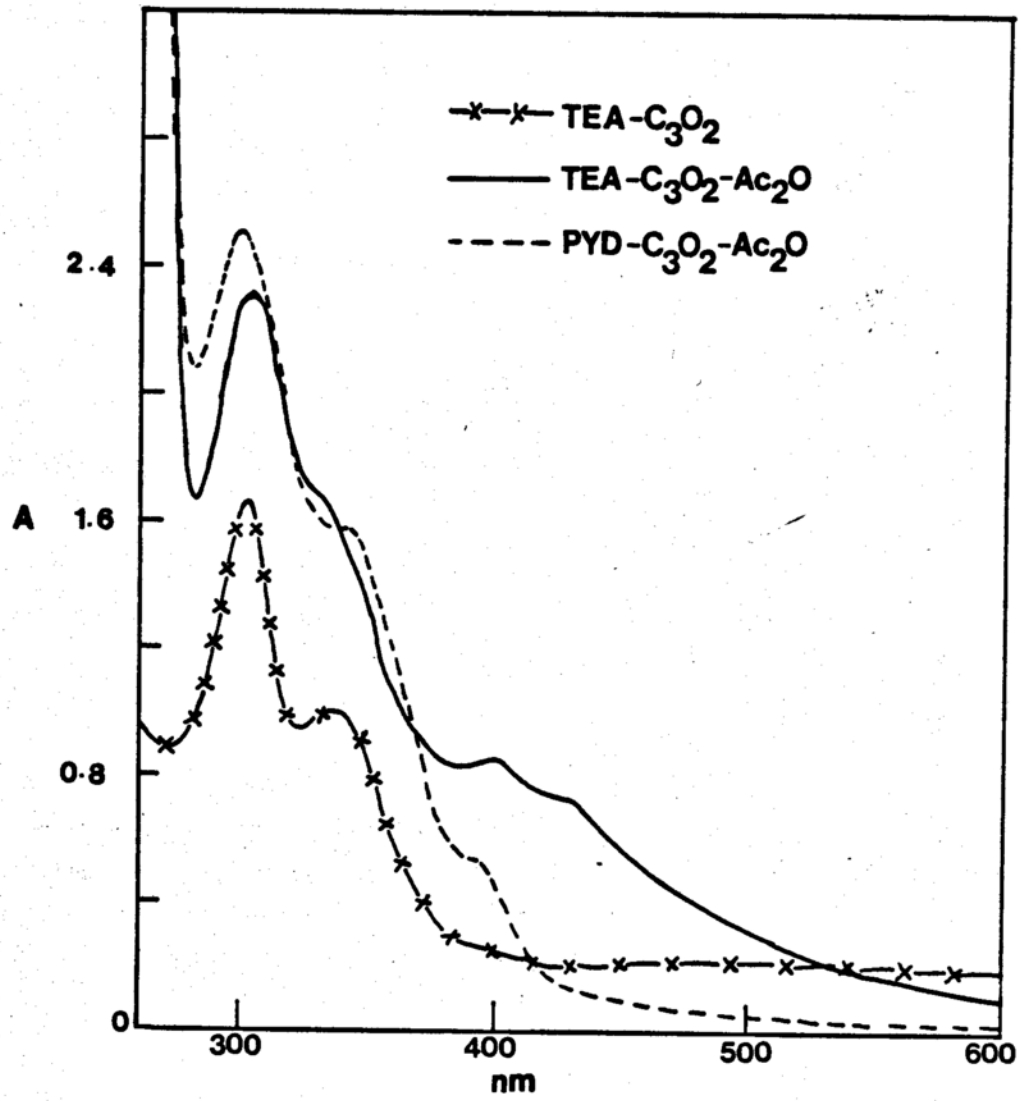


Figure 18. Absorption Spectra of Reaction Products.

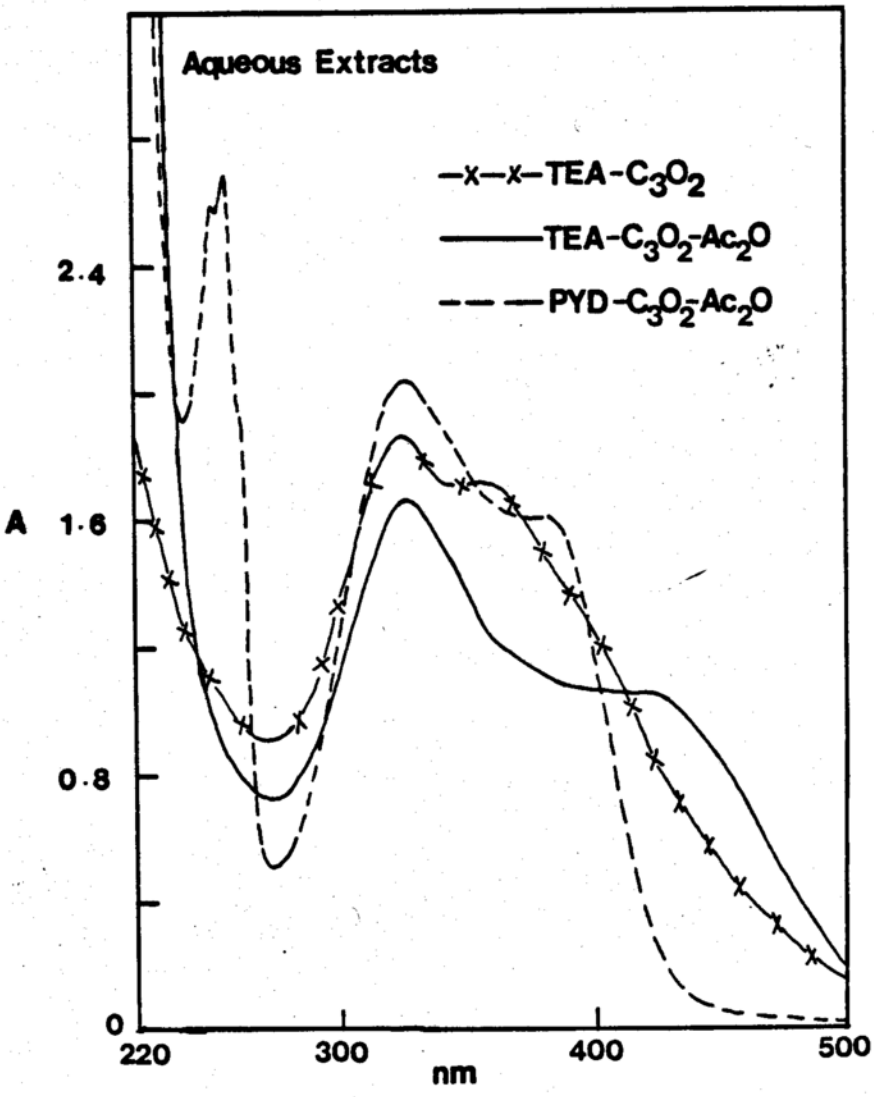


Figure 19. Absorption Spectra of Aqueous Extracts of Reaction Products.

extract at about 251 nm. Independent spectral evidence suggested the peak might be due to pyridine. A mass balance was not performed on the pyridine peak from the aqueous extract.

Spectral characterization of aqueous and methanol solutions of the precipitate formed in the reaction between triethylamine and carbon suboxide are shown in Figure 20. Spectral shift in the methanol solvent is less than in the aqueous solvent. It was observed also that the precipitate was less readily soluble in methanol when compared with its solubility in water. The observed spectral characteristics as well as the solubility behavior of the precipitate in these two solvents may be governed by the polarity of the solvents and of the reaction product(s).

The aqueous extracts were treated with 2 to 3 drops of either concentrated sulfuric acid, or a saturated solution of sodium hydroxide. Within the 30-minute period of spectral observation, acidification did not appear to change the spectral behavior of the products. However, alkalination led in each case to a gradual diminishing of the shoulder, which, in all of the absorption spectra, appeared in the visible region of the spectrum. But the absorbance of the peaks in the ultraviolet region appeared to remain stable.

Figure 21 is a comparison between the ultraviolet-visible absorption spectra of a reaction solution, of its acetonitrile-water solution, and of a high performance liquid chromatography (HPLC)-generated spectral tracing for the reaction of acetic

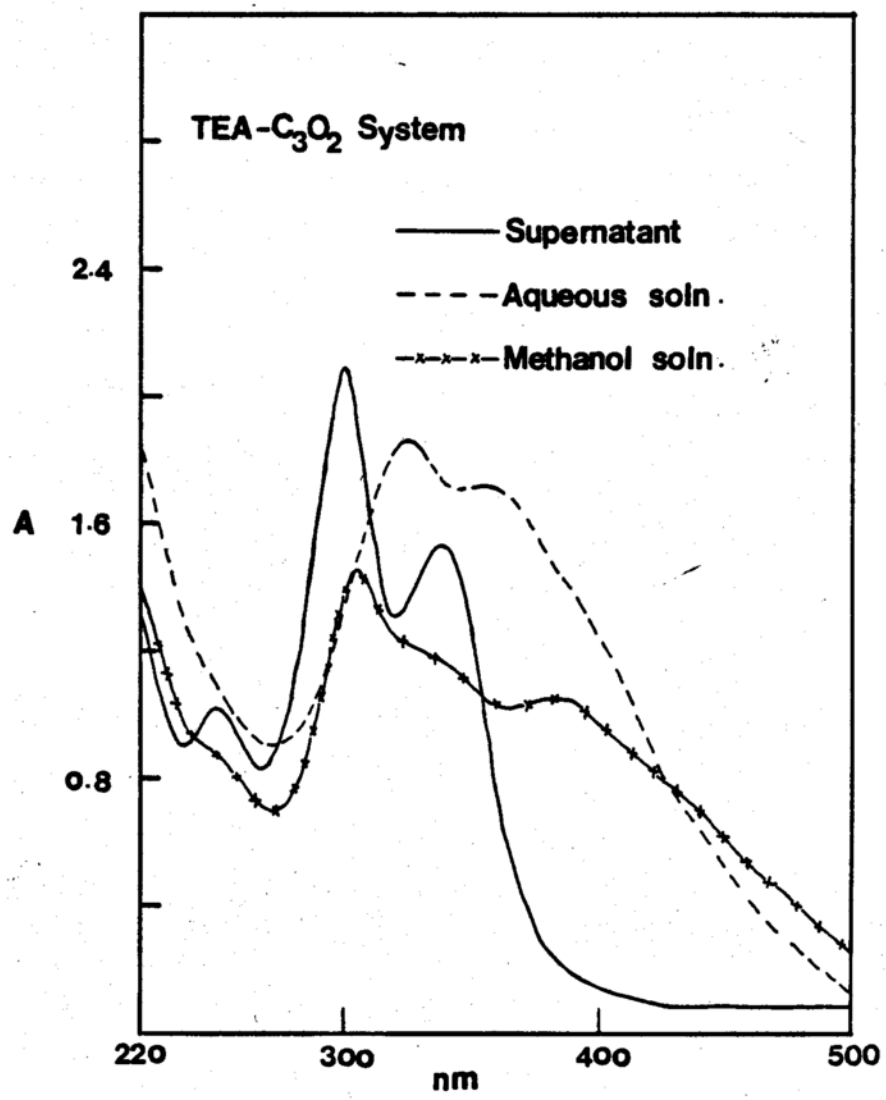


Figure 20. Methanol and Aqueous Extracts of Precipitate of the Reaction Between Triethylamine and Carbon Suboxide.

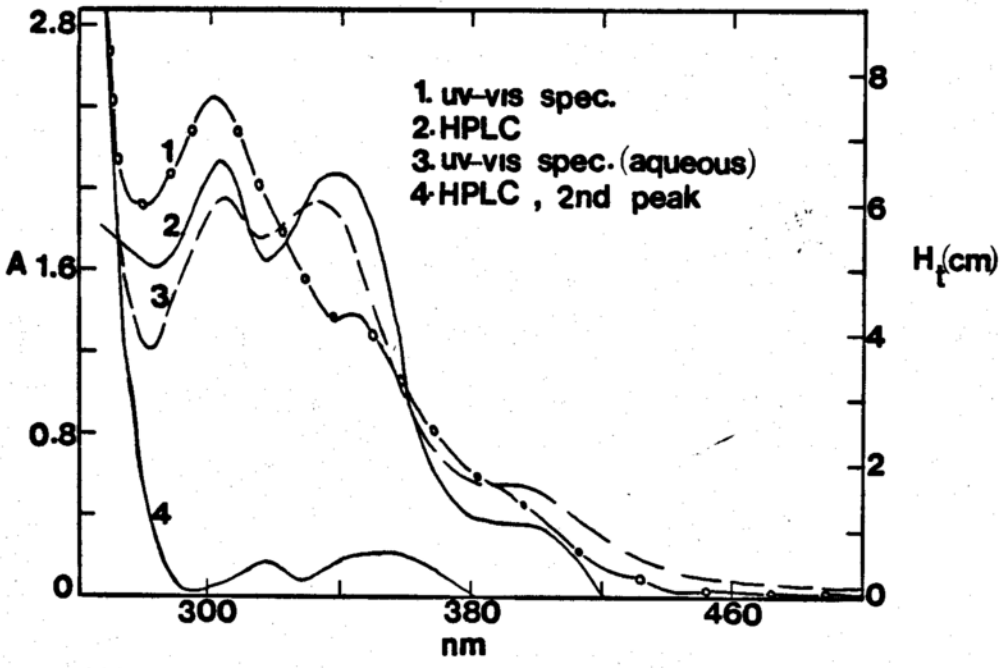


Figure 21. Comparison of Ultraviolet-Visible Absorption Spectra of Reaction Solution and Acetonitrile-Water (Aqueous) Solution with HPLC-generated Spectrum for the Reaction Between Acetic Anhydride Solutions of Pyridine and Carbon Suboxide.

anhydride solutions of pyridine with carbon suboxide. The absorption spectrum of the reaction mixture was obtained in the usual manner. The absorption spectrum of the acetonitrile-water solution was obtained by diluting the reaction mixture 1 to 10 ml with a 50% v/v acetonitrile in water, and recording the spectrum against an acetonitrile-water blank on a Varian 2200 UV-VIS spectrophotometer. In the HPLC experiment, peak heights (H_t) were obtained as a function of wavelength. For each wavelength a reaction system was set up and allowed to proceed at room temperature for approximately 30 min. One to 10 ml dilutions were made in the acetonitrile-water mobile phase before injecting into the HPLC. In order to minimize the residency time of the reaction product on the column, the flow rate (2.0 ml per min) and, therefore, the pressure (1000 p.s.i.) were increased. The good spectral correlation between direct ultraviolet-visible spectrophotometry and the HPLC result indicates a similarity in the absorbing species.

HPLC of reaction solutions were undertaken, and representative chromatograms are shown in Figures 22 to 25. One ml of acetic anhydride solutions of pyridine (10^{-2} ml), 3,4-lutidine (10^{-2}M), triethylamine (10^{-3}M), and n-tributylamine (10^{-3}M) were reacted with 2-ml aliquots of carbon suboxide (2.53×10^{-2}M) for approximately 90 min. A blank reaction was constituted with 2 ml of carbon suboxide and 1 ml of acetic anhydride. Reaction solutions were diluted 1 to 5 ml in the acetonitrile-water mobile phase; the mixtures were

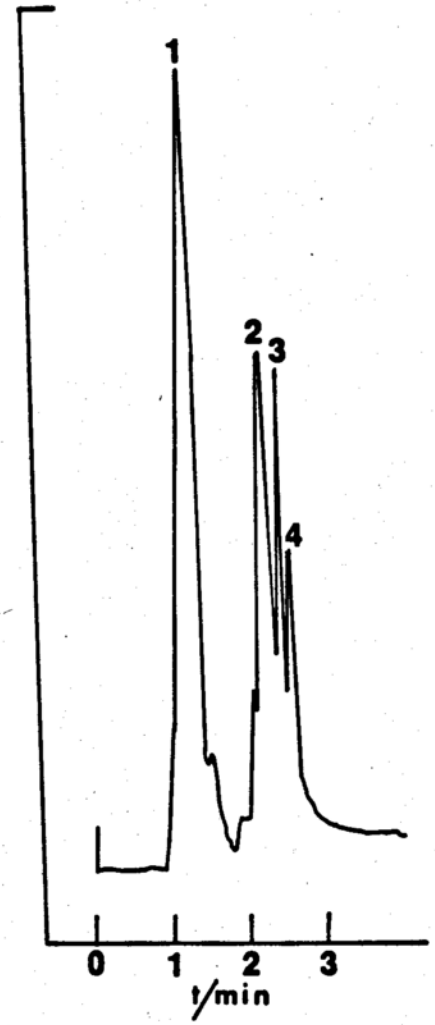


Figure 22. HPLC Chromatogram of Reaction Mixture Using Pyridine.

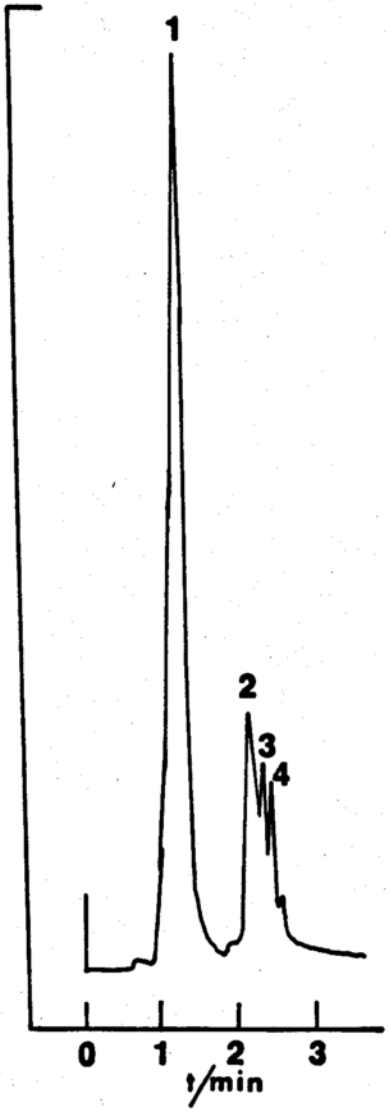


Figure 23. HPLC Chromatogram of Reaction Mixture Using 3,4-Lutidine.

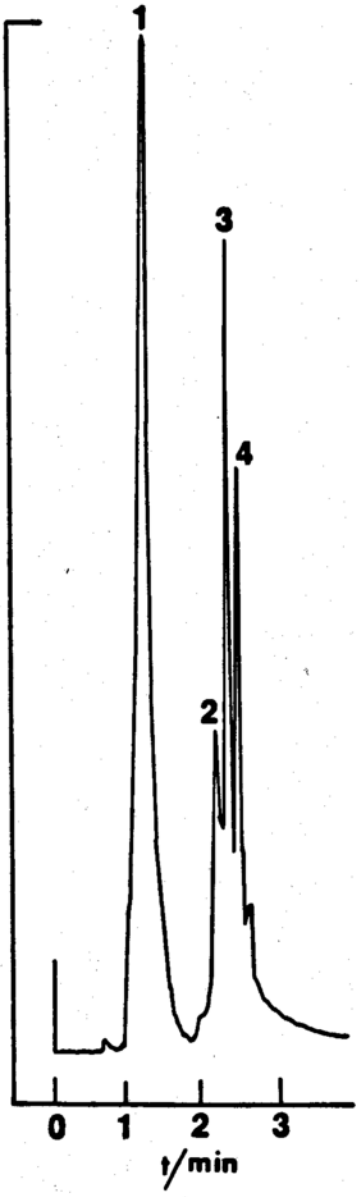


Figure 24. HPLC Chromatogram of Reaction Mixture Using Triethylamine.

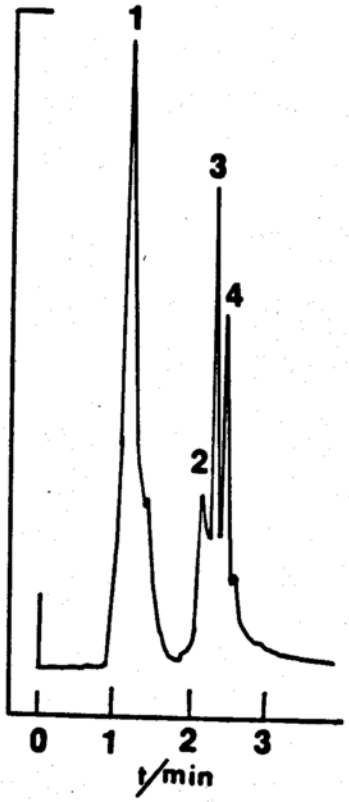


Figure 25. HPLC Chromatogram of Reaction Mixture Using n-tributylamine.

injected into the HPLC less than 60 sec after the dilution. No peaks were observed with the blank at the analytical wavelengths, which were 300 nm for the pyridine system, and 305 nm for 3,4-lutidine, triethylamine, and n-tributylamine systems.

Table VII gives a listing of the retention times of the peaks from the four reaction systems considered. The retention times appear to be the same for all corresponding peaks, but we hesitate to attempt to derive any meaningful conclusions from that fact. A comparison of the chromatograms indicates that for the aliphatic tertiary amine compounds, the intensities of the peaks were 1 > 3 > 4 > 2; for the aromatic amines, however, it was 1 > 2 > 3 > 4. The chromatograms are representative of systems that had been reacting for between 80 and 90 min. In the course of the kinetic investigation of the pyridine reaction system, it was observed that the chromatograms varied as a function of time; a fifth peak was observed beyond the 90-minute time point. The 'analytical peak', i.e. peak number 1 was first to appear after the lag time phase. This was followed by peaks 2, 3, and 4 in that order. But whether the emergence of peaks 2, 3, 4, and 5 was the result of formation of new products or the degradation of the so-called analytical peak, is not known. Preparative HPLC will be needed to test the purity and to establish the identity of these eluted species.

Thin layer chromatography (TLC) of the reaction mixture of acetic anhydride solutions of pyridine and of triethylamine on

Table VII. Retention Times of Eluents of Reaction Mixtures of Acetic Anhydride Solutions of Triethylamine (TEA), n-tributylamine (TBA), Pyridine (PYR), and 3,4-lutidine (3,4-LUT) with Carbon Suboxide Solution.

	Retention Time (t_R)/min			
Peak No.	TEA	TBA	PYR	3,4-LUT
1	1.16	1.13	1.27	1.30
2	2.10	2.06	2.23	2.17
3	2.26	2.23	2.47	2.33
4	2.40	2.40	2.60	2.43

silica gel plates, using 30% v/v methanol in chloroform solvent, revealed a single spot in both cases. This is a normal-phase separation technique; the lack of separation on the TLC plates may be a consequence of the limitation of the technique.

4. Mass Spectrometry of Product

Figure 26 shows the mass spectrum of the product of the reaction between triethylamine and carbon suboxide solution. The mass spectrum was obtained using a Finnigan 4000 gas chromatography-mass spectrometer with a model 6000 data system; the electron impact process was utilized for ion sampling. The conditions under which the spectrum was obtained, as well as how the sample was prepared are outlined in section B.5, Chapter II. A listing of the relative intensities of mass ions, and the possible empirical formulas and ion structures, is presented in Table VIII.

The ion structures proposed on the basis of the mass spectral data are very reasonable. There is an ambiguity in the presence of methanol, which could have led to its incorporation into the product. There is uncertainty concerning whether the fragmentation pattern is that of a single compound; HPLC data indicated there could be more than one product. A detailed interpretation and discussion of the mass spectrum will, however, be given in Chapter IV.

