I. STUDIES ON MIXED ANHYDRIDE SYSTEMS

II. STUDIES OF THE ALPHA CHYMOTRYPSIN CATALYZED HYDROLYSIS OF SPECIFIC SUBSTRATES

Thesis by

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ABSTRACT

The synthesis and purification of *p*-nitrobenzoic-acetic mixed anhydride, *p*-methoxybenzoic-acetic mixed anhydride and benzoic-acetic mixed anhydride is described. The thermodynamic constants for the disproportionation reaction have been determined. A mechanism for this reaction is discussed. Measurements of the interaction of acetic and benzoic acids with the acetic-benzoic anhydride system have been made.

The cleavage of the mixed anhydrides with nucleophilic agents is described.

The synthesis of the following compounds is described: N-trimethylacetyl-L-tyrosinhydrazide, N-dichloroacetyl-L-tyrosinhydrazide, N-formyl-L-tyrosinhydrazide, N-trichloroacetyl-L-tyrosinamide and N-acetyl-L-cysteic acid carboxylamide. The kinetic constants for the α chymotrypsin hydrolysis of these substances are reported.

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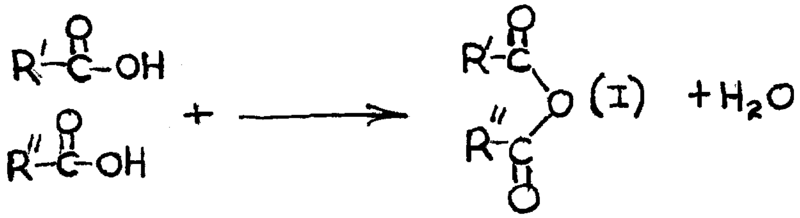
PART I. STUDIES OF MIXED ANHYDRIDE SYSTEMS

INTRODUCTION

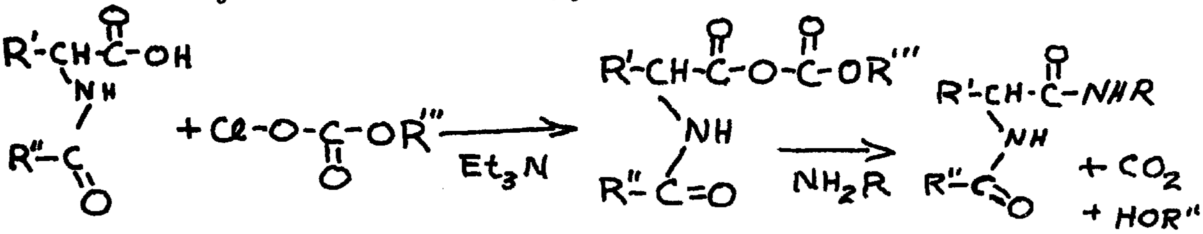
Mixed anhydrides I, formed by the loss of water from two different carboxylic acids, have been known or postulated for a long time. Until the advent of high vacuum techniques the liquid members of this class of compounds could not be prepared in a pure state. Any attempt at fractionation of these compounds at temperatures above one hundred degrees centigrade resulted in the production of a distillate, whose boiling range was continuous from the boiling point of one symmetrical anhydride to the boiling point of the other symmetrical anhydride. Therefore, it was not certain whether this material represented a new compound or a mixture of equal portions of the two symmetrical anhydrides. This point was partially clarified by Behal,(1) who prepared several crystalline solid mixed anhydrides and showed them to have discrete properties distinct from either of the symmetrical anhydrides. Other evidence for the existence of these substances was obtained by Kilpatrick,(2) who showed that acetic-proponic mixed anhydride hydrolyzed at a rate which was intermediate be-



tween the rate of hydrolysis of acetic and proponic anhydrides. The reaction accurately followed first order kinetics throughout its course, while an equimolar mixture of acetic and proponic anhydrides gave kinetics which was not homogeneously first order.

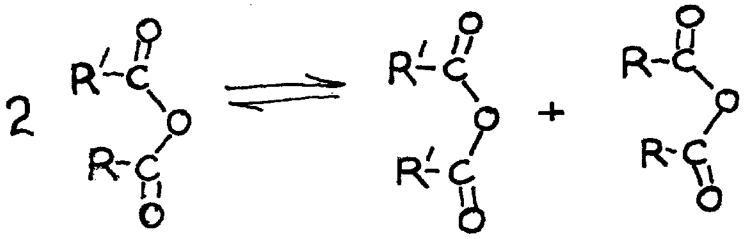
Except for their relationship to the Perkin synthesis(3) and certain acylation reactions,(4) interest in these substances has lagged until the last few years. Recent developments in the field of peptide synthesis have greatly increased the importance of these compounds and have stimulated efforts to understand their properties.

Wieland(5) and Vaughn(6) have developed new peptide syntheses based upon the conversion of an acylated amino acid or peptide into a mixed anhydride. The mixed anhydride is then allowed to react with the free amino group of another amino acid to form a peptide bond. It was soon found very convenient to form the acylated amino acid mixed anhydride with an alkyl chlorocarbonate.



The rate and the magnitude of equilibrium constant of the disproportionation reaction and the products obtained by heterolytic cleavage with nucleophilic agents are the properties of a mixed anhydride which one is most interested in being able to predict.