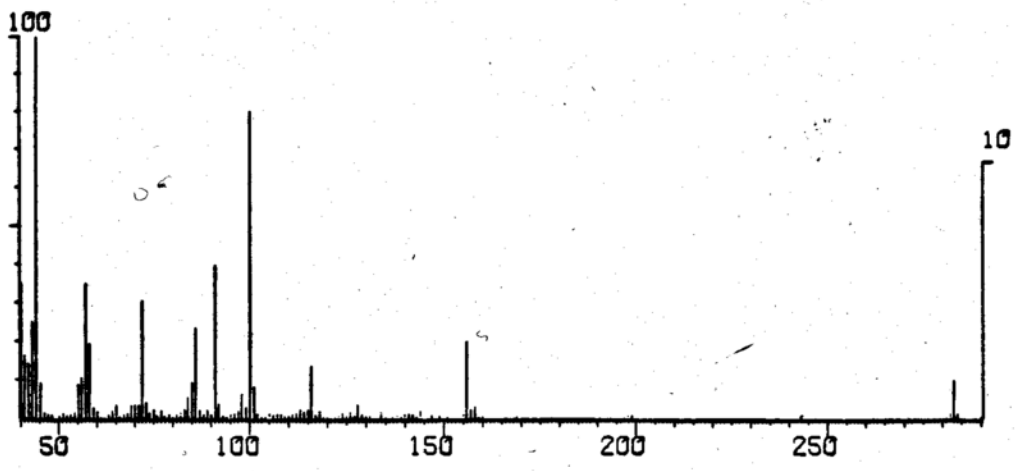


Figure 26. Mass Spectrum of Product of Reaction Between Triethylamine and Carbon Suboxide.

Table VIII. Mass Spectrum of Product Showing Possible Ions.

m/e	Relative Intensity	Empirical Formula	Possible Ion
40	35.7	C_2O	$(C \equiv C = O)^+$
41	16.6		
42	14.8		
43	25.6	C_2H_5N	$(N - C_2H_5)^+$
44	100	CO_2	$(O = C = O)^+$
45	9.11		
55	9.40	C_2HNO	$(O \equiv C - N \equiv CH)^+$
56	11.1	C_2H_2NO	$^+O \equiv C - N = CH_2$
57	35.5	C_2H_3NO	$(O = C = N - CH_3)^+$ or $(O \equiv C - \underset{\displaystyle H}{\underset{\vert}{N}} = CH_2)^+$
		C_3H_5O	$(O = C - CH_2CH_3)^+$
		C_2HO_2	
		C_4H_9	
58	19.5	C_2H_4NO	$O = C = \overset{\displaystyle +}{\underset{\displaystyle H}{\underset{\vert}{N}}} - CH_3$
		C_3H_8N	$H - \overset{\displaystyle +}{N}\!\!\begin{array}{l} \diagup\, CH_2CH_3 \\ \diagdown\!\!= CH_2 \end{array}$

Table VIII (continued)

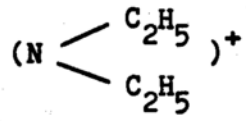
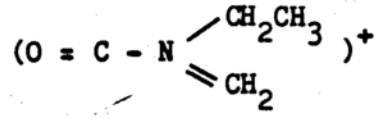
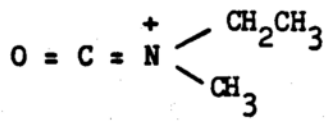
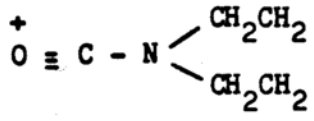
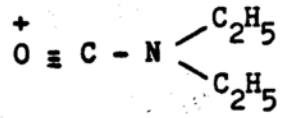
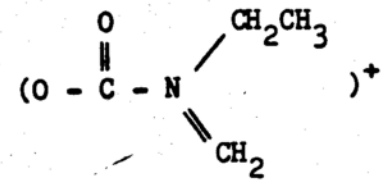
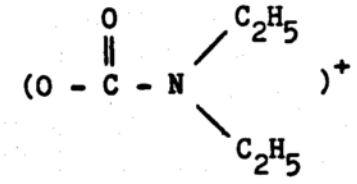
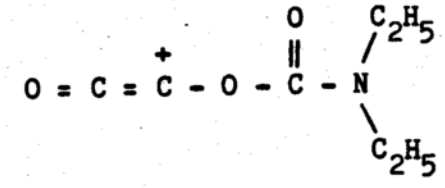
m/e	Relative Intensity	Empirical Formula	Possible Ion
68	1.63	C_3O_2	$(O \equiv C \equiv C \equiv C \equiv O)^+$
72	30.9	$C_4H_{10}N$	
84	5.90		
85	9.76	C_4H_7NO	
86	24.0	C_4H_8NO	
		$C_5H_{10}O$	
91	40.1	$C_2H_5NO_3$	
		$C_3H_9NO_2$	
		$C_4H_{13}NO$	No reasonable structures.
		$C_4H_{11}O_2$	
		$C_5H_{15}O$	
		C_5HNO	
		C_6H_5N	
		C_6H_3O	

Table VIII (continued)

m/e	Relative Intensity	Empirical Formula	Possible Ion
98	6.48	C_5H_8NO	
100	80.4	$C_5H_{10}NO$	
101	8.53	$C_4H_7NO_2$	
116	13.9	$C_5H_{10}NO_2$	
156	20.2	$C_7H_{10}NO_3$	
283	2.11	$(2C_3O_2+NEt_3+2CH_3OH-H_2O)$ $C_{14}H_{21}NO_5$ $(3C_3O_2+NEt_3-H_2O-2H_2)$ $C_{15}H_9NO_5$	No structures

C. Spectral Studies

1. Initial Rate and Lag Time

Absorption spectrophotometric studies of the reaction between acetic anhydride solutions of tertiary amines and carbon suboxide led to the observation of a lag time (induction time) phenomenon. We have investigated the dependence of the lag time and initial rates on various factors. But before we present the results of our findings, definitions of the initial rate and lag time are in order.

Figure 27 shows absorbance-time profiles of the reaction between pyridine and carbon suboxide in the presence of acetic anhydride. Two ml aliquots of carbon suboxide solution were reacted with 1 ml aliquots of freshly prepared acetic anhydride solutions of pyridine. In each case, the final concentration of pyridine in the cuvette was 10^{-2}M. The content of the cuvette was mixed thoroughly and measurements initiated less than 15 sec post mixing the reagents together.

In this family of curves, the lag time is defined as the intercept on the time axis of the tangent drawn to the absorption curve at the point where the absorbance is 0.2 absorbance units above the baseline. The initial rate is the measure of the slope, usually on the most linear portion of the absorption curve. These definitions are arbitrary, but for a given set of data, they provide a consistent measure of these parameters. Because of this arbitrariness, we have tried not to make extensive use of initial

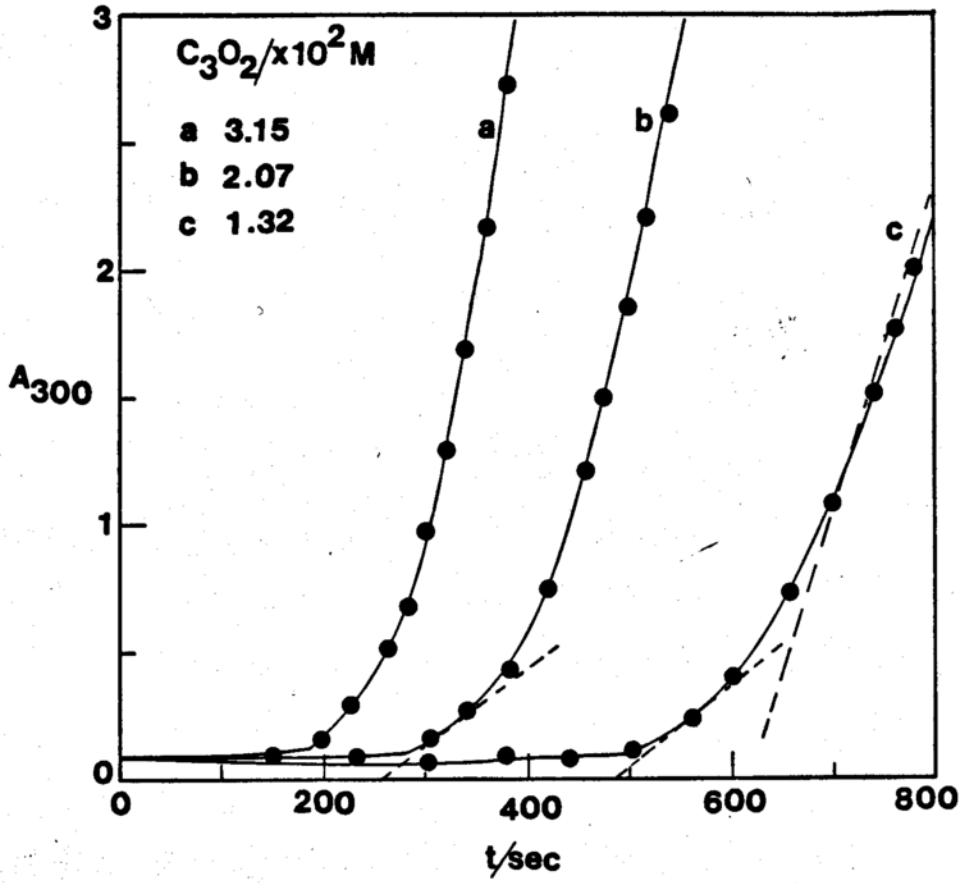


Figure 27. Absorbance-time Profiles of the Reaction between Pyridine and Ether Solutions of Carbon Suboxide in the Presence of Acetic Anhydride. Plot illustrates definition of initial rate and lag time.

rates, especially those derived from absorption spectrophotometric measurements of the kind shown in Figure 27. Where possible, different analytical methodologies were used to gather kinetic data so as to avoid making use of initial rates. However, we used initial rate measurements in the correlation of basicity of amine with reactivity; see Section D.2.

2. Dependency of Lag Time and Initial Rates on:

a. Concentration of Carbon Suboxide

Figures 28 and 29 show the dependence of lag time on the concentration of carbon suboxide using, in the one case, a constant concentration of pyridine (5.00×10^{-3}M), and in the other, a constant concentration of triethylamine (5.00×10^{-4}M). Table IX summarizes the lag times and initial rates for both reaction systems.

In these cases also, as in all subsequent investigations of the dependence of the lag times and initial rates on other factors to be presented in this section, the lag times were defined as the intercepts on the time axes of the tangents to the absorption curves at the points where the absorbance was 0.2 above baseline. The straight lines from which the initial rates (slopes) were obtained are also added in each case for clarity. In both of these reaction systems, there was a lag-time phase, the duration evidently being a function of the concentration of carbon suboxide. The duration of

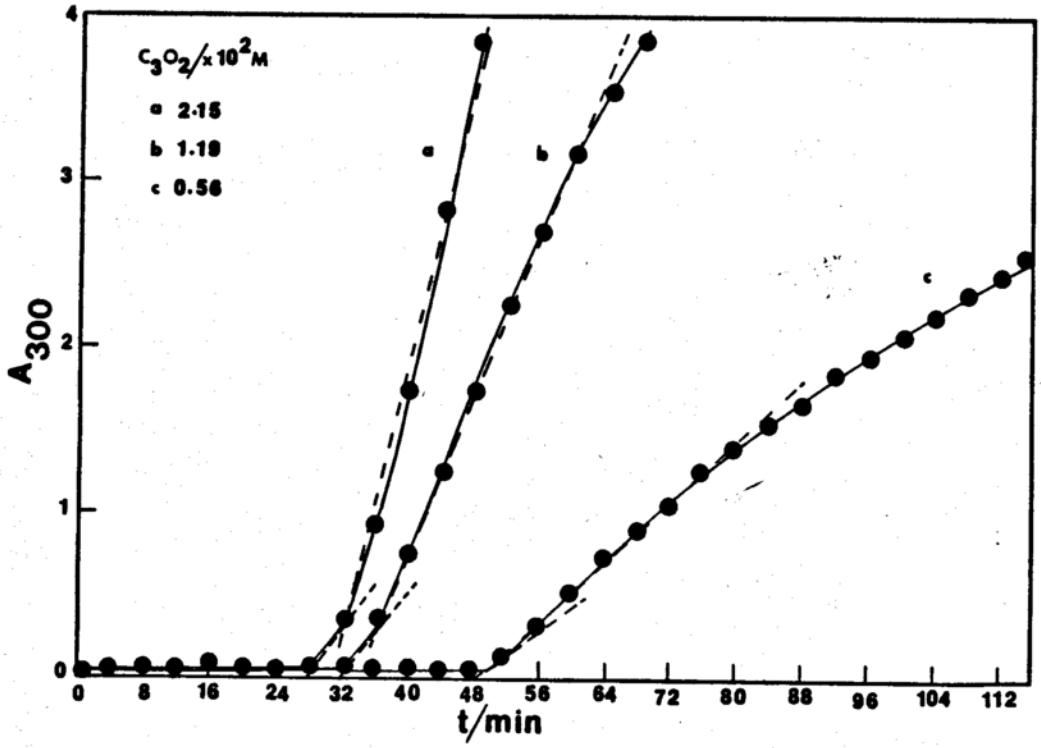


Figure 28. Absorbance-time Course of the Reaction between Carbon Suboxide and Pyridine at Constant Pyridine Concentration.

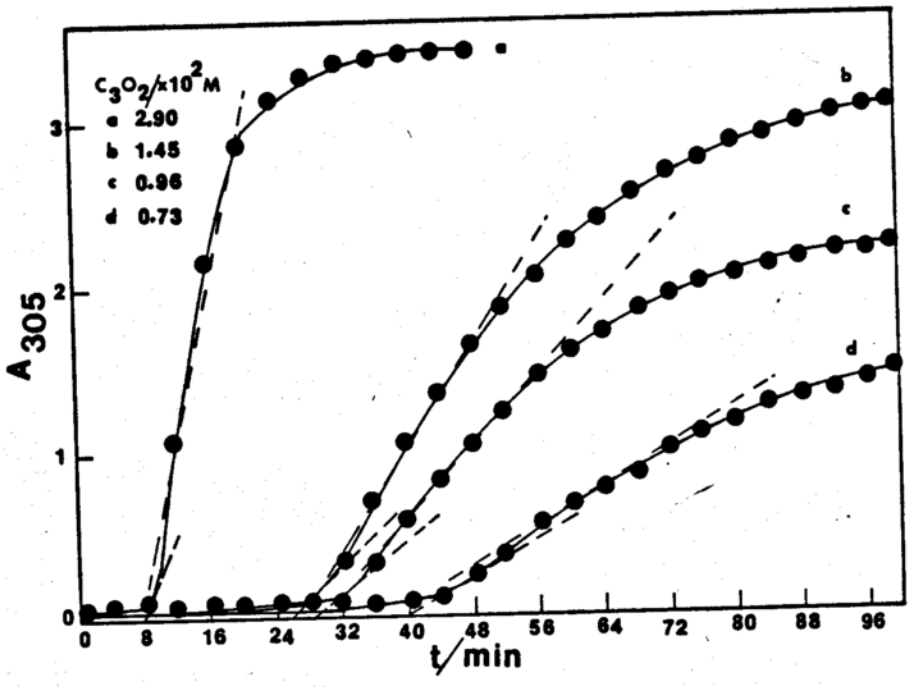


Figure 29. Absorbance-time Course of the Reaction between Carbon Suboxide and Triethylamine at Constant Triethylamine Concentration.

Table IX. Dependence of Lag Time and Initial Rates on the Concentration of Carbon Suboxide.

	5.00×10^{-3}M PYR		5.00×10^{-4}M TEA	
$C_3O_2 \times 10^2$/M	Initial Rate/ absorbance sec^{-1}	Lag Time/ sec	Initial Rate/ absorbance sec^{-1}	Lag Time/ sec
2.9	—	—	0.216	480
2.1	0.206	1680	—	—
1.4	—	—	0.0792	1560
1.2	0.118	1920	—	—
0.96	—	—	0.0547	1740
0.73	—	—	0.0306	2400
0.56	0.0462	2840	—	—

the lag time apparently has an inverse relationship to the concentration of carbon suboxide. Table X gives the logarithms of the concentrations of carbon suboxide and the inverse of the lag times. Figure 30 is a plot of the data from Table X. These plots are approximately linear for both reaction systems. The slopes are approximately 1.0 and 0.4 for the reaction with triethylamine and with pyridine, respectively. Although it is obvious that there is a dependency of the lag time on the concentration of carbon suboxide, it is not clear what relationship, if any, these slopes may have to the reaction order. The initial rates are linearly dependent upon the concentrations of carbon suboxide.

The experimental procedure involved diluting solutions of carbon suboxide using anhydrous diethyl ether to get an appropriate concentration. Two ml of the suboxide solution was then reacted with 1.0 ml of the acetic anhydride solution of amine. Absorbance measurements were performed on a Varian 2200 UV-VIS spectrophotometer; these were initiated less than 15 sec after mixing the reagents. The analytical wavelengths were 300 nm (pyridine), and 305 nm (triethylamine).

b. Identity of Tertiary Amine Compound

Figures 31 and 32 show the dependence of the lag time on the concentration of pyridine and of triethylamine, respectively. In each case, the lag time is rather sharply defined; apparently formation of the product does not begin for a substantial period of

Table X. Logarithmic Values of the Concentrations of Carbon Suboxide and the Inverse of the Lag-times.

Log $[C_3O_2]$	Log (1/lag-time)	
	PYR	TEA
-1.54	--	-2.68
-1.68	-3.22	--
-1.85	--	-3.19
-1.92	-3.28	--
-2.02	--	-3.24
-2.14	--	-3.38
-2.25	-3.45	--

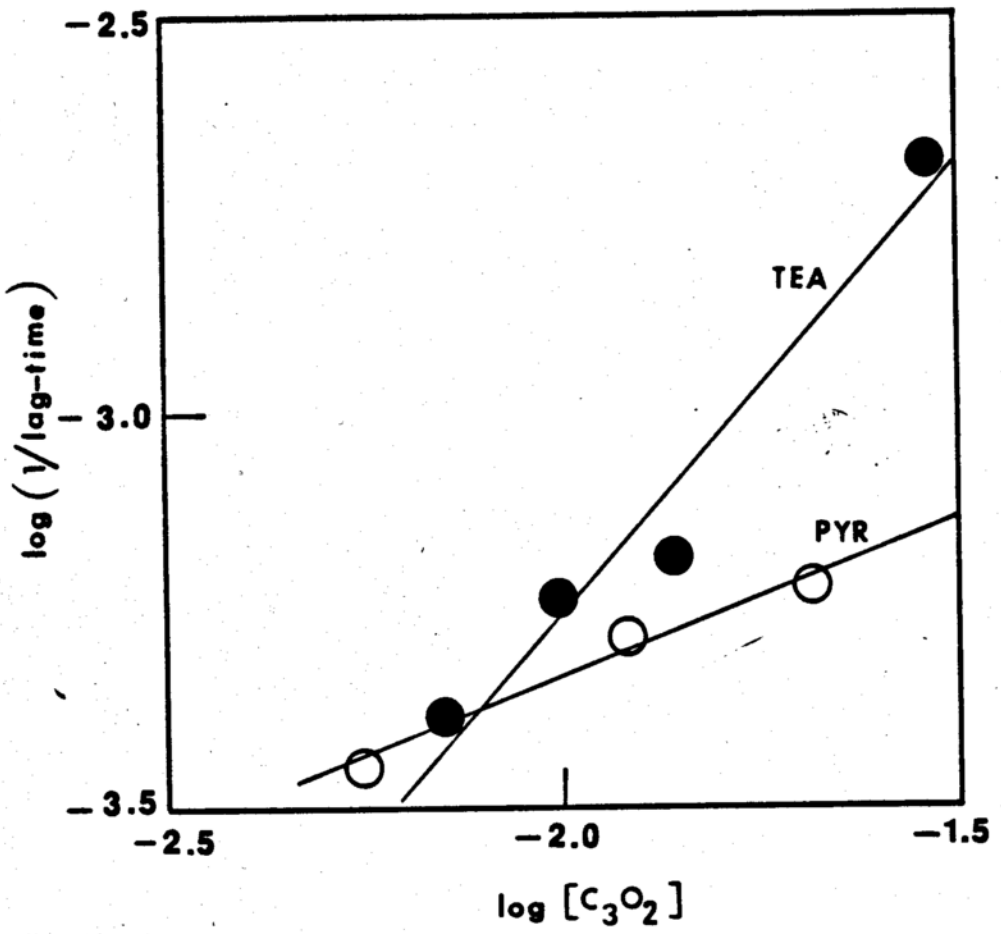


Figure 30. Plot of log $[C_3O_2]$ <u>versus</u> log (1/lag-time).

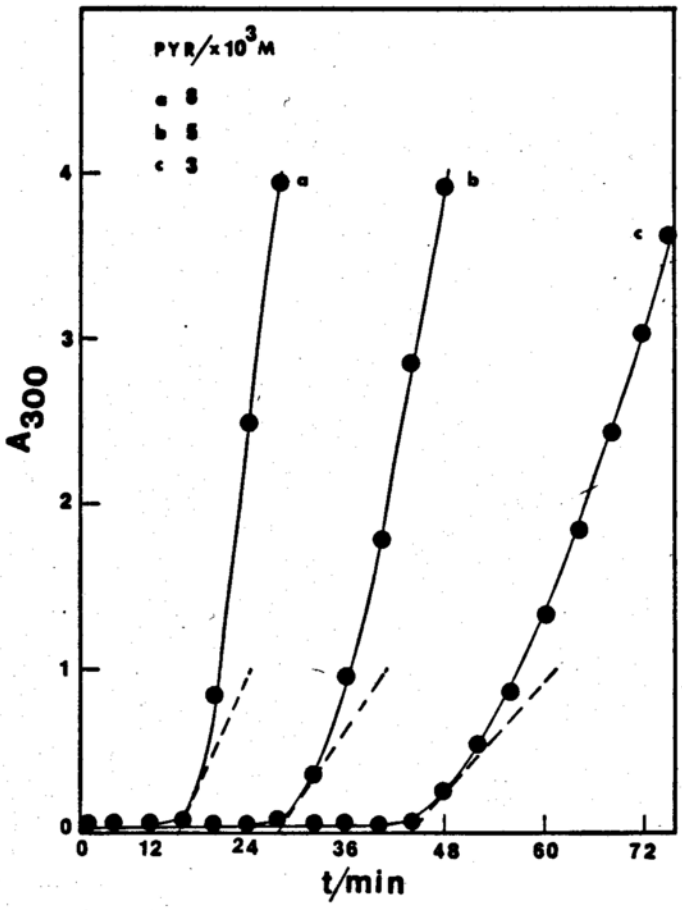


Figure 31. Absorbance-time Course of the Reaction between Carbon Suboxide and Pyridine. Concentration of carbon suboxide was 2.35×10^{-2}M.

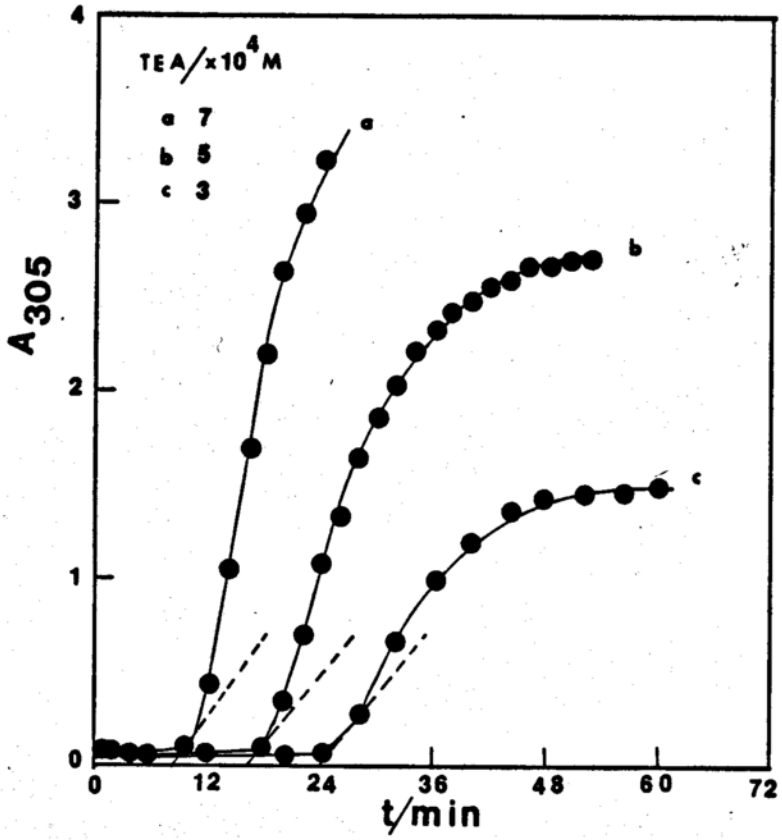


Figure 32. Absorbance-time Course of the Reaction between Carbon Suboxide (2.03×10^{-2}M) and Triethylamine.

time. The duration of the lag-time phase, evidently, depends upon the identity of the amine, being longer for pyridine than for triethylamine. For both of these compounds, a logarithmic plot of the inverse of the lag time as a function of the logarithm of the concentration of the amine, gives an approximately linear relationship with a slope of about 1; see Figure 33.

The conduct of the experiment in these cases involved reacting a constant molar concentration of carbon suboxide with varying molar concentrations of acetic anhydride solutions of the amines at $25^{\circ} \pm 0.1^{\circ}C$. In a related experiment, the dependence of the lag time on the amine pKa was investigated. We wished to assess for a given class of amine (aliphatic or aromatic) if the lag time, at fixed amine concentration, was dependent on the pKa. The experimental procedure involved reacting 2.0 ml aliquots of a constant molar concentration of carbon suboxide solution with 1.0 ml aliquots of acetic anhydride solutions of aliphatic ($10^{-3}M$) and aromatic ($10^{-2}M$) amines.

Tables XI and XII show the pKa values and lag times associated with aromatic and aliphatic amines, respectively. Figures 34 and 35 are plots of the logarithm of the inverse of the lag time as a function of pKa. For the aromatic amine series, this plot gives an approximately linear relationship for the closely related amines pyridine, 3-picoline, 4-picoline, and 3,4-lutidine. The least squares line through these points has a slope of about 1. The hindered amines 2,4-lutidine, 2,6-lutidine, and 2,4,6-collidine

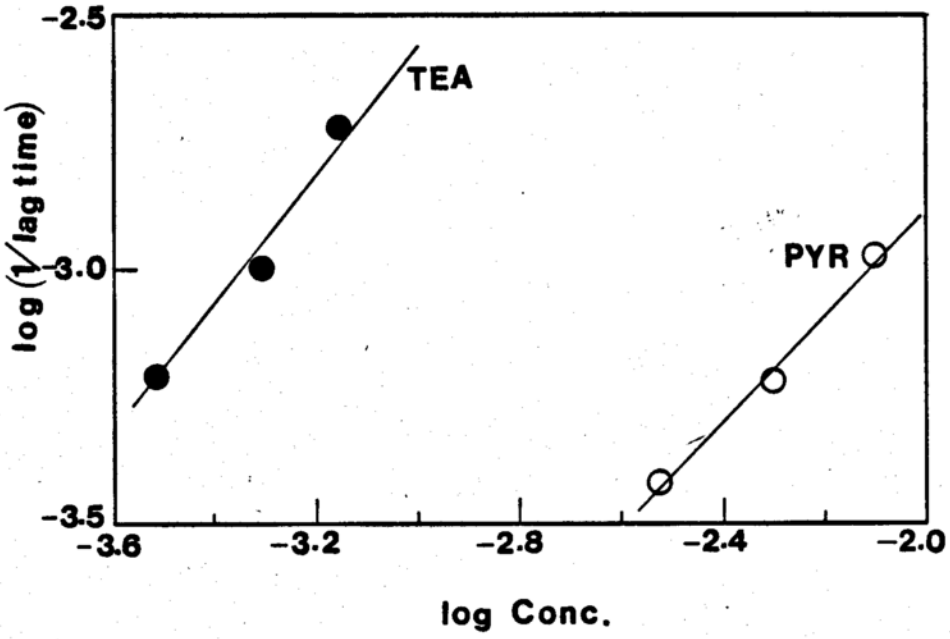


Figure 33. Plots of Logarithm of Amine Concentration versus log (1/lag-time). Key: a. triethylamine; b. pyridine.

Table XI. pKa Values and Lag-times for the Aromatic Amine Series.[a]

Amine	Abbreviation	pKa	Lag time/ sec	Log (1/lag-time)
Pyridine	PYR	5.23	910	-2.96
3-Picoline	3-PIC	5.73	420	-2.62
4-Picoline	4-PIC	6.04	120	-2.08
3,4-Lutidine	3,4-LUT	6.46	16	-1.20
2,6-Lutidine	2,6-LUT	6.72	7040	-3.85
2,4-Lutidine	2,4-LUT	6.63	5340	-3.73
2,4,6-Collidine	2,4,6-COL	7.59	1500	-3.18

[a]Concentration of carbon suboxide used was 1.75×10^{-2}M.

Table XII. pKa Values and Lag-times for the Aliphatic Amine Series.[a]

Amine	Abbreviation	pKa	Lag time/ sec	Log (1/lag-time)
Triethylamine	TEA	10.72	1370	-3.14
n-Tripropylamine	TPA	10.66	1410	-3.15
n-Tributylamine	TBA	10.89	1190	-3.07
Allyldimethylamine	ADMA	8.72	2200	-3.34
1-Dimethylamino-2-propyne	DMA-2-P	7.05	7010	-3.85

[a]Concentration of carbon suboxide used was 1.74×10^{-2}M.

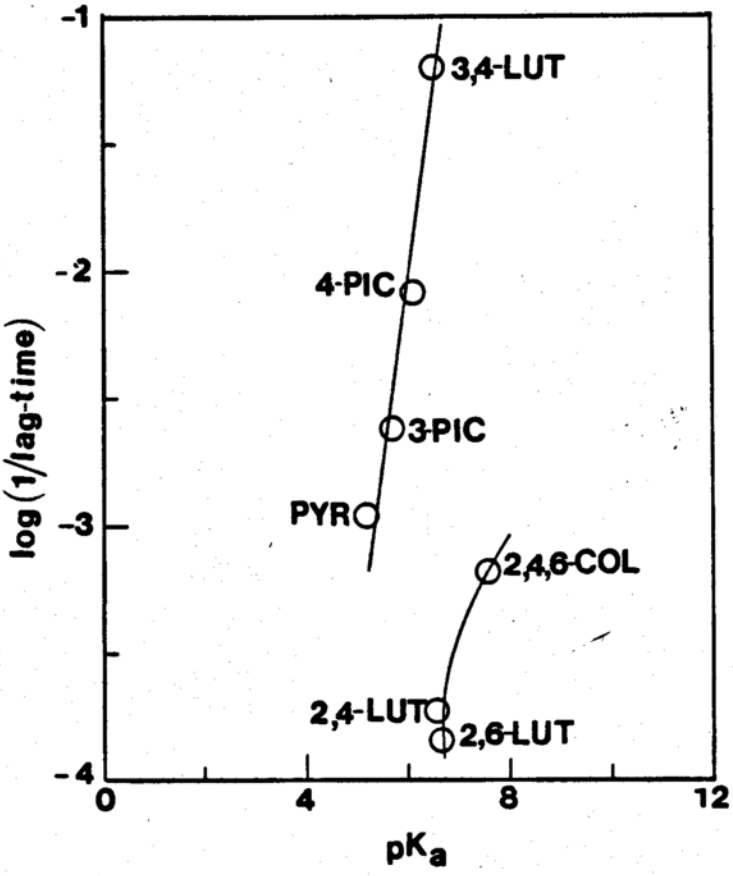


Figure 34. Plot of pKa versus Logarithm of the Inverse of Lag-time for the Aromatic Series.

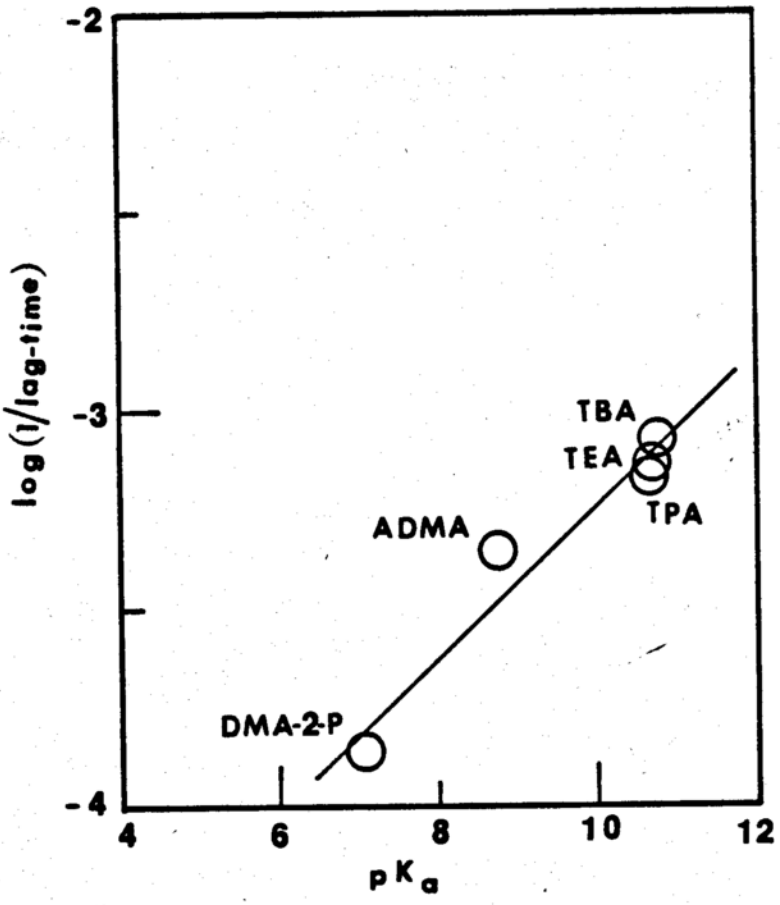


Figure 35. Plot of pKa versus Logarithm of the Inverse of Lag-time for the Aliphatic Series.

do not fall on the least squares line. The least squares line for the aliphatic amines, on the other hand, has a slope of about 0.2.

The effect upon the lag time when excess amine over carbon suboxide is used was also investigated. For a 0.10 molar acetic anhydride solution of pyridine reacting with carbon suboxide (0.0171 M), there was a lag-time of approximately 35 sec. No lag-time was seen when excess triethylamine (0.10 M) was used over carbon suboxide. Nor was there a residual lag-time when excess 3,4-lutidine (0.10 M) was used.

c. Concentration of Acetic Anhydride

The dependence of the lag time on the concentration of acetic anhydride at a given concentration of pyridine (10^{-2}M) and of carbon suboxide (1.93×10^{-2}M, 1.76×10^{-2}M, or 1.63×10^{-2}M) was undertaken. Solutions of acetic anhydride (9.00 M, 7.00 M, 6.00 M and 5.00 M) were prepared in anhydrous ether. Aliquots of these solutions were added to aliquots of solutions of carbon suboxide in a 1 to 2 volume ratio. The reactions were initiated by adding 2.40 µl of pyridine (approximately 10^{-2}M final concentration) to the cuvette.

Figure 36 is a representative plot of the absorbance-time data for the reaction system using 1.63×10^{-2}M carbon suboxide. Table XIII summarizes the findings of the investigation. There is a dependence of both the lag time and initial rate on the concentration of acetic anhydride for corresponding determinations.

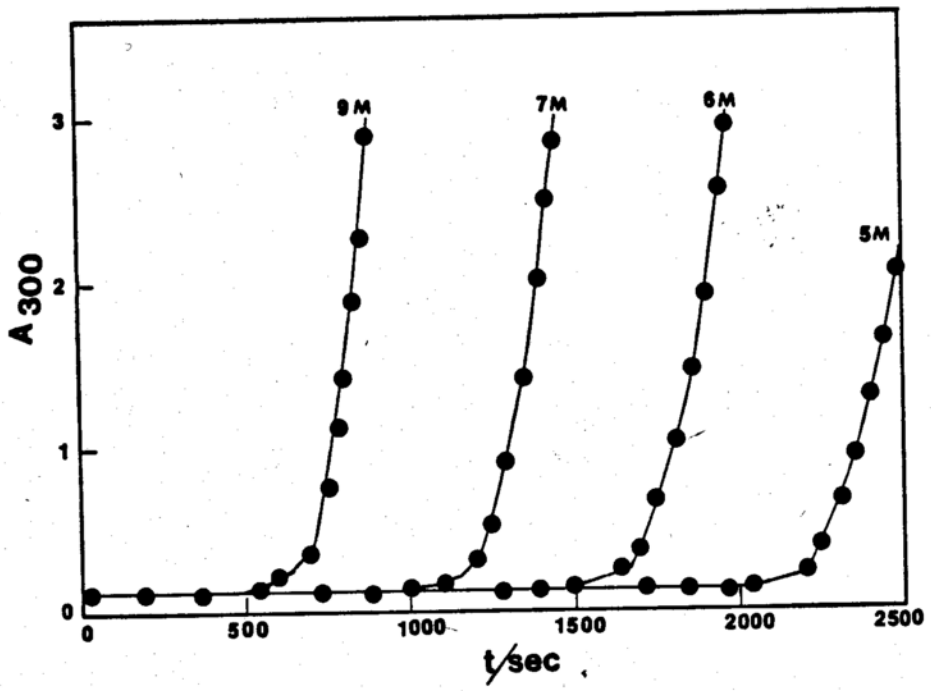


Figure 36. Representative Plot of Absorbance-time Data. Carbon suboxide concentration = 1.63×10^{-2}M.

Table XIII. Dependence of the Lag Time and Initial Rates on the Concentration of Acetic Anhydride.[a]

AC_2O/M		C_3O_2/M x 10^2	Lag Time/sec	Initial Rate/absorbance sec^{-1} x 10^3
	1.	1.93	505	18.7
9.00	2.	1.76	510	18.2
	3.	1.63	524	16.3
	1.	1.93	910	12.9
7.00	2.	1.76	1090	12.7
	3.	1.63	1130	12.6
	1.	1.93	1310	11.2
6.00	2.	1.76	1540	9.91
	3.	1.63	1600	10.6
	1.	1.93	1340	9.70
5.00	2.	1.76	2070	8.35
	3.	1.63	2120	8.07

[a]Corresponding rows 1, 2, or 3 are for determinations using the same carbon suboxide concentration.

Lower concentrations of acetic anhydride (1.00 M and 2.00 M) gave lag times in excess of 5000 seconds. As expected, for a given acetic anhydride concentration, the lag times and initial rates show a dependence on carbon suboxide concentration.

d. Preaging of Reagents

The lag time was always present under the conditions of our investigation. So far, on the basis of our observations, we have established dependence of the length of the lag time phase on factors such as the concentration of carbon suboxide and of acetic anhydride, as well as the concentration and base strength of the amine. In all of these cases, the lag time is sharply defined; apparently product formation does not begin prior to the end of the lag time phase. There is the possibility, however, of intermediate product accumulation during the lag time phase. Preaging of the reagents was carried out under identical, final conditions of acetic anhydride, carbon suboxide, and pyridine concentrations. The different combinations of preaged pairs of reagent solutions are given on p. 51. The preaging time for the samples was approximately 950 sec. We wished to compare the lag times, and to explore the possibility of its elimination under normal reaction conditions, i.e. the conditions under which a typical kinetic run could be carried out.

Table XIV lists the lag times and initial rates that were obtained. Although the preaged pair II has the lowest lag time

Table XIV. Lag Time, Initial Rate and the Preaging of Reagents.

Number[a]		C_3O_2/M x 10^2	Lag Time/sec	Initial Rates/absorbance sec^{-1} x 10^3
I.	1.	2.36	320	24.4
	2.	2.55	296	27.4
II.	1.	2.36	288	27.0
	2.	2.55	240	26.5
III	1.	2.36	364	25.0
	2.	2.55	288	25.0
IV	1.	2.36	332	25.0
	2.	2.55	284	27.2
V	1.	2.36	336	26.0
	2.	2.55	296	25.0
VI	1.	2.36	352	25.0
	2.	2.55	334	25.6
VII	1.	2.36	354	26.0
	2.	2.55	336	26.0

[a]The number designation here is for the aged reagent solution pair as given on p. 51. Furthermore, corresponding rows 1, or 2, are for determinations using the same carbon suboxide concentration.

compared to the control (pair VII), the pretreatment process has not completely eliminated the lag time. When pair II was aged for 60-70 min, the lag time (approximately 245 sec) was still present.

D. Catalysis and the Bronsted Relationship

1. Fate of the Amine

The extent of involvement of the tertiary amine compound in this reaction process was investigated. We wished to answer the question concerning whether or not the amine was consumed during the course of the reaction.

Gas chromatography (GC) was used to monitor the concentration of the amine compound in the reaction mixture as a function of time. In all cases, the injection port and interface temperatures on the GC instrument were maintained constant at 250° and 300°C, respectively. The column was a 10.5-foot length by 0.25-inch diameter gas tubing, packed with 10% by weight carbowax 20M on 80/100 mesh Gas-Chrom Q support. Nitrogen at 50 p.s.i. was used as the carrier gas. The volume of sample injected was maintained at approximately 3.0 µl. Representative chromatograms are shown in Figures 37 to 40.

Figure 41 is the plot of peak height (H_t) as a function of time for the reaction of pyridine (10^{-2}M) and carbon suboxide (2.47 x 10^{-2}M) in the presence of excess acetic anhydride. The column temperature was maintained at 100°C; the attenuation and range were set at 32 and 10, respectively. Under these conditions, the retention times were approximately 9 min for acetic anhydride and 7 min for pyridine. No change in the concentration of pyridine was observed. The fluctuation of peak heights about 1.05 cm is probably

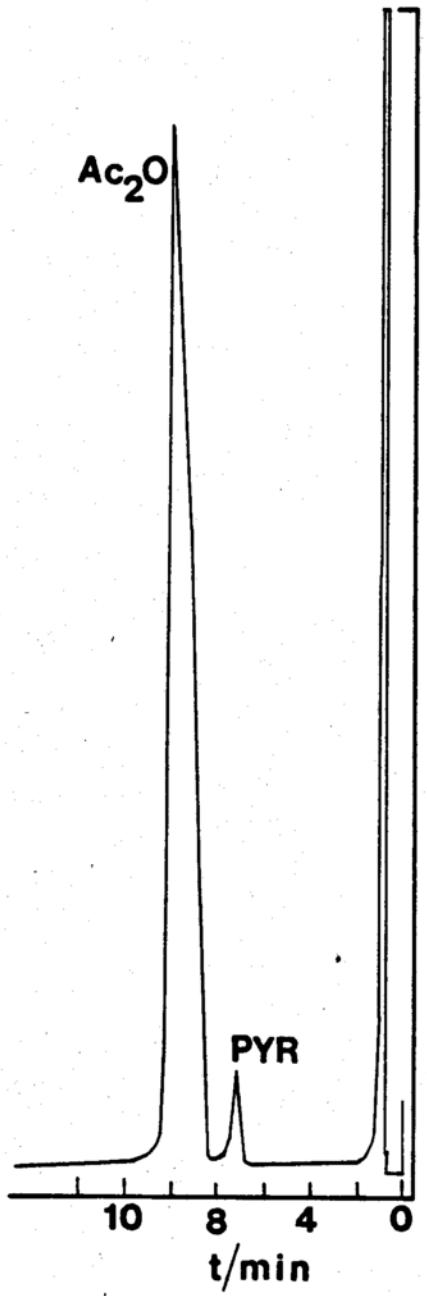


Figure 37. Representative GC Chromatogram of the Reaction between Pyridine and Carbon Suboxide in the Presence of Acetic Anhydride.

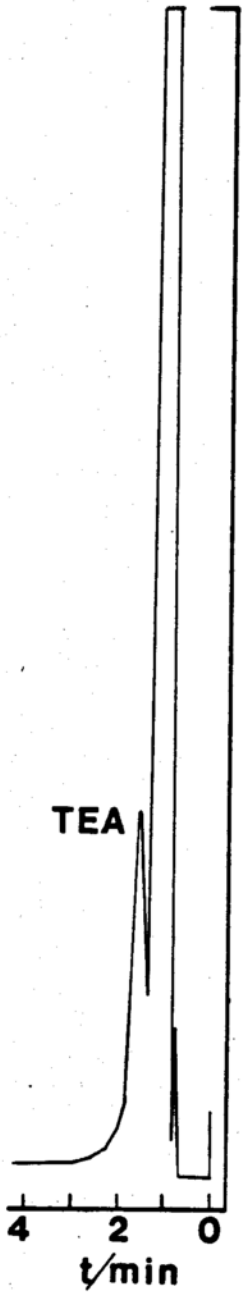


Figure 38. Representative GC Chromatogram of the Reaction between Triethylamine and Carbon Suboxide.

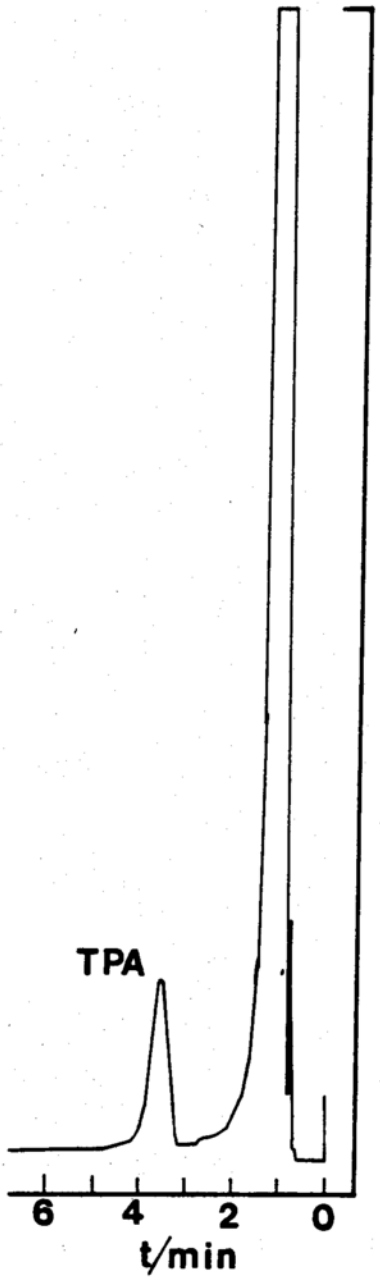


Figure 39. Representative GC Chromatogram of the Reaction between n-tripropylamine and Carbon Suboxide.

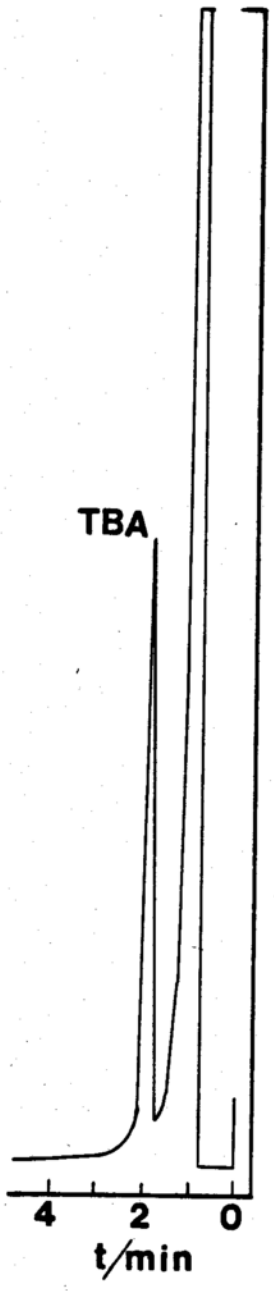


Figure 40. Representative GC Chromatogram of the Reaction between n-tributylamine and Carbon Suboxide.

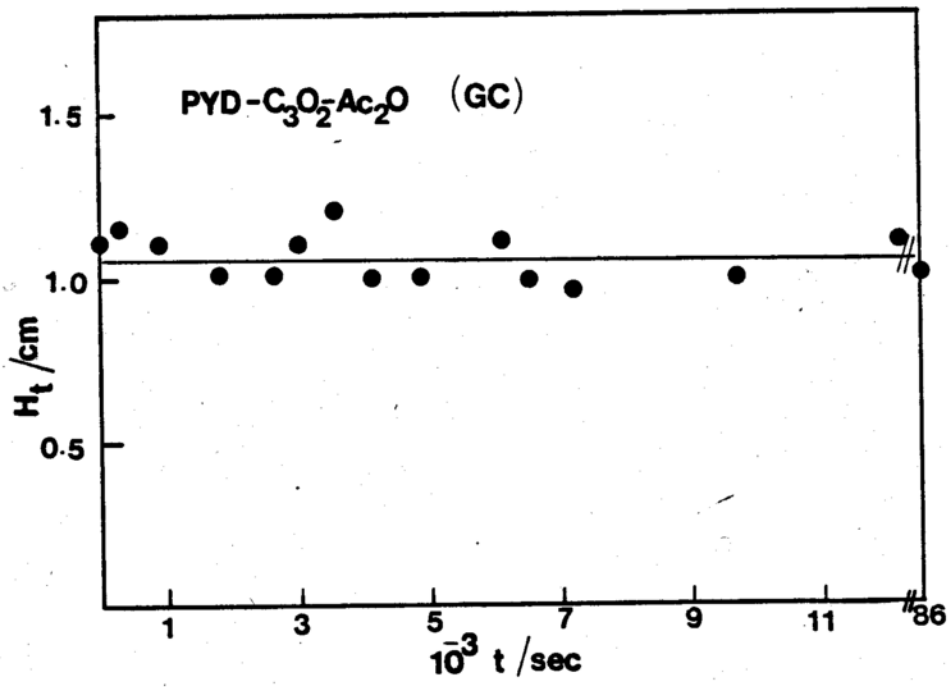


Figure 41. Concentration-time Dependency of Pyridine.

a consequence of slight differences in the volume of sample injected; no internal standard was used. It may also be the result of error associated with measurement of the small peak heights.

Figures 42, 43, and 44 are plots of peak heights as a function of time for the reaction of the aliphatic amines triethyl, n-tripropyl, and n-tributyl, respectively, with carbon suboxide. No acetic anhydride was used in these reactions. The concentrations of carbon suboxide used were 2.53×10^{-2}M (for triethylamine), and 2.13×10^{-2}M (for both n-tripropyl- and n-tributylamine). The initial concentration of amine in the reaction mixture was 10^{-3}M in all cases. For the triethylamine reaction system, the column temperature was maintained at 70°C; the attenuation and range were set at 32 and 10, respectively. This column temperature proved to be the optimum for the triethylamine reaction system, although it did not lead to a very good resolution of the triethylamine peak from the ether (solvent) peak. In the cases of n-tripropyl- and n-tributylamine reaction systems, the column temperatures were 80°C and 180°C, respectively. The attenuation and range for both reaction systems were set at 16 and 10. Under these conditions, peak retention times were approximately 1.5 min (triethylamine), 3.5 min (n-tripropylamine), and 2.0 min (n-tributylamine).

For all of the aliphatic amines considered, there was a decrease in the concentration of the amine in the reaction mixture as a function of time. But the concentration of amine did not go to zero in any of the reaction systems studied. The apparent

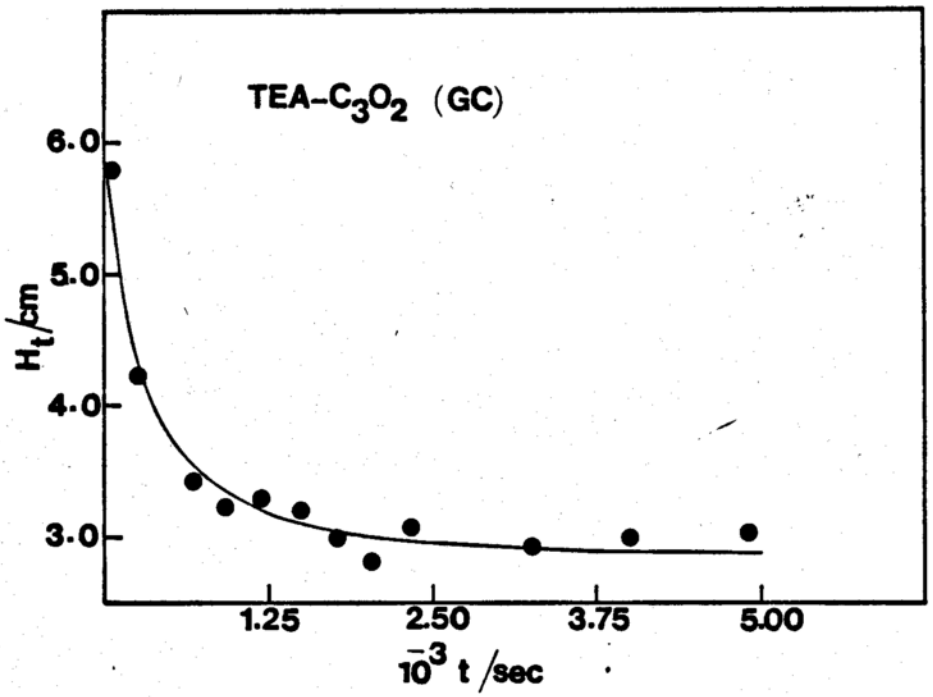


Figure 42. Concentration-time Dependency of Triethylamine.

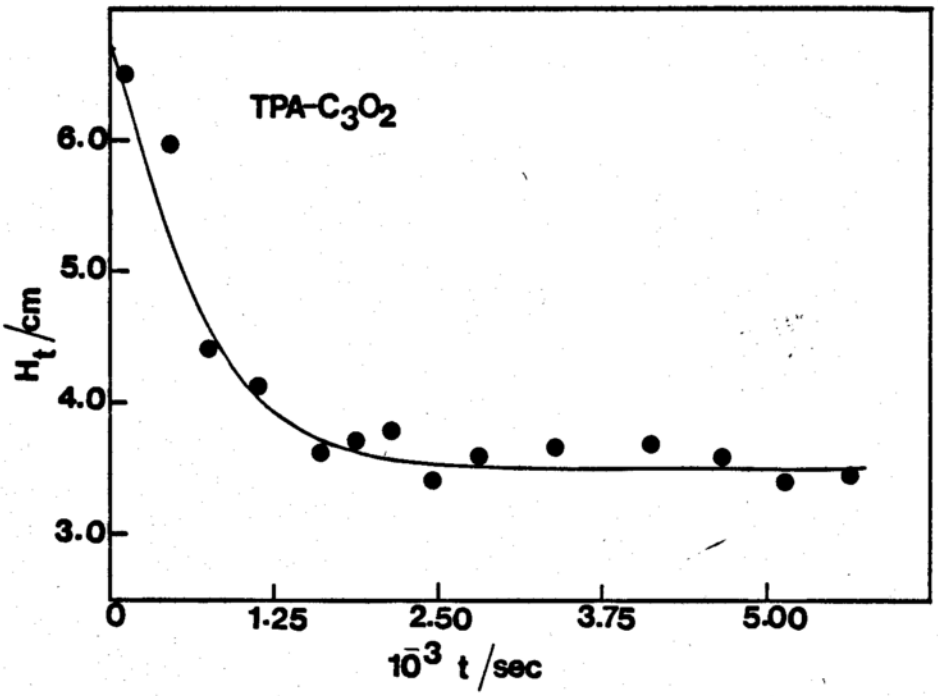


Figure 43. Concentration-time Dependency of n-tripropylamine.

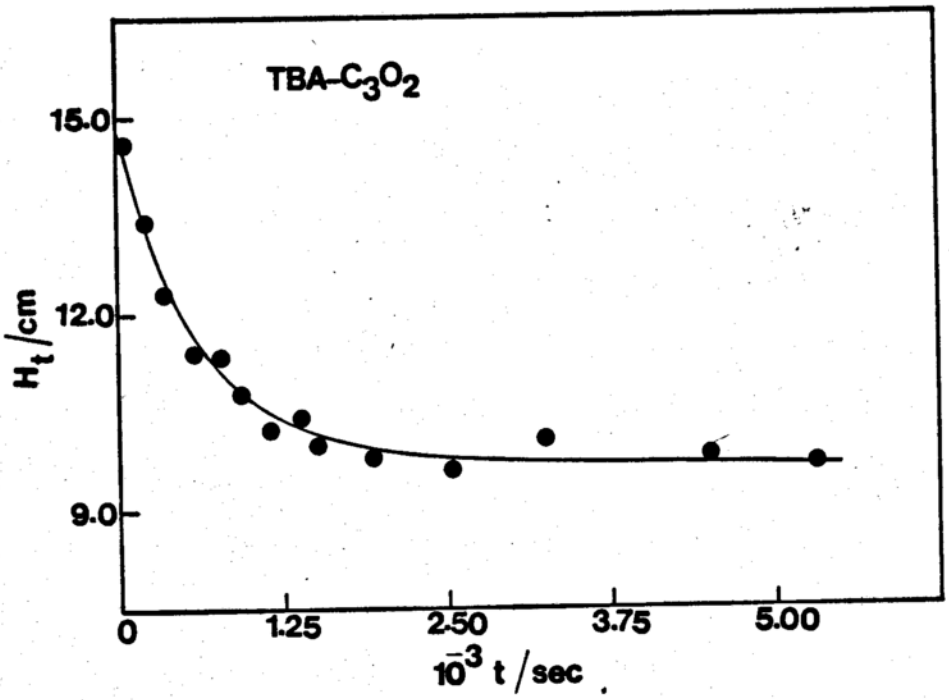


Figure 44. Concentration-time Dependency of n-tributylamine.

concentrations of unreacted amine in the ether supernatant were approximately 50, 60, and 70% of the initial concentrations for triethyl-, n-tripropyl-, and n-tributylamine, respectively.

2. Correlation of Basicity of Amine with Reactivity

Although strictly applicable to the description of reactions involving proton transfer, the Bronsted catalysis law, which is a relation between an equilibrium constant and a rate constant (87), has been applied successfully to reactions that do not involve proton transfer. The fundamental intent is to establish if a relationship exists between the basicity (or nucleophilicity) of the catalyst (pKa being a pertinent measure of basicity) and its catalytic activity. So far, we have evidence that leads us to suspect there may be a difference in behavior between aromatic and aliphatic amines in this reaction process. We have investigated the effect of the basicity of amine on reactivity.

Acetic anhydride solutions of the amines were reacted with solutions of carbon suboxide, and absorption measurements performed at $25^{\circ} \pm 0.1^{\circ}C$. Initial rate (slope) measurements were made from the absorption-time curves.

Figure 45 shows the absorption-time plots for the aliphatic amines. Table XV is a summary of the initial rates. Figure 46 is the Bronsted plot of the data from Table XV. The Bronsted plot is approximately linear with a slope of 0.12.

Figure 47 is the absorption-time profile for the aromatic amine

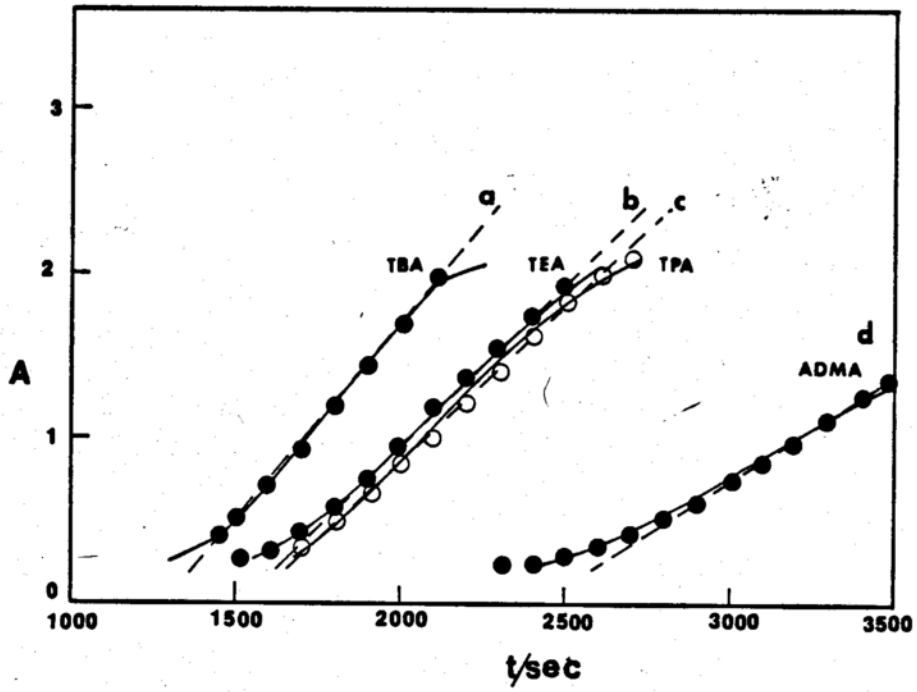


Figure 45. Absorption-time Plots for the Aliphatic Amines.

Key: a. TBA; b. TEA; c. TPA; d. ADMA

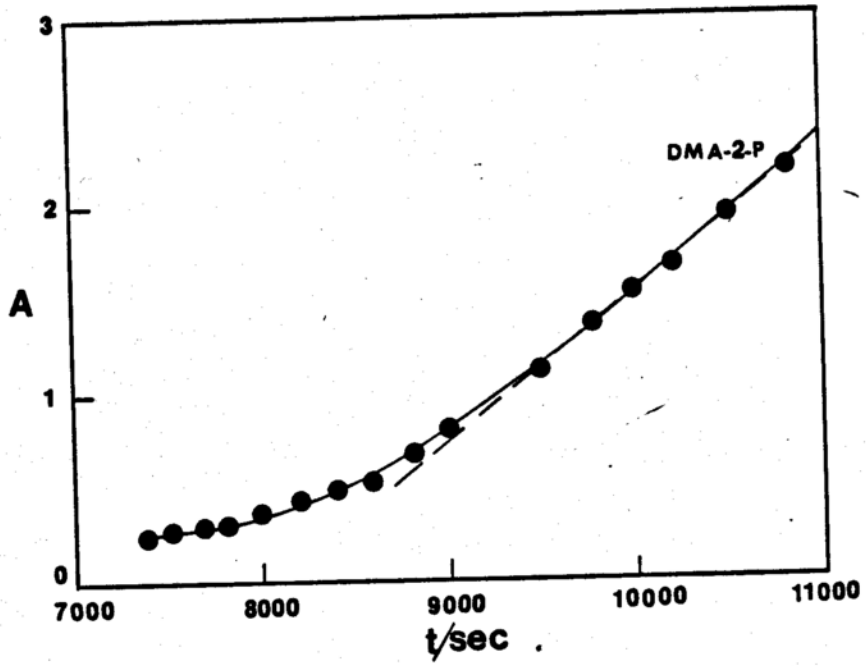


Figure 45 continued. Absorption-time Plot for the Aliphatic Amine, 1-Dimethylamino-2-propyne.

Table XV. pKa Values and Initial Rates for the Aliphatic Amine Series.[a]

Amine	pKa	Initial Rate/ Absorbance sec^{-1} x 10^3	Log (Initial Rate)
Triethylamine	10.72	1.96	-2.71
n-Tripropylamine	10.66	1.94	-2.71
n-Tributylamine	10.89	2.42	-2.62
Allyldimethylamine	8.72	1.29	-2.89
1-Dimethylamino-2-propyne	7.05	0.785	-3.10

[a]In all cases, 1.0 ml of a 10^{-3}M solution of amine in acetic anhydride was reacted with 2.0 ml of 1.86×10^{-2}M of carbon suboxide.

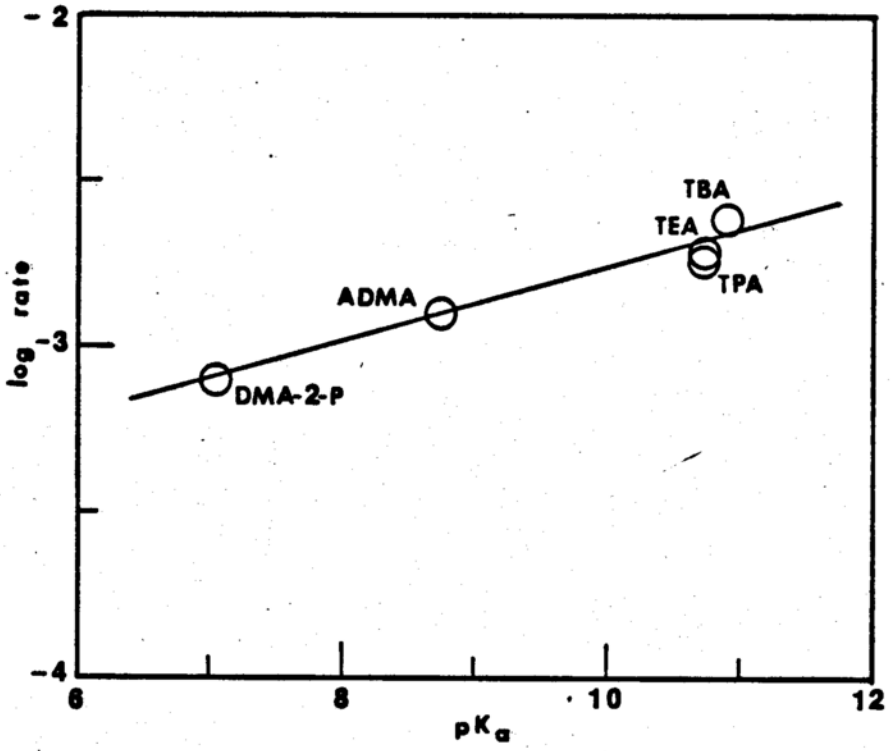


Figure 46. Bronsted Plot for the Aliphatic Amine Series.

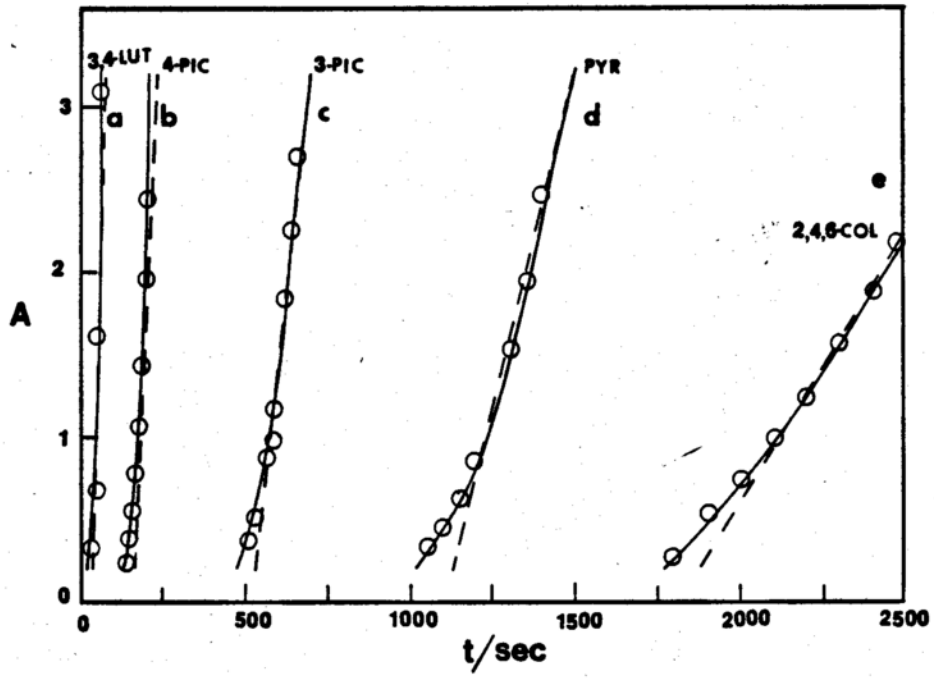


Figure 47. Absorption-time Plots for the Aromatic Amines.

Key: a. 3,4-lutidine; b. 4-picoline;

c. 3-picoline; d. pyridine; e. 2,4,6-collidine.

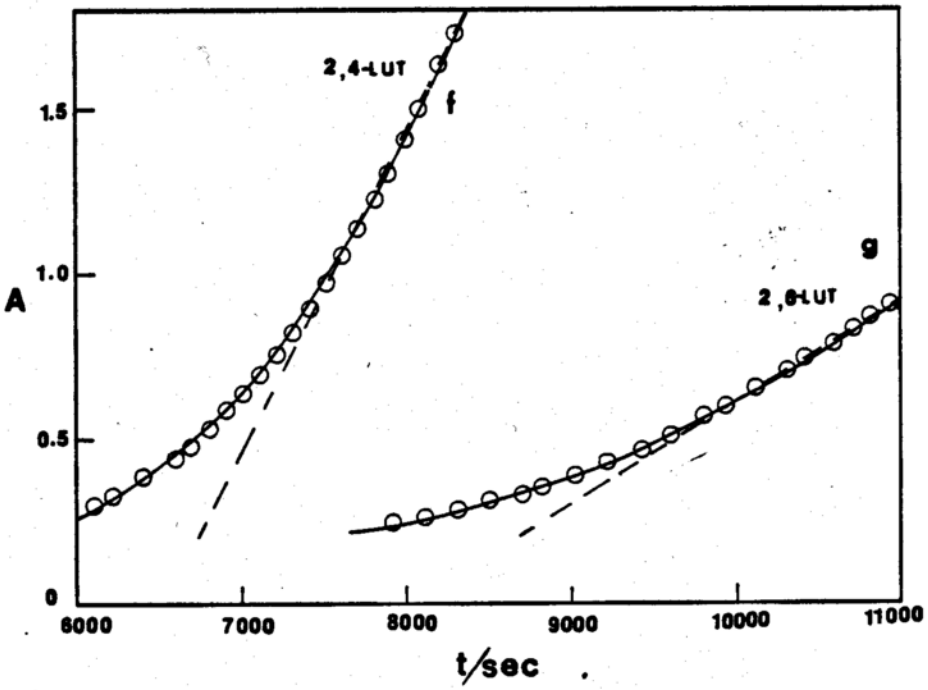


Figure 47 continued. Absorption-time Plots for:

f. 2,4-lutidine; g. 2,6-lutidine.

compounds. Table XVI summarizes the initial rates, while Figure 48 is the Bronsted plot of these data. The Bronsted plot for the aromatic amine (pyridine and substituted pyridines) series presents two families of curves. The sterically hindered amines 2,4-lutidine, 2,6-lutidine and 2,4,6-collidine deviate markedly from the linearity exhibited by pyridine, 3-picoline, 4-picoline, and 3,4-lutidine. The least squares line through pyridine, 3-picoline, 4-picoline, and 3,4-lutidine has a correlation coefficient of 0.99, and a slope of 1.0.

Table XVI. pKa Values and Initial Rates for the Aromatic Amine Series.[a]

Amine	pKa	Initial Rate/ absorbance sec^{-1} x 10^3	Log (Initial Rate)
Pyridine	5.23	8.45	-2.07
3-Picoline	5.73	18.2	-1.74
4-Picoline	6.04	46.3	-1.33
3,4-Lutidine	6.46	139	-0.858
2,4-Lutidine	6.63	0.974	-3.01
2,6-Lutidine	6.72	0.310	-3.51
2,4,6-Collidine	7.75	3.26	-2.49

[a]In all cases, 1.0 ml of a 10^{-2}M acetic anhydride solution of the amine was reacted with 2.0 ml of 1.75×10^{-2}M of carbon suboxide.

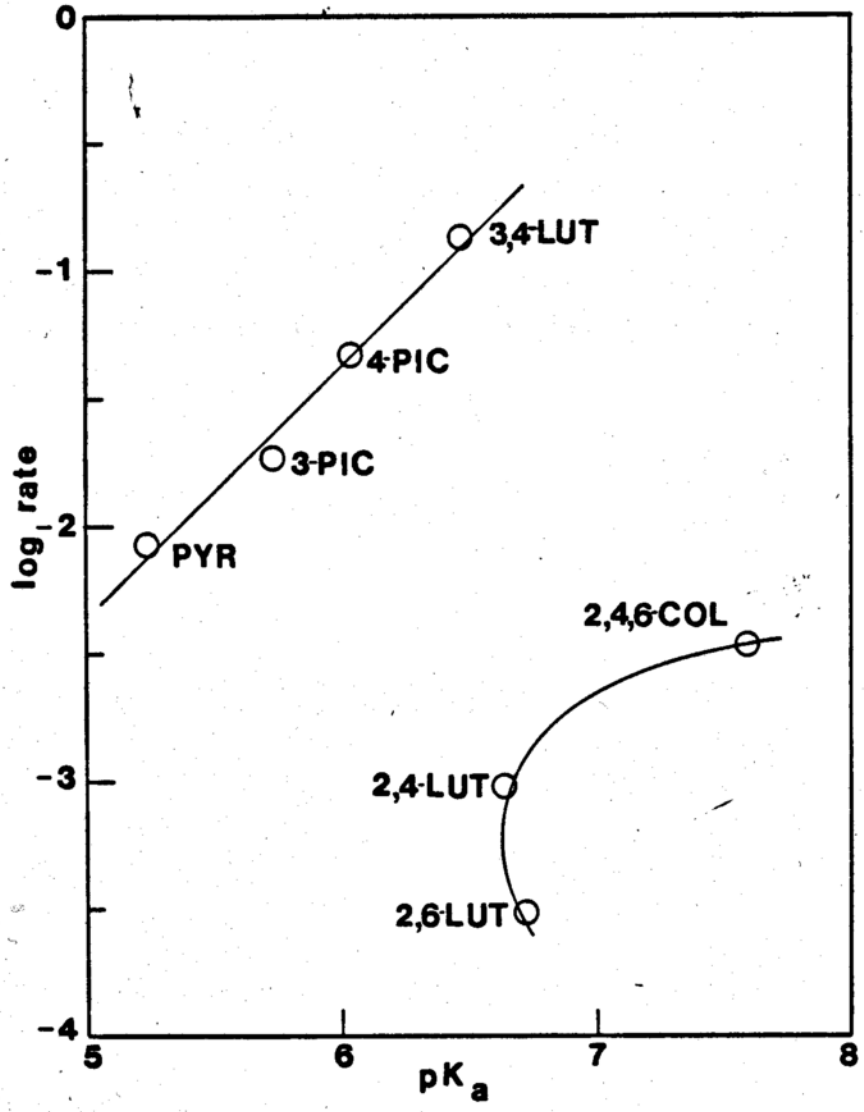


Figure 48. Bronsted Plot for the Aromatic Amine Series.

E. Concentration and Time-Dependence Investigations

Various analytical methodologies, including GC, HPLC, and UV-visible spectrophotometry, were used to monitor the appearance of product, or the disappearance of reactants, as a function of time. In all the concentration and time-dependence studies, the concentration of the amine compound was always smaller than that of carbon suboxide. When a reaction system involved use of acetic anhydride, it was always present in huge excess. The details of experimental conditions are presented in Chapter II.

1. Kinetics of the Triethylamine Reaction System

Figure 49 shows absorbance-time plots for four concentrations of triethylamine (7.0×10^{-4}M, 6.0×10^{-4}M, 5.0×10^{-4}M, and 3.0×10^{-4}M) reacted with carbon suboxide (2.25×10^{-2}M) in the presence of excess acetic anhydride. Initial rates were measured on the linear portions immediately beyond the lag time phase of these absorbance-time plots; the least squares line from which the initial rates (slopes) were obtained have been included in the figure. Table XVII gives the initial rate values as a function of the concentration of triethylamine, as well as the logarithmic values of the initial rates. Figures 50 and 51 are plots of the data from Table XVII.

Color development in the reaction system takes place only in the presence of the amine compound. The conditions of the reaction

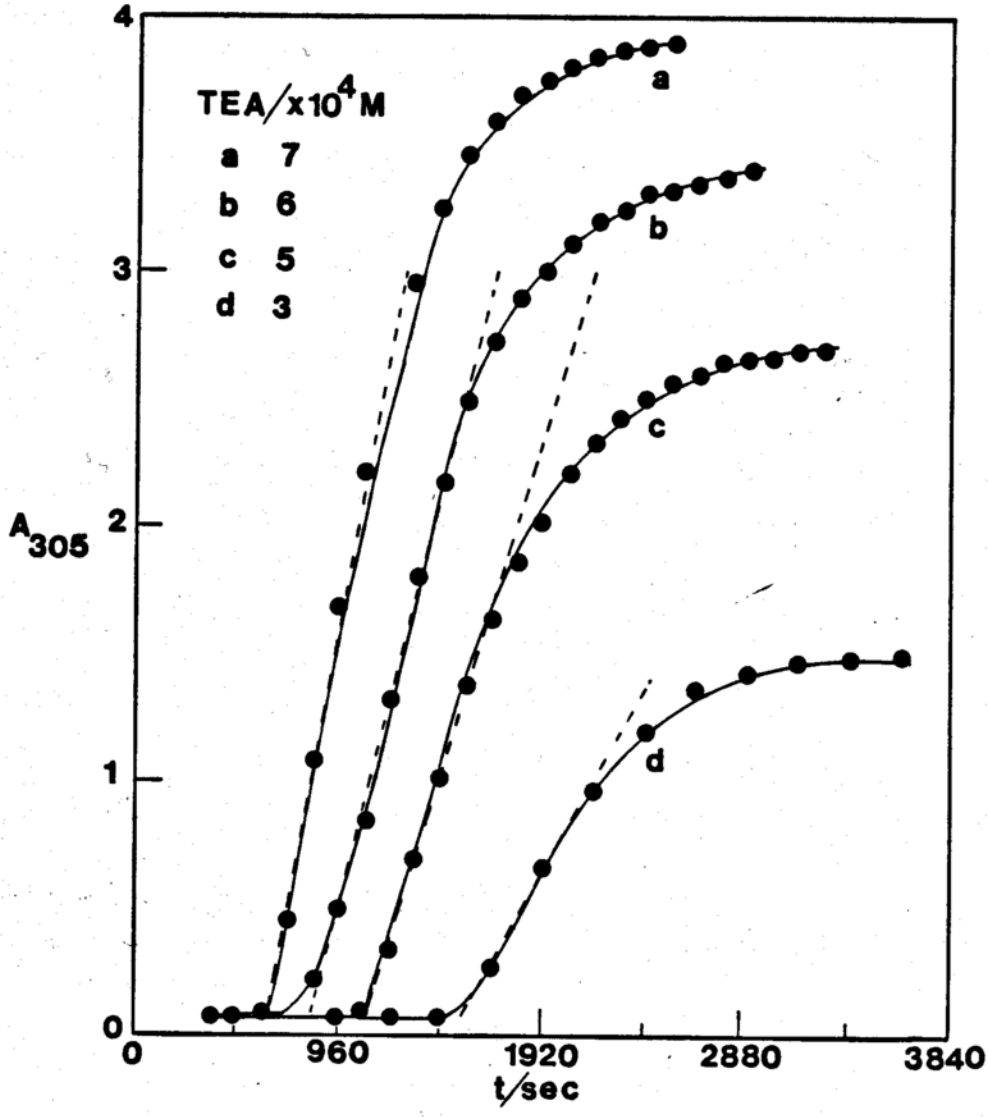


Figure 49. Absorbance-time Plots for the Reaction between TEA and Carbon Suboxide in the Presence of Acetic Anhydride.

Table XVII. Initial Rate (k_{obs}^{in}) Values as a Function of Triethylamine Concentration for the Reaction between Triethylamine and Carbon Suboxide in the Presence of Acetic Anhydride.

TEA/M x 10^4	k_{obs}^{in}/absorbance sec^{-1} x 10^3	$\frac{k_{obs}^{in}}{[C_3O_2]}$	log k_{obs}^{in}
7.0	4.58	0.20	-2.34
6.0	3.56	0.16	-2.45
5.0	2.72	0.12	-2.56
3.0	1.48	0.066	-2.83

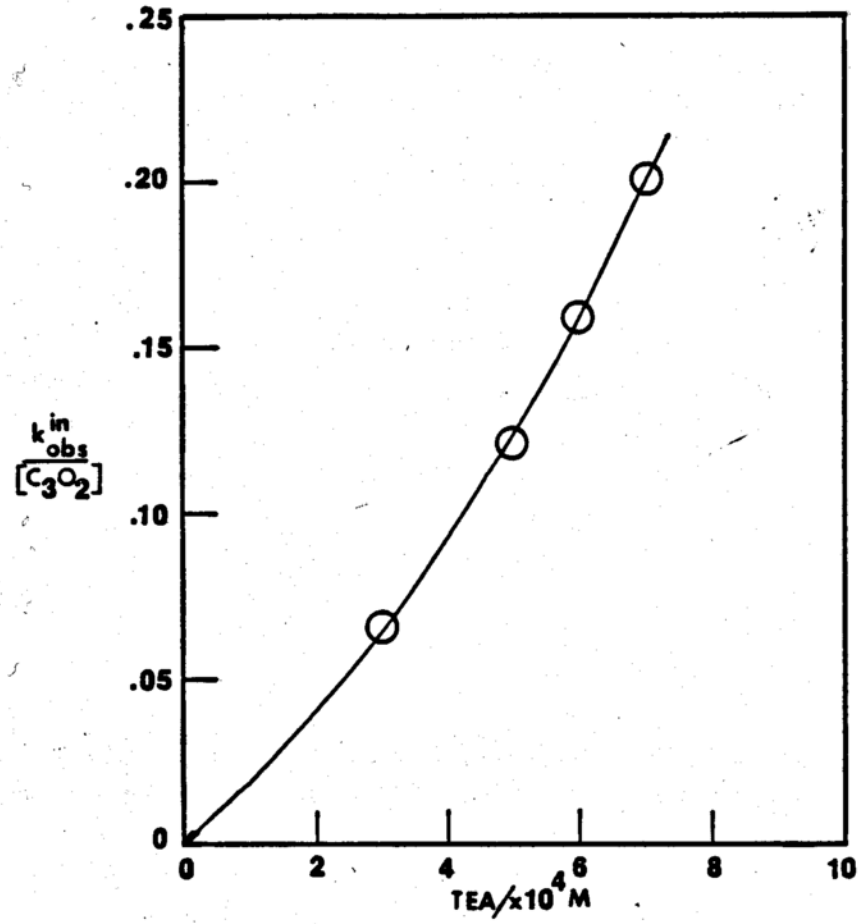


Figure 50. Plot of the Ratio $k^{in}_{obs}/[C_3O_2]$ as a Function of TEA Concentration.

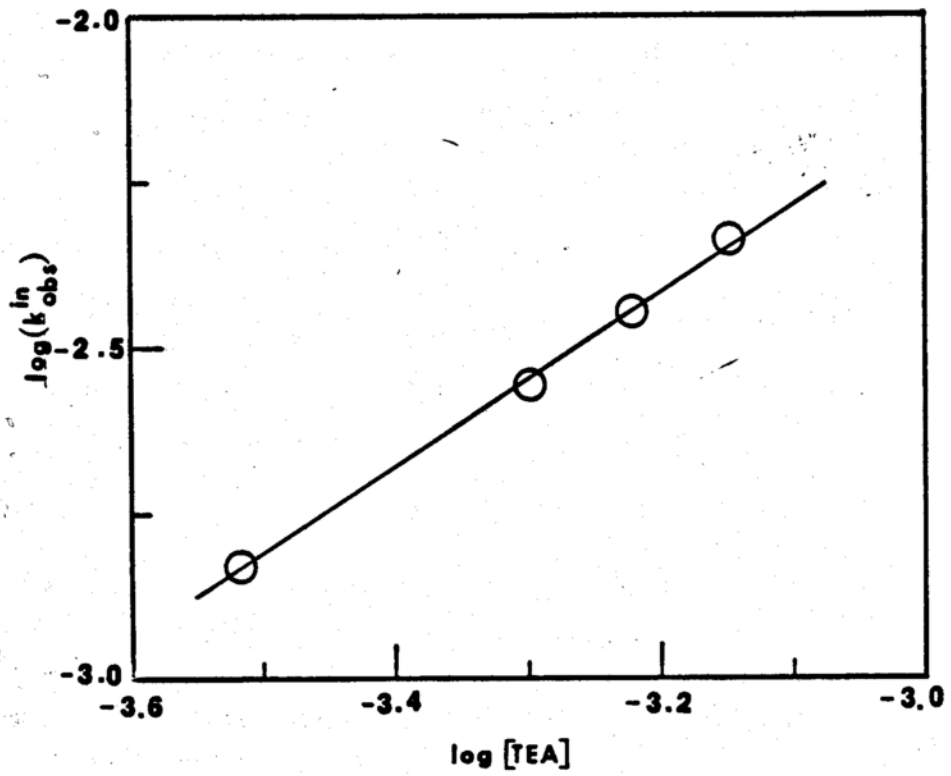


Figure 51. Plot of Log k_{obs}^{in} as a Function of log [TEA].

are such that the suboxide concentration is much larger than the triethylamine concentration. Therefore, the observed initial rate (k_{obs}^{in}) may be expressed as

$$k_{obs} = (k_0 + k_1 [TEA]^n) [C_3O_2] \qquad (1)$$

where k_0 is the rate constant at zero triethylamine concentration, and n is the order with respect to triethylamine. Based upon our knowledge of the reaction system, k_0 is zero. Figure 50 shows the non-linearity exhibited when the ratio $k_{obs}^{in}/[C_3O_2]$ is plotted as a function of triethylamine concentration. The least squares line through the points in the plot of log (k_{obs}^{in}) as a function of log [TEA], Figure 51, has a correlation coefficient (r^2) of 0.99, and a slope of 1.3, which is the apparent order (n) with respect to triethylamine.

We also undertook a kinetic investigation of the reaction between triethylamine and carbon suboxide in the absence of acetic anhydride. The disappearance rate of carbon suboxide was monitored at 265 nm; equal volumes (1.5 ml) of ether solutions of the reagents were mixed in a cuvette to initiate the reaction. Table XVIII gives the molar concentrations of carbon suboxide as a function of time in the reaction between carbon suboxide and four triethylamine concentrations. Table XIX gives the points on the tangent line drawn to the concentration-time plots at t = 0. The initial velocities (v_0), as well as their logarithmic values, obtained as a

Table XVIII. Molar Concentrations of Carbon Suboxide as a Function of Time in the Reaction between Carbon Suboxide and Four Triethylamine Concentrations.

	TEA/M x 10^3			
t/sec	3.0	2.55	2.35	2.00
0	0.0117	0.0117	0.0117	0.0117
150	0.0104	0.0106	0.0111	0.0113
300	0.00957	0.00983	0.0103	0.0105
450	0.00915	0.00932	0.00970	0.00996
600		0.00894	0.00936	0.00953
750				0.00919

Table XIX. Points on the Tangent Line Drawn to the Concentration Time Plot at t = 0 for Four Triethylamine Concentrations.[a]

	TEA/M x 10^3			
t/sec	3.0	2.55	2.35	2.00
0	0.0117	0.0117	0.0117	0.0117
75	0.0110	0.0111	0.0113	0.0114
150	0.0103	0.0106	0.0108	0.0110
225	0.00960	0.0100	0.0104	0.0106

[a]The numbers in the table refer to the molar concentrations of carbon suboxide.

function of triethylamine concentration, are listed in Table XX. Figure 52 is a log-log plot of the data from Table XX. The least squares line through the data points has a correlation coefficient of 0.98 and a slope of 1.7, the apparent order with respect to triethylamine.

2. Kinetics of the Pyridine Reaction System

HPLC was used to study the kinetics of the reaction between pyridine (12.0×10^{-3}M, 8.0×10^{-3}M, 5.0×10^{-3}M, and 3.0×10^{-3}M) and carbon suboxide (2.52×10^{-2}M) in the presence of excess acetic anhydride. Portions of the reaction mixture were diluted 1 in 5 ml with the acetonitrile-water mobile phase prior to injecting into the HPLC column. Figure 53 shows the variation of peak height (H_t) as a function of time for the four pyridine concentrations. Figure 54 is the semi-logarithmic pseudo first-order plot from which the rate constants (k_{obs}), shown in Table XXI, were evaluated. Although each of these lines is reasonably linear (given the uncertainty in peak height estimates), their slopes, and therefore k_{obs}, vary with initial concentration, hence the reaction cannot be first-order in pyridine. A log-log plot gives, from the slope (Figure 55), an apparent order of 0.63. Figure 56 shows the data plotted in another way: this apparent 'saturation' behavior is suggestive of a rate-controlling complexation.

Table XX. Initial Velocities as a Function of Triethylamine Concentration.

TEA/M x 10^3	v_0/M sec^{-1} x 10^6	log v_0
3.00	9.3	-5.03
2.55	7.5	-5.13
2.35	5.6	-5.25
2.00	4.7	-5.33

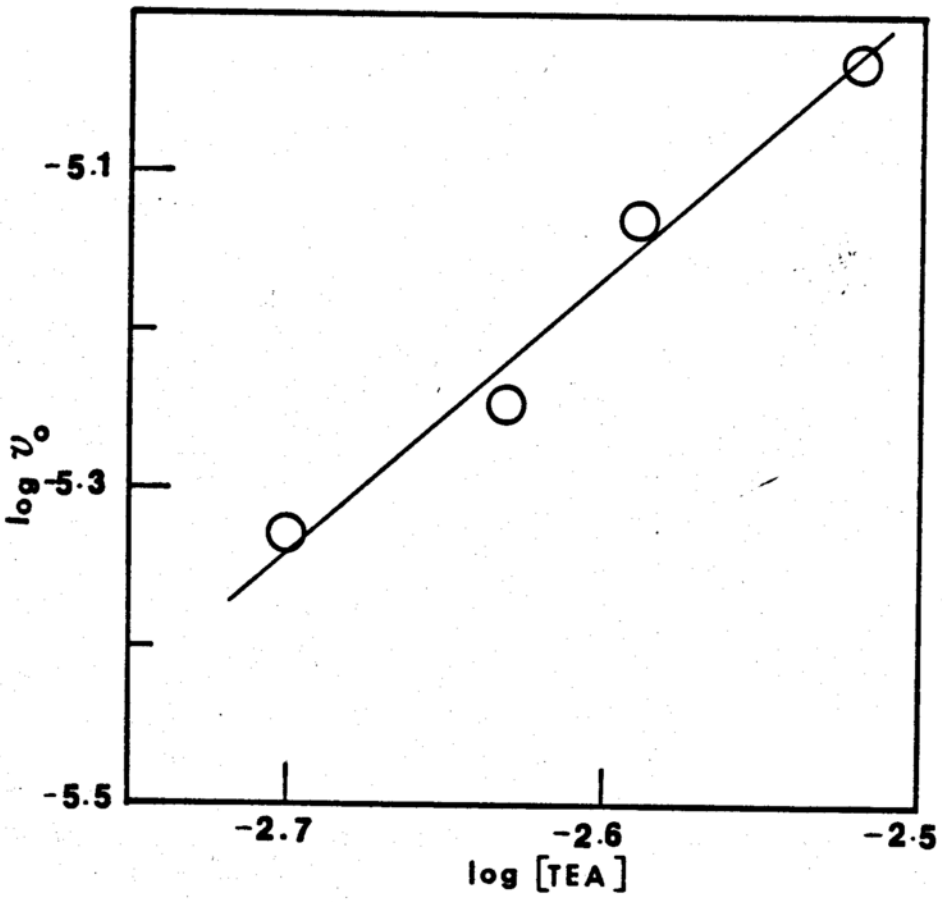


Figure 52. Plot of log v_0 as a Function of log[TEA].

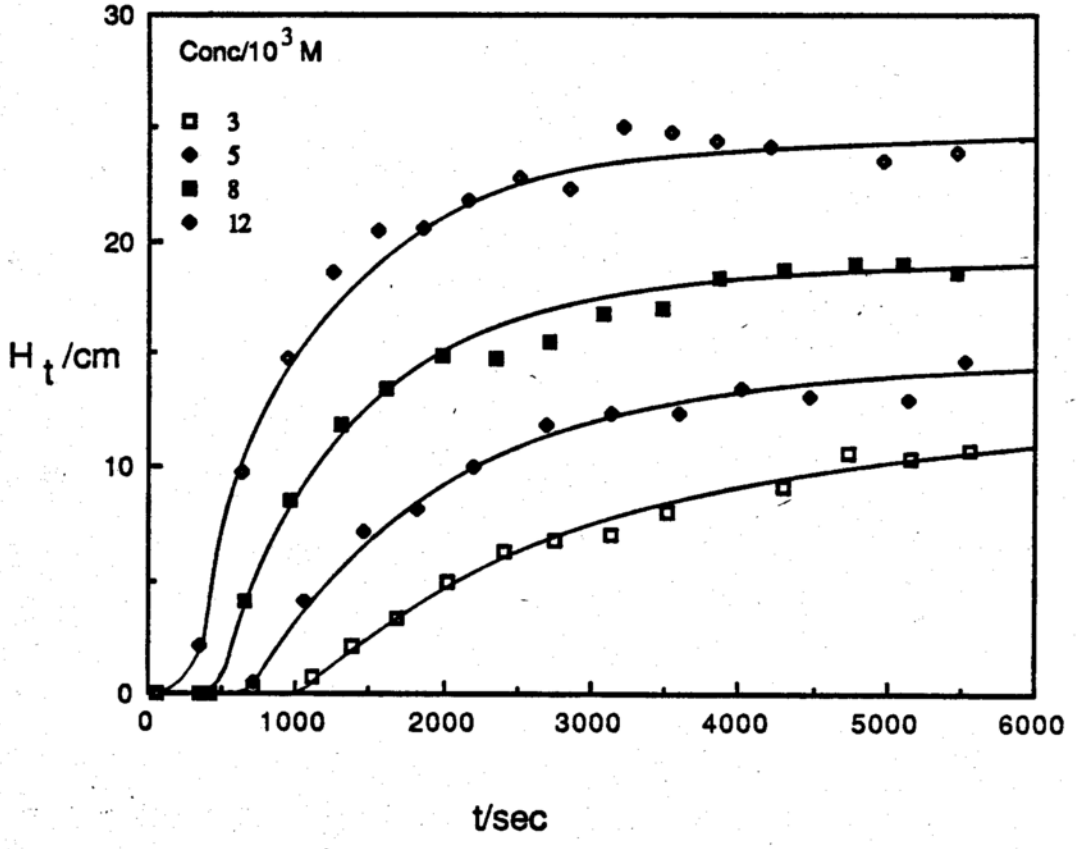


Figure 53. HPLC Data on the Reaction between Pyridine and Carbon Suboxide in the Presence of Acetic Anhydride.

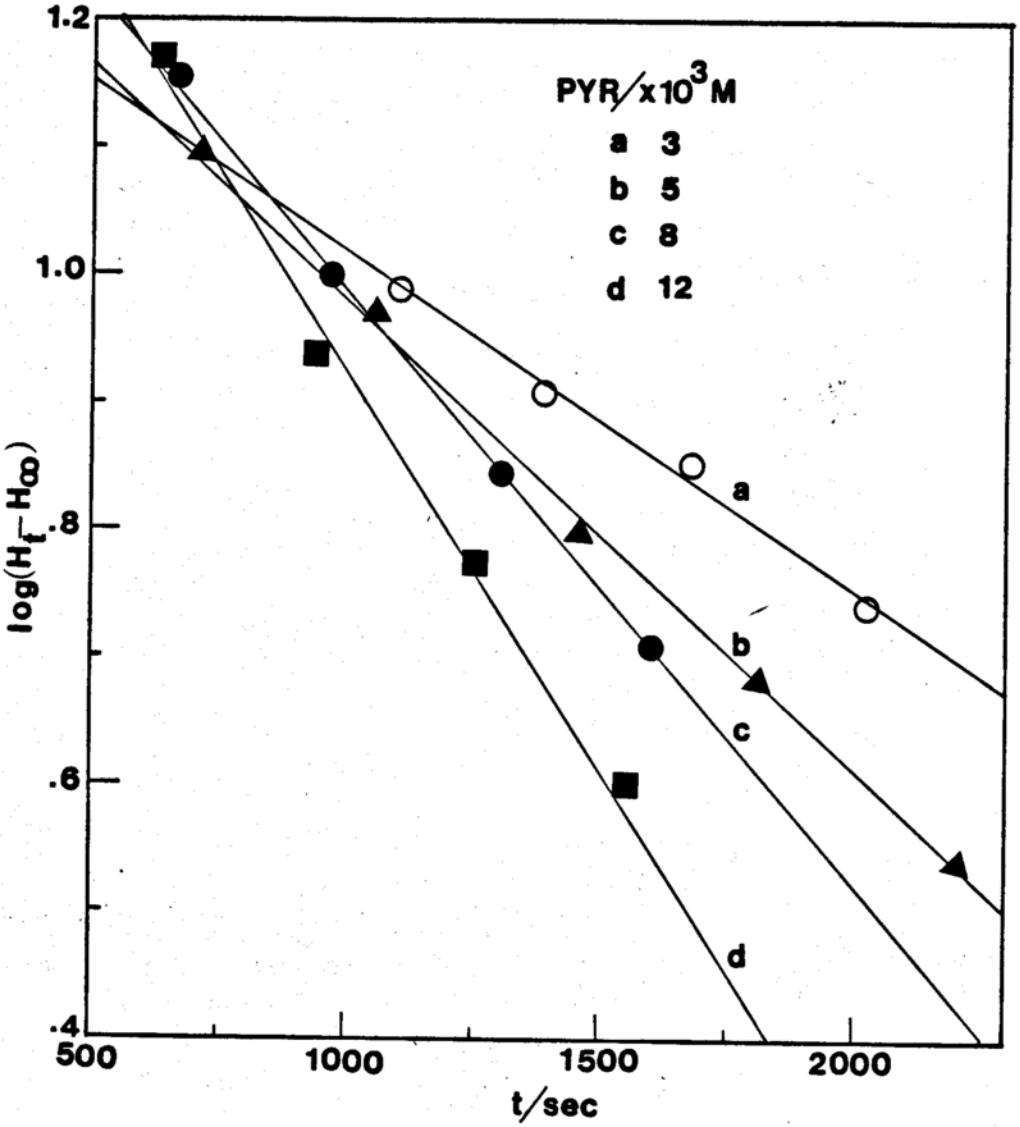


Figure 54. Semi-logarithmic Pseudo First-order Plot of the Pyridine Data.

Table XXI. Rate Constants for the Reaction of Pyridine with Carbon Suboxide in the Presence of Acetic Anhydride.

PYR/M x 10^3	k_{obs}/sec^{-1} x 10^4	log k_{obs}
3.0	5.99	-3.22
5.0	8.75	-3.06
8.0	11.0	-2.96
12.0	14.7	-2.83

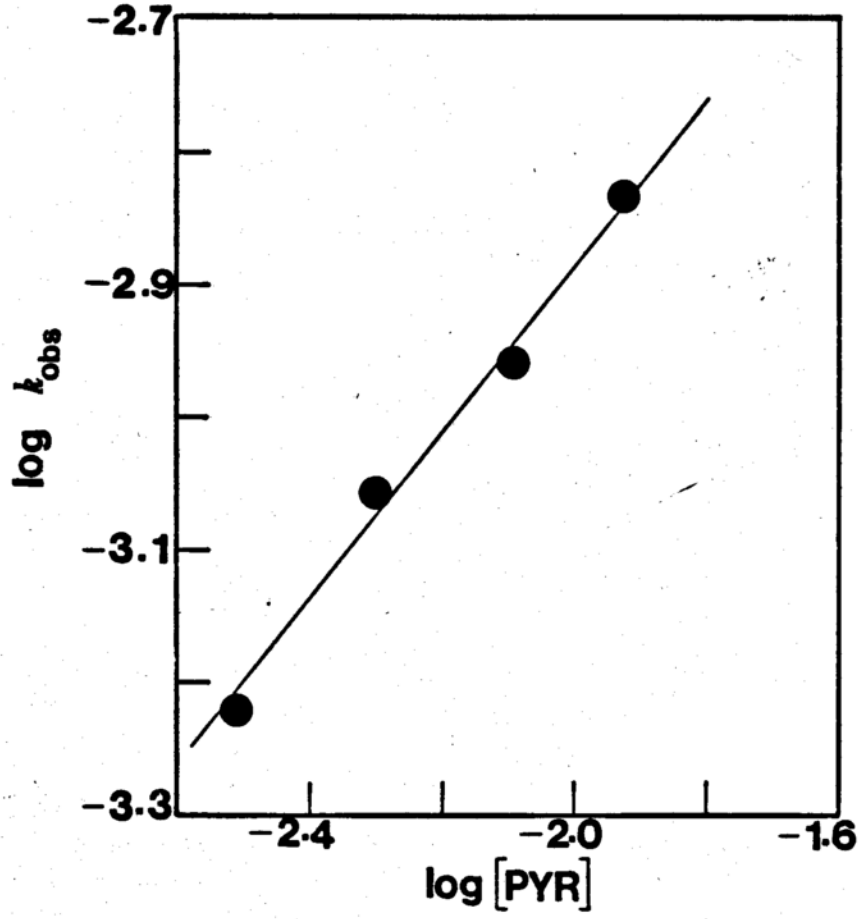


Figure 55. Plot of log k_{obs} as a Function of log [PYR].

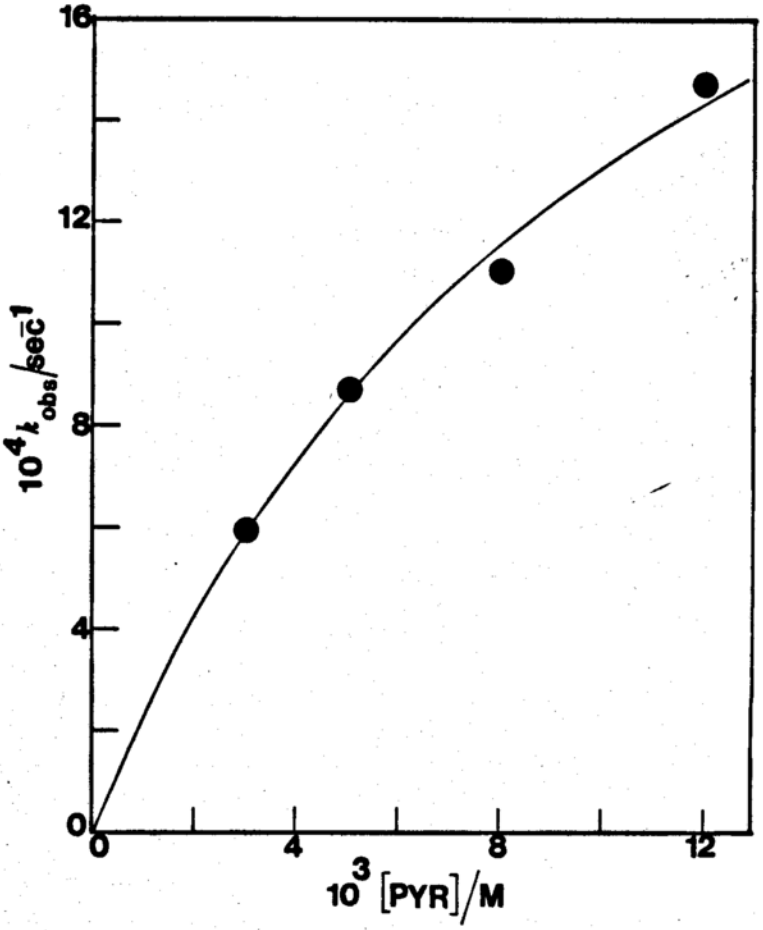


Figure 56. Plot of k_{obs} as a Function of Pyridine Concentration.

3. Kinetics of n-tripropylamine and Carbon Suboxide Reaction Systems

The isolation technique was used to investigate the order with respect to the concentration of both n-tripropylamine (TPA) and carbon suboxide. In both cases, the decrease in the concentration of carbon suboxide as a function of time was monitored at 265 nm. Initial rate (velocity) measurements were used to evaluate the apparent reaction orders. In one case, ether solutions of TPA (5.0×10^{-3}M, 4.5×10^{-3}M, 4.0×10^{-3}M, and 3.0×10^{-3}M) were reacted with carbon suboxide (2.66×10^{-3}M) solutions in equal volume ratios. In the other case, varying concentrations of carbon suboxide (2.61×10^{-2}M, 1.22×10^{-2}M, 2.39×10^{-2}M, 1.86×10^{-2}M, and 1.57×10^{-2}M) were reacted with a constant initial concentration of TPA (10^{-2}M).

Figure 57 shows plots of the concentration-time behavior of the reaction system at different TPA concentrations. The tangent lines, drawn to the concentration-time plots at $t = 0$, have also been inserted for clarity. The slopes of the tangent lines were used as estimates of the initial velocity (v_0). Figure 58 is a log-log plot of v_0 as a function of TPA concentration. The apparent reaction order with respect to the concentration of TPA is 1.4. Figure 59 shows the variation of initial velocity as a function of TPA concentration. The initial velocity appears to be linearly dependent upon the concentration of TPA.

Figure 60 is a plot of the concentration-time behavior of the

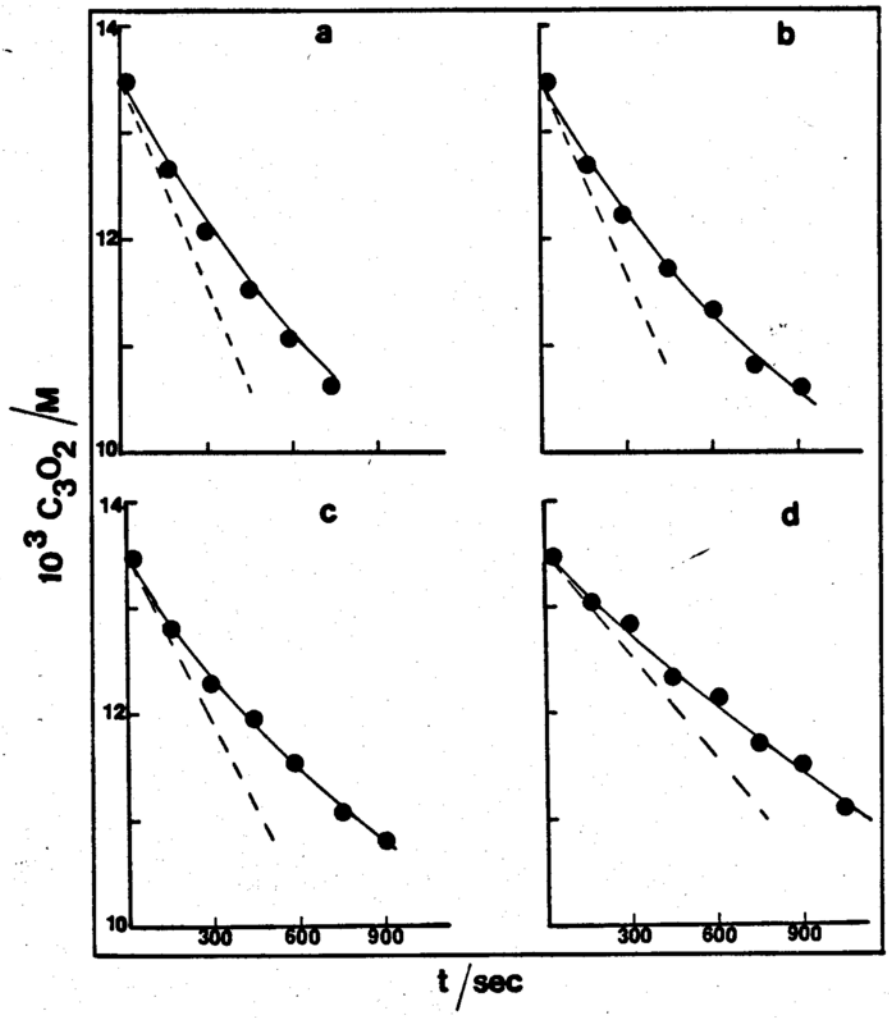


Figure 57. Concentration-time Behavior for the Reaction between Carbon Suboxide and Four TPA Concentrations. Key: a. 5.0×10^{-3}M; b. 4.5×10^{-3}M; c. 4.0×10^{-3}M; d. 3.0×10^{-3}M.

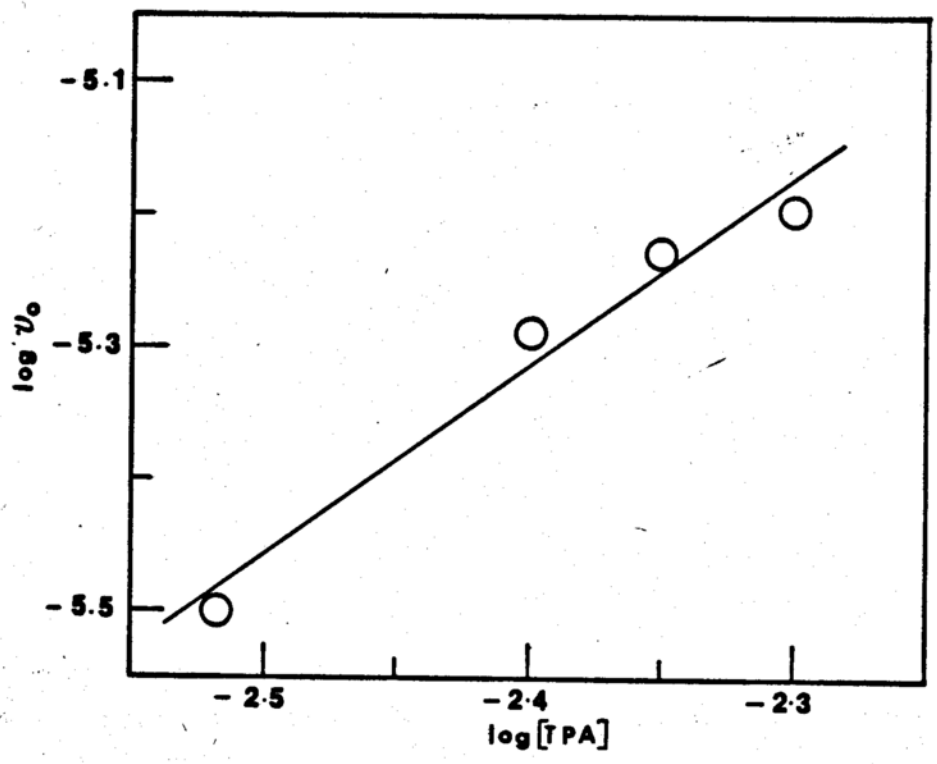


Figure 58. Log v_0 <u>versus</u> log [TPA].

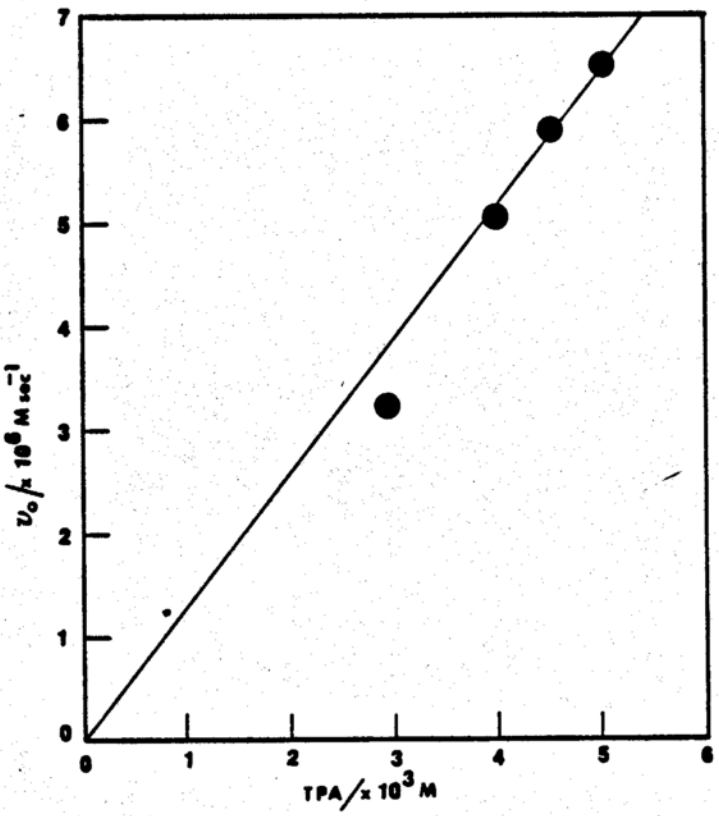


Figure 59. Variation of Initial Velocity as a Function of TPA Concentration.

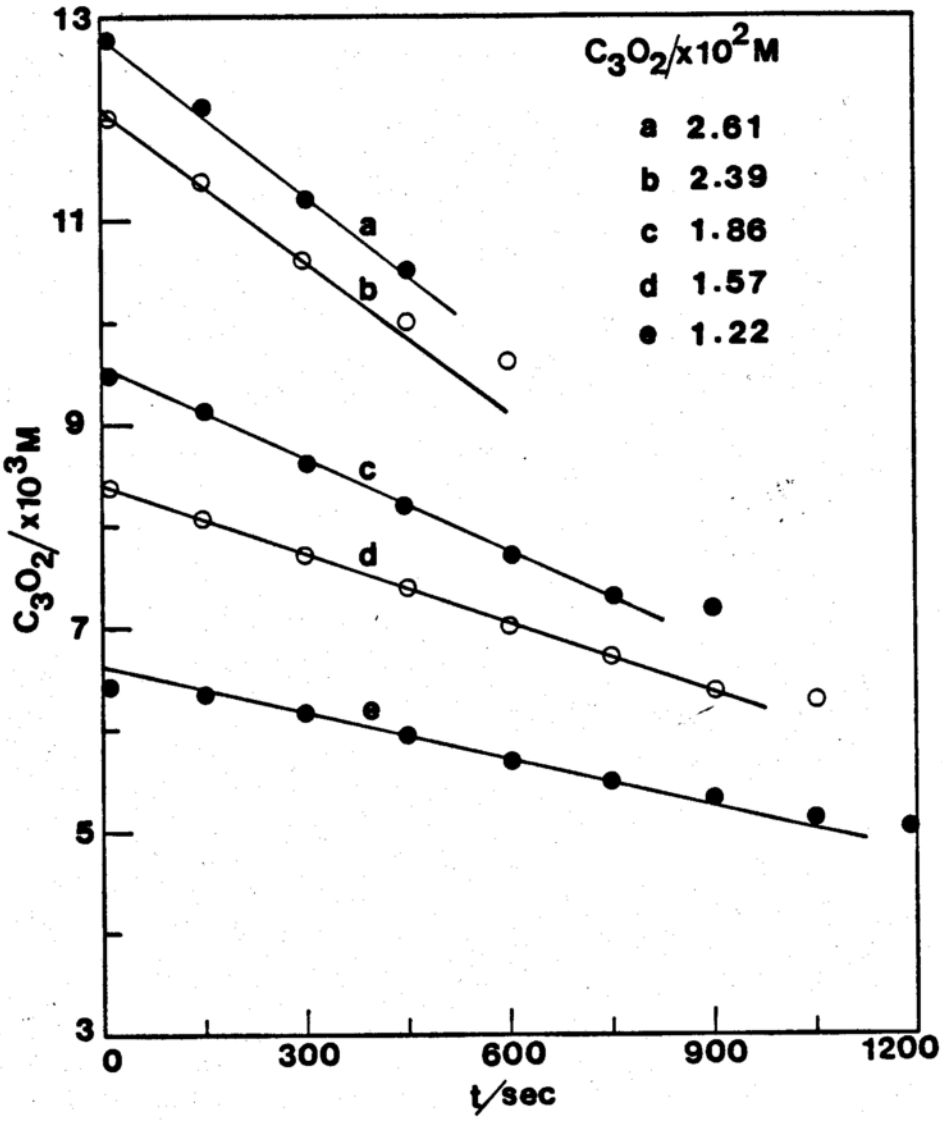


Figure 60. Concentration-time Behavior of the Reaction between TPA and Five Carbon Suboxide Concentrations.

reaction between TPA and different carbon suboxide concentrations. There is a slight curvature in the concentration-time profile, which is to be expected. Estimates of the initial velocity were based upon the slope of the least squares line through the concentration-time points prior to the curvature. These least squares lines are inserted in the figure. Figure 61 is a plot of the logarithm of these initial velocities as a function of the logarithm of carbon suboxide concentration. The least squares line through these points has a slope of 2.0, the apparent order with respect to carbon suboxide.

4. Kinetics of the n-tributylamine Reaction System

Kinetics of the reaction between n-tributylamine (TBA) and carbon suboxide were studied using gas chromatography. Conditions for the gas chromatography are given in section D.1, Chapter III. Reactions were initiated by adding microliter quantities of TBA to aliquots of carbon suboxide (2.60×10^{-2}M) to give these initial concentrations in TBA: 10×10^{-3}M, 8.4×10^{-3}M, 5.6×10^{-3}M, and 3.5×10^{-3}M. Samples were withdrawn from the reaction mixture as a function of time and injected into the GC instrument.

Figure 62 is a plot of peak height as a function of time. Semi-logarithmic pseudo first-order plots of the data appeared to be linear, given the variability in peak heights. The slopes (observed rate constants) of these plots, see Table XXII, were constant with a mean value of $1.08 \times 10^{-3} \pm 6.52 \times 10^{-5}$ sec^{-1}.

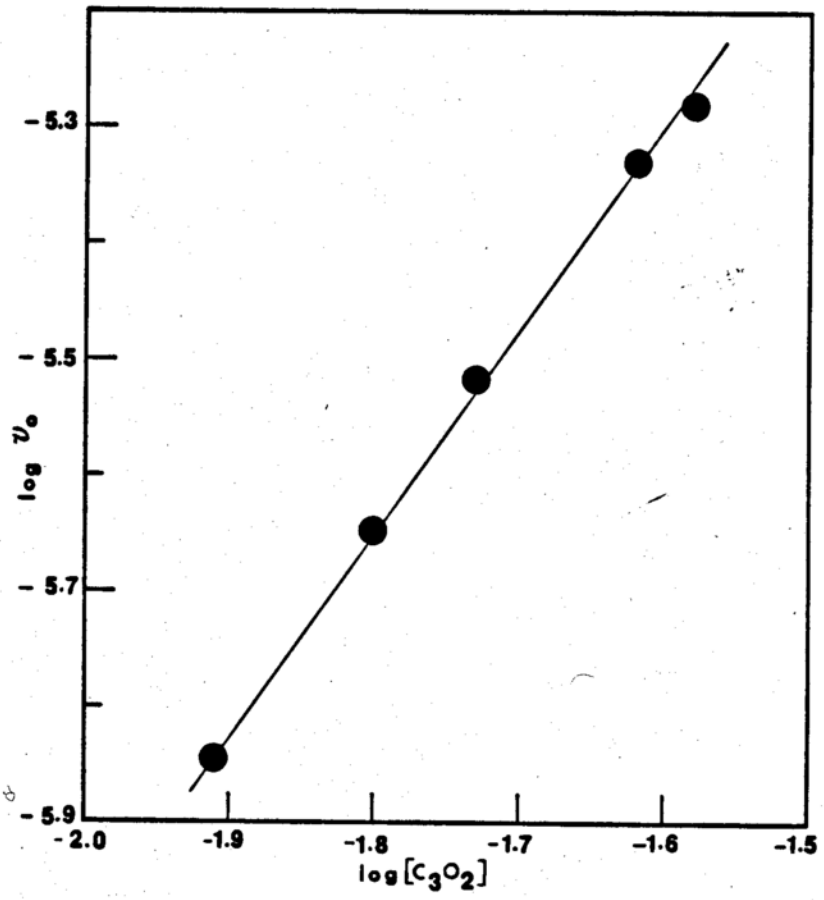


Figure 61. Log v_0 <u>versus</u> log $[C_3O_2]$.

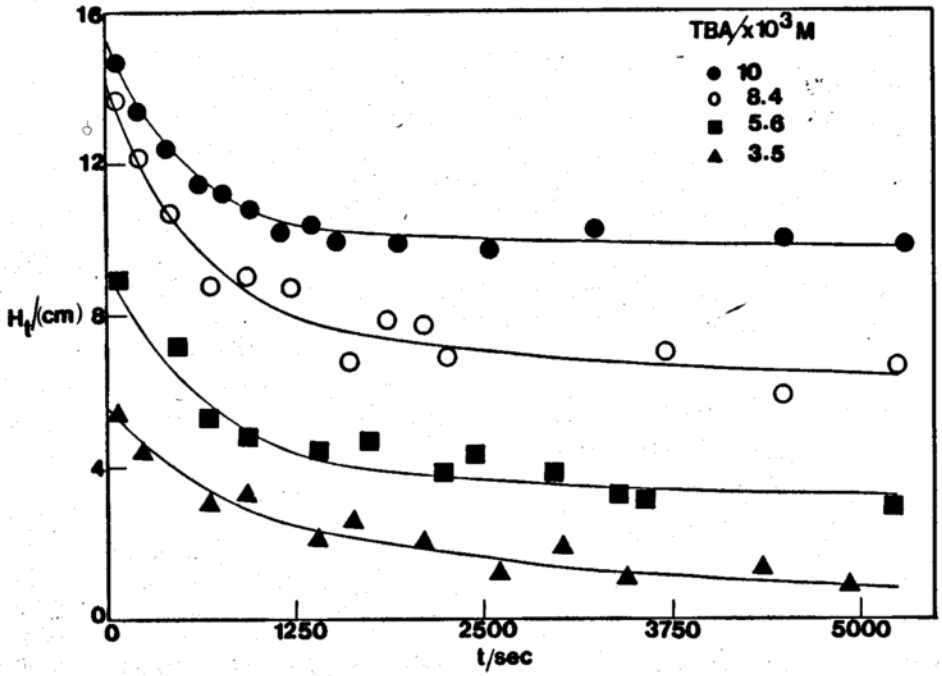


Figure 62. Concentration-time Profile of TBA.

Table XXII. First-order Rate Constants for the Reaction between TBA and Carbon Suboxide.

TBA/M x 10^3	k_{obs}/sec x 10^3
10	1.70
8.4	1.02
5.6	1.15
3.5	1.07

IV. DISCUSSION

As demonstrated previously, a number of organic polycarboxylic acids are capable of forming a colored product when reacted with a tertiary amine compound in the presence of acetic anhydride. The reaction mechanism and pathway leading to formation of the colored product(s) probably varies as a function of the identity of the organic acid, and of the tertiary amine compound. Given the strongly dehydrating conditions under which these analytical reactions are conducted, it is possible that the initial reaction pathway goes through the anhydride species of the organic acid. Experimentation with the reaction between malonic anhydride (carbon suboxide) and tertiary amine compounds with or without acetic anhydride, indicates that the mechanism probably is complex. There may be other side reactions in addition to the reaction process leading to color formation.

A. Kinetic Observations

1. Fate of the Amines

Figures 41 to 44 show gas chromatographic data concerning the

fate of some of the amine compounds that have been used. There is a clear distinction in the fate of the aromatic and aliphatic amines. The gas chromatographic evidence indicates that there is a decrease in the concentration of the aliphatic amines TEA, TPA, and TBA as a function of time; see Figures 42 to 43. Thus these amines are probably becoming incorporated as part of the product molecule(s). The concentration of the aromatic amine, pyridine, on the other hand, appears not to have changed as a function of time.

There is an apparent conflict between the gas chromatographic finding that pyridine is not consumed and the results of the kinetic investigation of the reaction between carbon suboxide and pyridine in the presence of excess acetic anhydride using HPLC. In light of the GC finding, it is probably surprising that the HPLC-kinetic data reach apparent limiting values proportional to the concentration of pyridine. It is possible, however, that although we are treating this as a kinetic phenomenon, the controlling factors during the time-invariant period of the absorbance-time curve may not be influenced predominantly by the concentration of pyridine. But the concentrations of the two other components of the reaction mixture, carbon suboxide and acetic anhydride, are much higher than pyridine. So that, unless there are other side reactions, which are using up carbon suboxide, a situation that is very probable, it is difficult to conceptualize how else these apparent limiting values are attained. A plot of the observed rate constant as a function of pyridine concentration, Figure 56, showed an apparent 'saturation'

behavior, which is suggestive of a rate-controlling complexation. The phenomenon of 'saturation' and, therefore, complexation does not have to involve pyridine; it can occur with either the catalyst (pyridine) or other reactant(s) (87).

2. Orders of Reaction

The apparent orders of reaction with respect to the concentration of amine and of carbon suboxide are given in Table XXIII. Although pseudo first-order conditions in the amine concentration were applied in all reaction systems, except for the reaction of TBA with carbon suboxide, none of these reaction systems appeared to be first order in the amine concentration. The apparent order with respect to TEA concentration is 1.3 in the TEA-C_3O_2-AC_2O reaction system, while it is 1.7 in the TEA-C_3O_2 reaction system. It is unclear if these numbers do in fact represent fractional orders. Moreover, not enough is known concerning the events during the lag time phase.

For the reaction systems TEA-C_3O_2, TPA-C_3O_2, and TBA-C_3O_2, the apparent orders of reaction are 1.7, 1.4, and 1.0, respectively. Although there may not be a significance to the numbers as far as being representative of the apparent orders, the trend appears to suggest the extent of incorporation of the amine compound into the product. Gas chromatography of supernatants of reaction mixtures similarly constituted indicated that the extent of consumption of amine in the reaction process was approximately 50, 40, and 30% of

Table XXIII. Apparent Orders of Reaction with Respect to Amine and to Carbon Suboxide.

Reaction System[a]	Apparent order with respect to	
	Amine	C_3O_2
TEA-C_3O_2-Ac_2O	1.3	-
TEA-C_3O_2	1.7	-
PYR-C_3O_2-Ac_2O	0.63	-
TPA-C_3O_2	1.4	-
C_3O_2-TPA	-	2.0
TBA-C_3O_2	1.0	-

[a]Only the concentration of the first listed compound was varied during the reaction process.

initial concentration for TEA, TPA, and TBA, respectively; see Figures 42 to 43.

3. The Lag-time Phenomenon

The analytical reaction of carbon suboxide with amine compounds in the presence or absence of acetic anhydride is preceded by a lag time (induction) phase; an induction period had previously been reported during the thermal polymerization reactions of carbon suboxide (64, 66). The lag time is sharply defined; the 'sharpness' being approximately equally defined for both aromatic and aliphatic amines. We have established the dependence of the lag time phase on the concentrations of carbon suboxide, of pyridine and triethylamine, of acetic anhydride, and on the basicity (pKa) of aliphatic and aromatic amine compounds.

Figure 63 is a combined plot showing the dependence of lag time on the basicity of aliphatic and aromatic amines. It is interesting to note the similarity between this plot and the Bronsted-type plot; see section A.4(b). Figure 63 presents three families of curves: the aliphatic amines fall on a least squares line having a slope of approximately 0.2, pyridine and the sterically unhindered pyridine derivatives fall on a least squares line having a slope of approximately 1, while the sterically hindered pyridines fall somewhere between the two mentioned classes. This empirical correlation may have some implication concerning the mechanism of catalysis by these compounds.

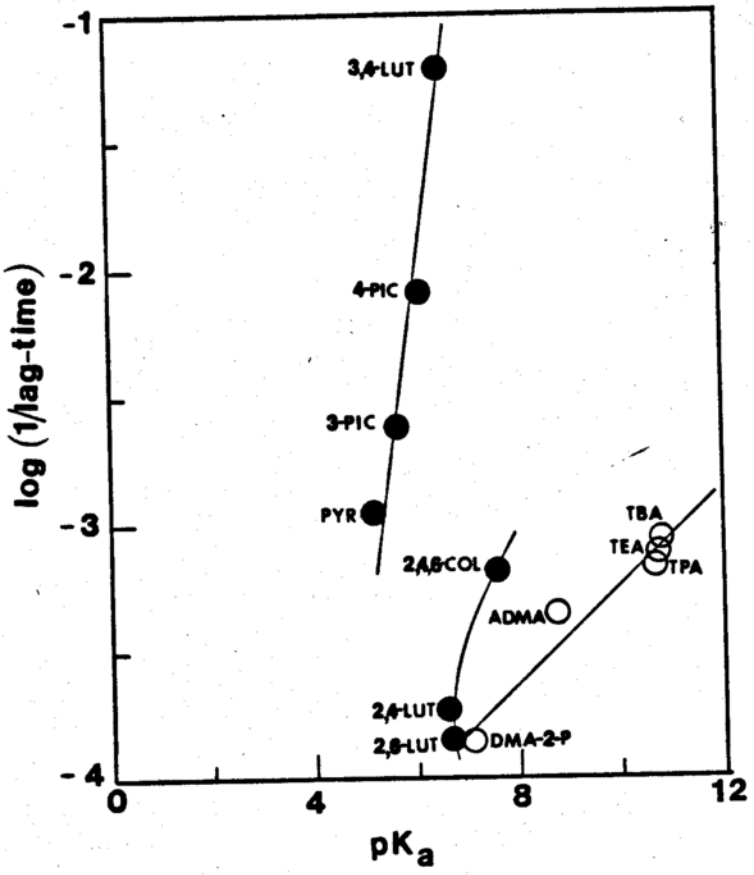


Figure 63. Combined Plot of log(1/lag/time) as a Function of pKa for Aromatic and Aliphatic Amine Compounds.

The empirical correlation of the logarithm of the inverse of the lag time as a function of the logarithm of the concentration of the various chemical entities upon which the lag time depends, possibly has the significance of a log (k_{obs}) *versus* log (concentration) plot. That is, the slope of such a plot may possibly have the significance of an 'apparent reaction order.' Plots of log (1/lag-time) as a function of log (concentration) were presented in Figures 30 and 33. A summary of the slopes of these plots is given in Table XXIV. The slope ('apparent order') with respect to carbon suboxide concentration differs depending on the identity of the amine, being 0.41 with pyridine and 1.1 with triethylamine. However, the 'apparent order' with respect to the concentration of pyridine and of triethylamine is 1.0 and 1.3, respectively. It is not clear whether or not this is a reflection of the true kinetic process during the lag time phase.

4. Catalysis and the Bronsted Relationship

a. General Considerations

The Bronsted catalysis law is strictly applicable to the description of catalysis involving proton transfer. But it has also been applied successfully to reactions that do not involve transfer of protons. If correlation is restricted to a limited range of reactivity and to structurally similar nucleophiles, there is usually a close correlation of nucleophilic reactivity with basicity

Table XXIV. Summary of Slopes of Plots of log (1/lag-time) as a Function of log (concentration).

Reaction System[a]	Slope[b] (apparent order)
C_3O_2-TEA-AC_2O	1.1
C_3O_2-PYR-AC_2O	0.41
TEA-C_3O_2-AC_2O	1.3
PYR-C_3O_2-AC_2O	1.0

[a,b]Only the concentration of the first listed compound was varied during the reaction. The apparent order, therefore, is with respect to the concentration of that compound.

for some reactions (88). Bruice and Lapinski (89) have shown such a correlation for the reaction of p-nitrophenyl acetate with a series of imidazoles, pyridines, anilines and anionic oxygen nucleophiles. Over a restricted range of basicity there is a good correlation of basicity or nucleophilicity with reactivity, but different classes of nucleophiles follow different lines.

The Bronsted catalysis law for basic compounds may be expressed by

$$k_B = G_B(1/K_a)^{\beta} \qquad (2)$$

where B and β refer to a base, and where K_a is the acid dissociation constant of the acid conjugate to the base B, or by

$$\log k_B = \beta pKa + \text{constant} \qquad (3)$$

Equation 3, which is applicable to nucleophilic as well as to general base catalyzed reactions, indicates that a plot of log k_B as a function of pKa should be linear with slope β. The magnitude of β provides an indication of the sensitivity of the reaction to the nucleophilicity (basicity) of the catalyst. It also provides insight into the extent of bond formation and charge development in the transition state. A large β value (>0.5) shows that the dependence on basicity is large, that basicity is a good model for the transition state, and that there is a large amount of positive

charge developed on the attacking nucleophile in the transition state (88). The reverse is true when the value of β is small.

Often, there is ambiguity concerning whether a catalyst is acting as a proton transfer agent (general base) or a nucleophile. For example, a Bronsted plot, i.e. a plot of the logarithm of the rate constant as a function of pKa, for a series of nucleophiles or bases could be characteristic of either a general base-catalyzed reaction or a nucleophile-catalyzed reaction. A Bronsted plot for a family of general bases adheres, with few exceptions, to a single line, independent of the structural characteristics of the bases making up the plot. On the other hand, a Bronsted-type plot of a series of structurally similar nucleophiles of differing steric requirements can show considerable deviations from a single line (87). In other words, in general base catalysis, basicity (as measured by pKa), and not steric requirements, determines where a compound would fall on the line in a Bronsted-type plot (90). The common ion effect sometimes is used as a criterion to distinguish between general basic and nucleophilic catalysis. For example, the addition of acetate ion to pyridine-catalyzed hydrolysis of acetic anhydride, significantly decelerates reaction, indicating nucleophilic catalysis by pyridine (91). But perhaps the strongest evidence that could be used to distinguish general base-catalyzed reactions from nucleophile-catalyzed reactions is the observation of a transient intermediate that could be identified as the intermediate of nucleophilic catalysis. The observation may be by

isolation of the hypothetical intermediate or by observation of the appearance and disappearance of the transient. Failure to detect an intermediate that should be observable by independent evidence is probable evidence for general base catalysis (87).

b. Bronsted-type Plot

In Figure 64 the logarithmic values of the initial rate constants for the reaction of various amine compounds with carbon suboxide in the presence of acetic anhydride are plotted versus pKa in the conventional Bronsted manner. A single line does not suffice to correlate all of the data for the pyridines. The least squares line through the structurally related pyridines has a slope (β value) of 1.0, which may indicate possible bond formation and high charge development in the transition state. The high β value also could possibly be an indication of a nucleophilic catalytic mechanism. But we hesitate to place any emphasis on this statement since we do not have independent evidence in support of nucleophilic catalysis. The use of a common ion (acetate ion) effect here is not possible because the acetate ion could, in this system, function as a base and give coloration via a different mechanism. The hindered pyridines, despite being more basic (higher pKa), deviate markedly from this line. The positions of these deviant amines on the Bronsted plot do not fall on the line for the aliphatic amines either.

It is quite possible that the pyridines are acting as

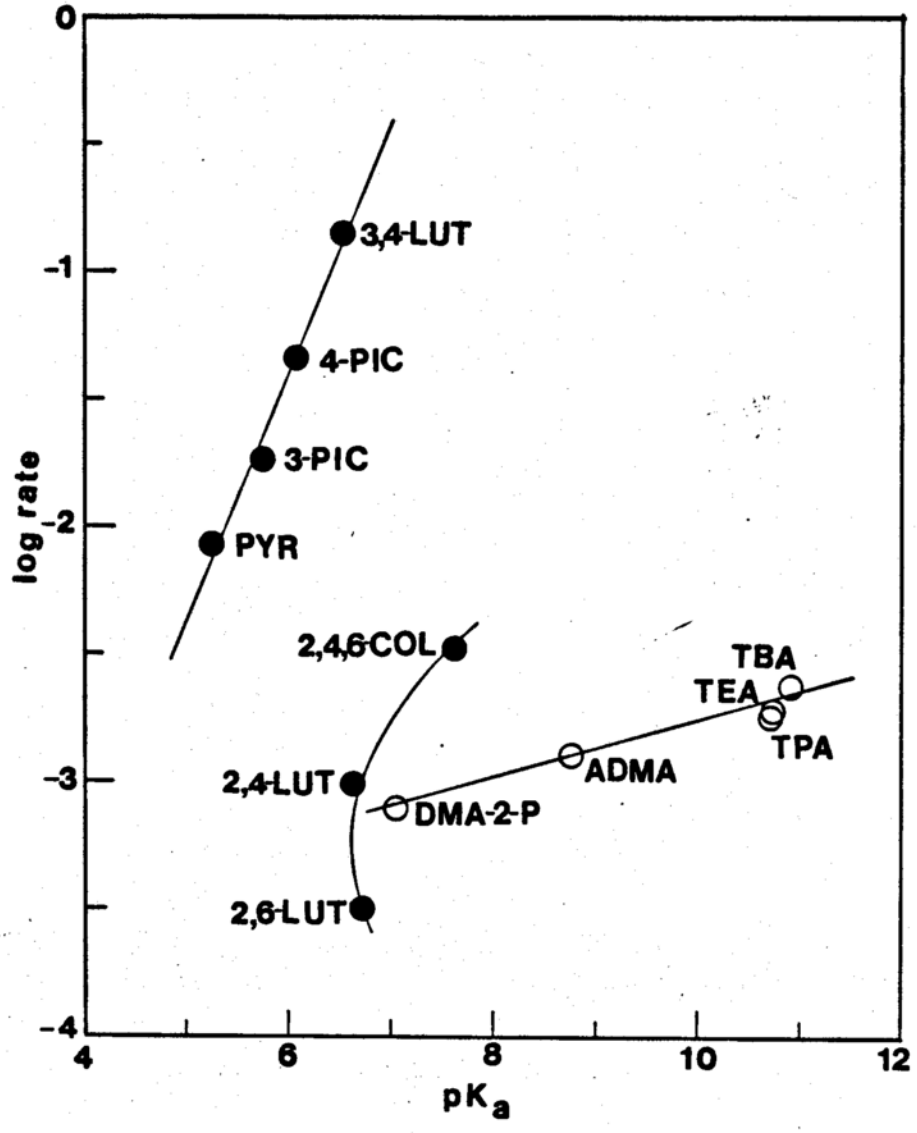
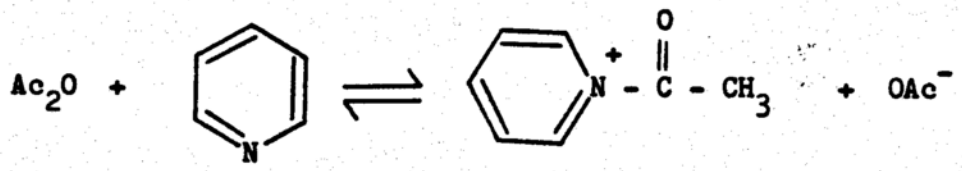


Figure 64. Combined Bronsted Type Plot for Aliphatic and Aromatic Amine Compounds.

nucleophilic catalysts in this reaction system. For one thing, they are not consumed in the overall reaction and, for another, there is the appearance of an approximate apparent half-order in pyridine; see Table XXIII. The acetylpyridinium ion is known to be formed from pyridine and acetic anhydride in the presence of other reactants (92, 93). On the basis of this, the apparent half-order in pyridine could be explained using the following equation:



$$A + B \rightleftharpoons I + OAc^-$$

Thus the concentration of I and of OAc^- are the same. Therefore,

$$K = \frac{[I]\ [OAc^-]}{[A]\ [B]}$$

$$= \frac{[I]^2}{[A]\ [B]}$$

Or

$$I = \sqrt{K\ [A][B]}$$

Thus, if the rate is first-order in I, it is half-order in B. But again, we cannot verify this inference using the common ion effect for the reason already advanced.

The least squares line through the aliphatic amine compounds has a correlation coefficient (r^2) of 0.99 and a slope (β) of 0.11. The low value of β may be indicative of a general base catalysis. But we note also that the aliphatic amines are not acting solely as catalysts.

B. Structural Observations

1. Spectrophotometry and Chromatography

The realization that aliphatic tertiary amines could react with carbon suboxide in the absence of acetic anhydride was a major breakthrough in this investigation. Not only was it possible to monitor the disappearance rate of carbon suboxide, but it also enabled us to study a wider absorption spectral region of the reaction mixture without the attendant interference from acetic anhydride. Figures 15 and 16 show the absorption spectral behavior of the reaction between carbon suboxide and two aliphatic amines. There is an initial decrease of the peak of 265 nm, i.e. a decrease in the concentration of carbon suboxide, a phenomenon probably associated with colorless intermediate species formation. This is followed by the sequential appearance of peaks at about 260 nm and 305 nm in that order. The appearance of these peaks is abrupt and rather rapid, much the same as the phenomenon of a lag-time phase followed by a sharp increase in absorbance. In other words, the absorption spectral evidence shows a lag-time phase during which an intermediate species is possibly being formed and a very rapid production of at least two reaction products, probably sequentially. It appears that this sequence of events leading to formation of products may be the same in the presence of acetic anhydride. Figure 17 shows the absorption spectral behavior of the reaction between TEA and carbon suboxide in the presence of acetic anhydride.

The concentration of acetic anhydride is only about 1/300th its usual concentration. Although the peak at 260 nm is obscured by the swamping absorption of acetic anhydride, we nonetheless observe an initial decrease in the concentration of carbon suboxide, a lag time, followed by the absorption at about 305 nm.

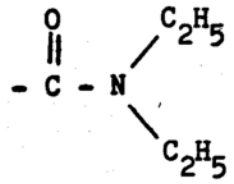
We were unable to observe this sequence of events using pyridine in the absence of acetic anhydride, suggesting perhaps the possibility of a difference in the reaction pathway.

Although absorption spectrophotometric evidence suggests the possibility of two reaction products, there could be more. High performance liquid chromatography of some of the reaction mixtures, Figures 22 to 25, indicated the presence of four, possibly more, reaction products. Although we have retention times for these peaks, Table VII, no meaningful information on structure could be derived from them. There are no standards to which these peaks could be compared; nor has their purity been established. Suffice it to say only that the HPLC data together with the absorption spectral information concerning the sequential appearance of peaks indicate there could be more than one product of reaction.

2. Mass Spectrometry

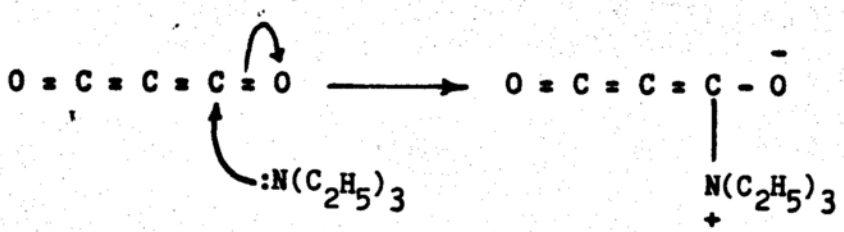
The mass spectral data on the product of the reaction between triethylamine and carbon suboxide were presented in Chapter III. There is a one-to-two, possibly more, mole ratio in the reaction between triethylamine and carbon suboxide. The apparent molecular

ion at m/e 283 may be due to incorporation of 3 molecules of carbon suboxide with one molecule of triethylamine, with the loss of a water molecule and 2 molecules of hydrogen gas. It may also have been due to incorporation of two molecules of methanol into the product consisting of 2 molecules of carbon suboxide and a triethylamine molecule with the loss of a water molecule. The mass spectral fragmentation pattern lends considerable support to the presence of the N,N-diethylamido group (XXII); see m/e 100, 116,



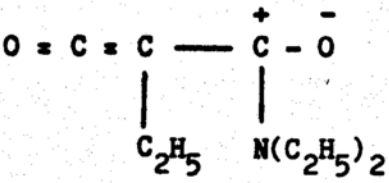
XXII

156, and possibly 85 and 101. This would indicate that there is a covalent attachment of triethylamine to carbon suboxide. Moreover, gas chromatographic data indicate that the concentration of aliphatic tertiary amines changed during the course of the reaction. Therefore, it is reasonable to propose structures which incorporate triethylamine as part of the molecule. It is possible, in this case, as a first step, there is attack at the carbonyl carbon of the suboxide to give XXIII as follows:

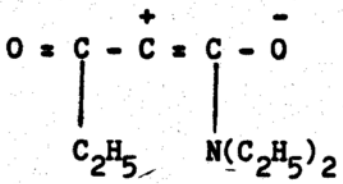


XXIII

This is possibly followed by a dealkylation and a carbonium ion shift to give either XXIV or XXV, which renders the molecule

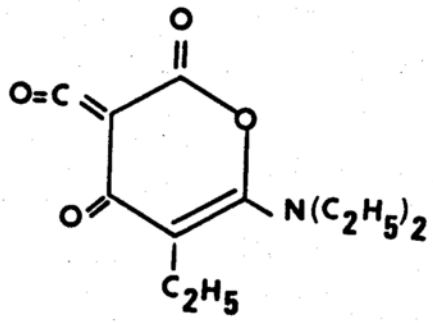


XXIV

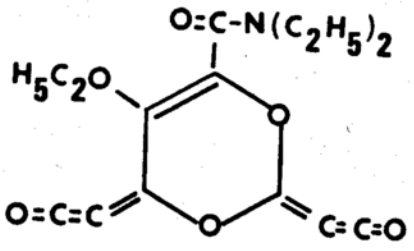


XXV

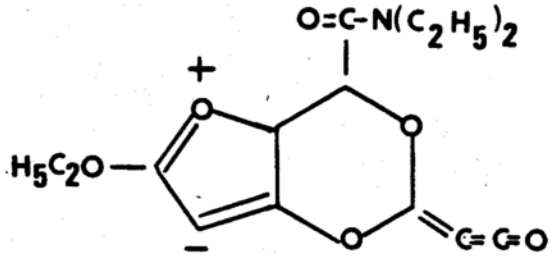
susceptible to attack by a second molecule of carbon suboxide. The attacking molecule of carbon suboxide could do so using one of its carbonyl groups, or the central carbon atom. Cyclization could follow leading to structures such as XXVI, XXVII, or XXVIII.



XXVI

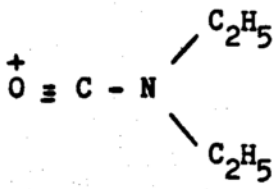


XXVII



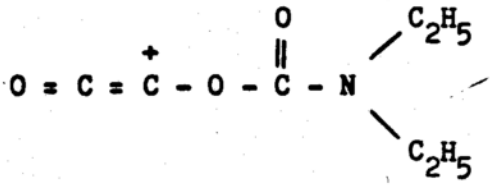
XXVIII

Although the mass spectral evidence does not enable us to conclusively determine the structure(s) of the product molecule(s), it is considerable enough, especially when taken together with the GC data, to suggest that triethylamine, and probably the other aliphatic amines, is incorporated as part of the product molecule(s). The peak at m/e 100 possibly is:



XXIX

If so, corresponding peaks would be expected at m/e 128 and m/e 156 for n-tripropyl- and n-tributylamine, respectively. Furthermore, with triethylamine the important peak at m/e 156 is possibly due to XXX, which would suggest corresponding peaks at m/e 184



XXX

and 212 for TPA and TBA, respectively. However, application of the same reaction conditions to the TPA and TBA reaction systems failed to yield a solid precipitate, possibly a consequence of the increased hydrophobicity of the products. We were unable, therefore, to rigorously verify these inferences.

C. Analytical Implications

1. Carbon Suboxide as a Reagent

It appears that the effective reactant in the malonic acid - acetic anhydride reaction system is carbon suboxide. Apparently this compound was generated in situ in the so-called classical analytical methods, i.e. methods that involved either aging or heating malonic acid in acetic anhydride. The recognition of carbon suboxide as the reactant, and its production external to the reaction medium, has been a tremendous improvement over the so-called classical methods. This has not only simplified the system, but has led to the discovery that aliphatic tertiary amine compounds do not require the presence of acetic anhydride to react.

Although fresh samples of carbon suboxide were distilled for each analysis, it appears that the compound has good stability in anhydrous diethyl ether (63); see Figure 3. It is quite possible that if stored at refrigeration temperatures ($4^{o}C$) ether samples of carbon suboxide could remain stable for at least one month.

2. The Preaging Process

The preaging experiments were performed with the intention of assessing their effect on the lag-time. It was thought that the day-to-day variability of the lag-time possibly had to do with the variable degree of carbon suboxide sensitization by the acetic anhydride, and possibly trace amounts of water, which the carbon

suboxide is in contact with prior to distilling into ether. The order of mixing the reagents was also thought to play a role. There is an obvious dependence of the lag time on the concentration of acetic anhydride; see Table XIII. However, as Table XIV shows, although the order of mixing has some effect on the duration of the lag-time phase, it is not clearcut and does not define the necessary components of a preaged 'analytical reagent.' Some of the reagent pairs shown in Table XIV involve treatment of carbon suboxide with acetic anhydride. In these pairs too the lag-time is still present. It is possible that the carbon suboxide may be entering into a non-covalent intermolecular interaction with diethyl ether molecules, which, in part, could also explain the good stability carbon suboxide has in ether. The lag-time phase then would be the time it takes for the abstraction of carbon suboxide from the molecular complex and to form the reactive intermediate.

3. Applicability

It will be desirable to eliminate the lag-time phase. For example, if this reaction were to be applied in post-column HPLC analysis of tertiary amines, the lag-time phase, especially its variability, will be less than desirable. The extent of the sensitivity of the analytical method is probably impaired by the presence of the lag-time phase. We deliberately attempted to avoid using amine concentrations that would result in lag-times in excess of one hour. The method appears to be more sensitive towards the

aliphatic amines than the aromatic amines. The absorbances are proportional to the initial tertiary amine concentrations, as they should be for a useful analytical technique. However, there is some ambiguity concerning why that should be the case with pyridine, and probably other aromatic tertiary amine compounds; these appear not to be consumed during the reaction process. Apparently, the aromatic amine could be catalyzing more than one reaction, some of which may be depleting the concentration of carbon suboxide in proportion to the amount of aromatic amine present.

D. Kinetic Schemes

Concerning concentration-time curves, we have not succeeded in constructing a kinetic model that is capable of reproducing all of the kinetic effects described for these systems in the Results chapter. We expect that a kinetic scheme that is applicable to these very complicated systems may be too complex to admit of an analytical solution, so we have made use of a general technique for the solution of a set of differential equations describing a kinetic scheme. This is the Monte Carlo technique of simulation, in which an ensemble of identical molecules is represented by a collection of identical letters in numbered cells, and 'reaction' of a molecule is simulated by the probabilistic action of random number selection. If the random number selected matches the number of a cell containing a molecule, the molecule 'reacts,' and is placed into another cell as a product molecule. This process could be applied to any kinetic scheme, however complex.

The number of selections applied to the ensemble is proportional to the rate constant for the reaction step. After each sequence of selections, the numbers of each 'type' of molecule (represented either by a letter or number) are summed, and these sums are plotted against the number of selection cycles to generate the 'concentration-time' profiles for all reactants, intermediates, and products in the scheme. This simulation technique is well-known (94, 95, 96). Of course, the technique will not provide closed form

rate equations for these complex systems; it provides qualitative insights into the relationship among concentrations, rate constants, and time for reactants, intermediates and products. In our preliminary trials we have used a manual version of the technique. Any refinement would more effectively be carried out with a computer.

Our efforts at simulating the concentration-time behavior of these complex reaction systems takes into account these experimental observations:

a. There is a lag-time in the appearance of product(s). The product appears abruptly after an apparently featureless lag period, and absorbance rises very quickly.

b. The loss of the amine is approximately first-order in the amine.

c. For the aliphatic tertiary amines, the amine is consumed, but its final concentration is not zero. Mass spectral data indicate the incorporation of TEA, and possibly other aliphatic amines, into product.

d. It appears that the initial rate is second-order in C_3O_2, but the rate of product appearance immediately following the lag-time could be first-order in C_3O_2. However, our simulations assume $[C_3O_2]_0 >> [\text{Amine}]_0$, so the order with respect to C_3O_2 has not been investigated.

e. Absorption spectral evidence shows that C_3O_2 is lost from $t = 0$.

f. Mass spectral evidence calls for 2 or 3 molecules of C_3O_2 per molecule of product.

A lag-time can be generated simply by interposing enough intermediates between the reactants and the product states, these intermediates having the effect of 'storing' material for some time before its appearance as products. Concentration-time profiles resembling those obtained experimentally can be generated for the aliphatic tertiary amines if reversible reactions are introduced at every stage, so that the final product is in equilibrium with starting materials. An alternative, that each product formed produces one H^+ that inactivates an amine molecule (general base catalysis), would require that exactly half the initial concentration of amine be left at $t = \infty$. The abruptness of product appearance ('initial rate') is a remarkable feature that we have not fully reproduced, but we will show some schemes and results that are suggestive.

1. Scheme I

The kinetic scheme that best accounts for the aliphatic amine (B) concentration-time profile, with consumption of 3 molecules of carbon suboxide (C) and a lag-time, is as follows

$$B \underset{k_{-1}}{\overset{k_1 C}{\rightleftharpoons}} I$$

$$I \underset{k_{-2}}{\overset{k_2C}{\rightleftharpoons}} X$$

$$X \underset{k_{-3}}{\overset{k_3C}{\rightleftharpoons}} P$$

where I and X represent intermediates and P the product. The scheme assumes implicitly that $C_0 >> B_0$. Figure 65 shows the Monte Carlo simulation with $B_0 = 100$, and $k_1C:k_{-1}:k_2C:k_{-2}:k_3C:k_{-3}$ = 10:10:10:10:10:10 for a total time (cycles) of 33 (arbitrary time units). This simulation indicates that the pair $B \rightleftharpoons I$ and the pair $X \rightleftharpoons P$ individually apparently equilibrate faster than the overall system (the equilibrium number of molecules should be 25 given the initial conditions). This may be an artifact of the sequence of selections. For example, B and P selections do not follow one another, or rather, there is no direct transfer from B to P. But this is, of course, physically correct.

2. Scheme II

The kinetic scheme simulated in Figure 66 is not ruled out. This kinetic scheme is represented by

$$B \underset{}{\overset{k_1C}{\rightleftharpoons}} I$$

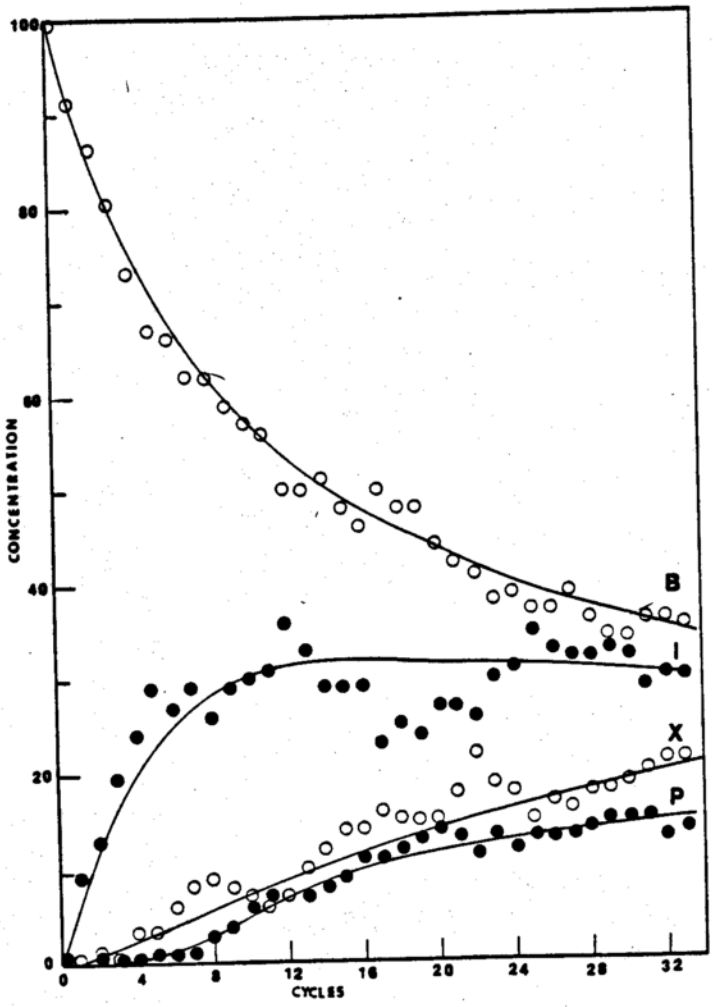


Figure 65. Monte Carlo Simulation for Scheme I.

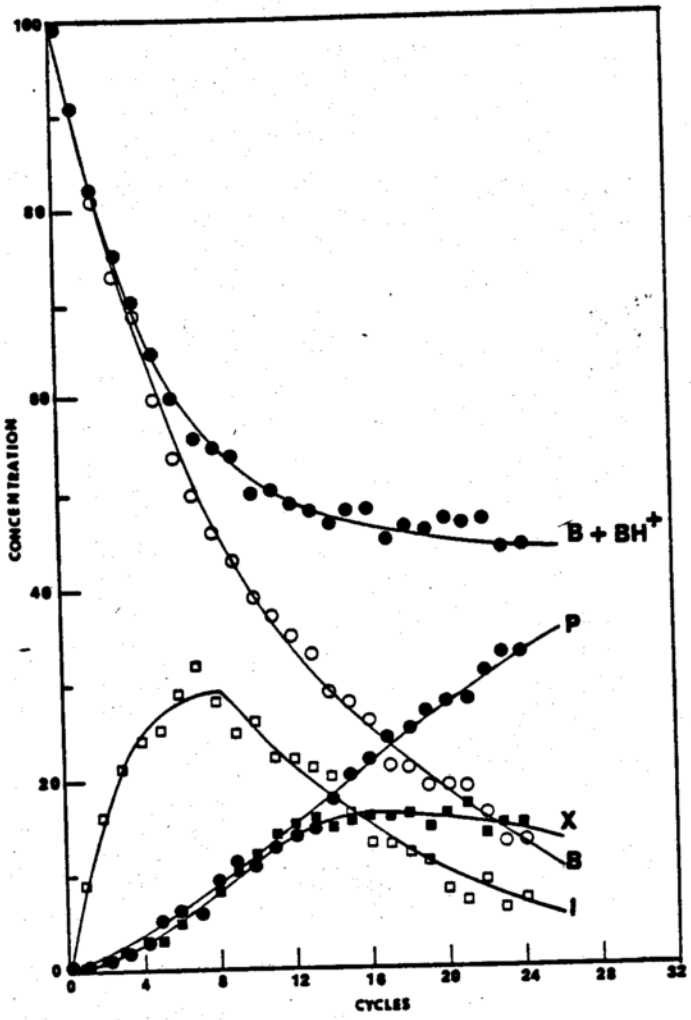


Figure 66. Monte Carlo Simulation for Scheme II.

$$I \xrightarrow{k_2 C} X$$

$$X \xrightarrow{k_3} P + H^+$$

$$H^+ + B \xrightarrow{\text{fast}} BH^+$$

the conditions being that $C_0 >> B_0$ and $k_1C:k_{-1}:k_2C:k_3 = 10:10:10:10$. That is, each time a P is produced one B is eliminated by protonation. Ultimately, the concentration of amine should approach a limiting value approximately half of the initial concentration. However, this is not evident at the times simulated, which may be a consequence of error associated with simulations with small numbers of molecules. The mass balances for this system are:

$$B_0 = B_t + I_t + X_t + P_t + BH_t^+$$

$$P_t = BH_t^+$$

$$P_\infty = BH_\infty^+$$

Since at $t = \infty$ there can be no X or I left (because of the irreversibility of the k_2 and k_3 steps), all of B must be likewise depleted. So at t_∞

$$B_0 = P_\infty + BH^+_\infty$$

and

$$P_\infty = 0.5\ B_0$$

$$BH^+_\infty = 0.5\ B_0\ .$$

3. Scheme III

The best lag-time behavior is predicted with this kinetic scheme,

$$B \xrightarrow{k_1 C} I$$

$$I \xrightarrow{k_2} X$$

$$2X \xrightarrow{k_3} P + X$$

the condition being that $C_0 >> B_0$ and $k_1C:k_2:k_3 = 10:5:20$ ($B_0 = 70$). This scheme requires, however, that the concentration of amine ultimately go to zero. Two selections were made for X. For each successful 'collision' a molecule of P was generated but only one X eliminated, the other being a catalyst. The 'concentration-time' profile generated is shown in Figure 67.

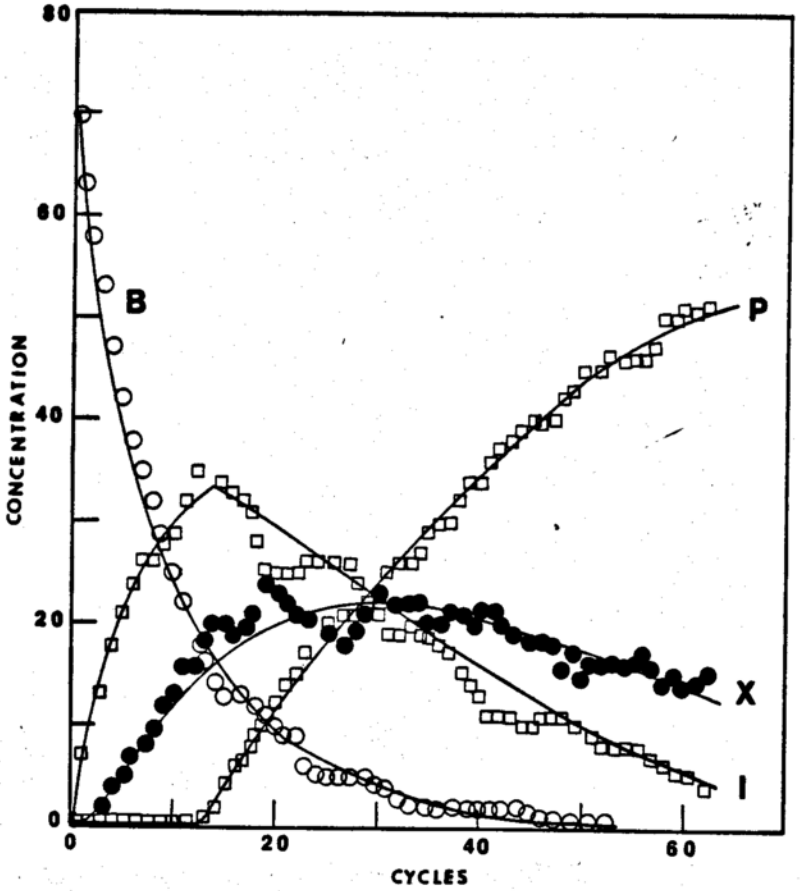


Figure 67. Monte Carlo Simulation for Scheme III.

E. Summary and Future Work

There are many organic polycarboxylic acids that are capable of taking part in this analytical reaction. Our grouping and classification of these acids indicates that the ability of the acid to generate coloration may be related to its anhydride molecule. We have studied the malonic acid system, which is a special case, being the only member among members of a homologous series capable of generating a coloration in this reaction process. We have prepared and identified carbon suboxide and have demonstrated that color formation by malonic acid in the analytical reaction is probably linked to the C_3O_2 molecule. The production of C_3O_2 external to the analytical reaction medium has led to the discovery that aliphatic tertiary amines behave differently than aromatic tertiary amines in their ability to produce coloration. Whereas the aromatic amines require the presence of acetic anhydride to react, the aliphatic amines do not. It is possible that the aromatic amines are acting only as catalysts, possibly via a nucleophilic mechanism. The aliphatic amines apparently are catalysts, but are also consumed in the reaction process.

At the present time it is fair to state that our studies have clarified many aspects of the malonic acid-acetic anhydride-tertiary amine analytical system, but a full understanding of this complex system requires additional studies along the lines revealed by the present work. Preparative HPLC could be used to collect fractions

of the eluted products, which would be tested for purity and identified, given that they are stable. The recent introduction of fast atom bombardment mass spectrometry (97-99), which is used for the analysis of dynamic processes such as detection of short-lived intermediates in reaction solutions, should make it possible to obtain real-time measurements on the appearance of intermediates and products. The reaction of citric and aconitic acids, probably via aconitic anhydride, is another interesting system for which the chemistry is yet to be elucidated. These reaction systems have the potential of being generally applicable to the determination of the tertiary amine functionality if their chemistry could be characterized. Chemical characterization would enable the analyst to have a better control of the analytical reaction.

It appears that a number of products are formed during the reaction process. We have provided mass spectral data in support of postulated product structures. Although our structural identification is not complete, it certainly provides a firmer basis for further investigation. The proposed structure of Groth and Wallerberg (46) in the reaction of acetic anhydride solutions of malonic acid and of pyridine did not take cognizance of carbon suboxide as being the probable reactive intermediate. The proposed aliphatic structure (XI, p. 25) prior to aromatization is a mixed anhydride of malonic acid and acetic anhydride.

The kinetics of the reaction process is very complex. Monte Carlo simulations of possible kinetic models have been attempted,

which may lay the ground for firmer mechanistic propositions, based probably upon intermediate and product identification.

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