Mixed anhydrides all show a tendency to disporportionate into the two symmetrical anhydrides



It is possible to show that this reaction is reversible and that an equilibrium state is reached. However, quantitative measurements of this equilibrium mixture were not possible until the advent of infrared spectophotometric techniques, since it was not possible to devise a chemical test which would quantitatively distinguish between the mixed anhydride and an equal molar mixture of the two symmetrical anhydrides.

Brown and Trotter(7) were the first to carry out quantitative measurements on a mixed anhydride system. They showed that if a mixture of acetic and butyric anhydrides is allowed to stand and its infrared spectrum examined as a function of time, the spectrum changes from an additive mixture of acetic and butyric anhydrides to that of a mixture of the two symmetrical anhydrides and a third substance. During this change a prominent new peak appears in the 8-9 micron region [the region of the C-O-C stretching frequency of anhydrides]. The appearance of this peak is accompanied by a corresponding decrease in the peaks associated with either symmetrical anhydride. Therefore, the new peak is assigned the C-O-C stretching frequency of acetic-butyric mixed anhydride. It was shown that after several hours the mixed anhydride peak attains constant dimensions and that it is invariant with changes in temperature. From this fact it was determined that the change in enthalpy for the disproportionation reaction was zero and that only entropy considerations should affect the over-all free energy change. Thus the disproportionation reaction seems to be similar to certain processes of ester interchange, of metal alkyl interchange and of the distribution of halogens between glycol dihalides. These reactions were first recognized by Calingaert(8) and called redistribution reactions. they are characterized by a statistical distribution of organic radicals about a central moiety.

If the mixed anhydride disproportionation reaction is a redistribution reaction, then the equilibrium constant should be four. This hypothesis was tested and found to be consistent with the data obtained for the acetic-butyric anhydride system.

Thus Brown and Trotter were able to measure the equilibrium constant for the acetic-butyric anhydride system without ever having isolated or purified the mixed anhydride. In like manner, they were able to measure the rate of disproportionation of the acetic-butyric mixed anhydride and found the second order rate constant to be

$$k = 4.125 \text{ Liters moles}^{-1} \text{ min.}^{-1} \text{ at } 398 \text{ K}$$

One of the most important reactions of mixed anhydrides is their cleavage by nucleophilic agents. This reaction constitutes the basis for many acylation reactions.

By the work of Gold(9) it has been shown that for most mixed anhydrides this reaction can be considered as an S_N2 attack by the nucleophilic reagent upon the carbonyl carbon-atom which carries the greatest positive charge and which is the least sterically hindered for such an attack. Often it is found that the factors which increase the positive charge of the carbonyl carbon also increase its steric shielding. Thus in the series: acetic-monochloroacetic, acetic-dichloroacetic, acetic-trichloroacetic mixed anhydride, it is the monochloroacetic mixed anhydride which has the best combination of characteristics to give the highest yield of α haloacylation. Often the effective steric size of substituents on the α carbon will vary with the reaction medium. If there is a delicate balance between the susceptibility of the two carbonyls to nucleophilic attack, then striking variations in product composition can be obtained by changing the reaction medium. Thus, in benzene, chloroacetic-acetic mixed anhydride gives six times as much chloroacetylation as acetylation, but in aqueous solvents two times as much acetylation as chloroacetylation is obtained.

In the absence of strongly protolytic solvents it is believed that acylium ions do not play an important part in the reactions of carboxylic anhydrides. However, Tedder *et al.*(10) have shown that trifluoroacetic mixed anhydrides seem to constitute an exception to this rule. In this case some reactions seem to proceed by a preliminary ionization into trifluoroacetate and acylium ions.

STUDIES ON SYSTEMS OF PARA SUBSTITUTED BENZOIC-ACETIC MIXED ANHYDRIDES

Aim and Scope of the Studies

The purpose of this work is to study the properties of three para substituted benzoic-acetic mixed anhydride systems; the benzoic-acetic, the p-nitrobenzoic-acetic, and the p-methoxybenzoic-acetic mixed anhydride systems. The investigation of the disproportionation reaction and the heterolytic cleavage with nucleophilic reagents was undertaken to learn whether the behavior of these substances was compatible with the ideas that were advanced in the introduction. In addition these studies were extended to include the interaction of acetic and benzoic acids with the benzoic-acetic mixed anhydride system.

It is hoped that the results of this work will be of some help in the design of future synthetic procedures in the field of amino acid chemistry.

The Disproportionation Reaction

Introduction

The work of Brown and Trotter(7) suggested that the nature of the disproportionation reaction might be relatively independent of the structure of the mixed anhydride and regardless of the character of the organic rest attached to the carbonyl carbon, the equilibrium constant would be four and the temperature independent.

To test this hypothesis, benzoic-acetic, p-nitrobenzoic-acetic, and p-methoxybenzoic-acetic mixed anhydrides were prepared in a pure state. These compounds differ from one another in regard to the positive character of the phenyl carbonyl carbon atom and should demonstrate any effect which arises from this cause. It is fortunate that these mixed anhydrides are of sufficient stability to allow their purification. This allows one to remove any doubt as to the interpretation of the change in the infrared spectrum that occurs when the two symmetrical anhydrides are heated together. The presence of the pure mixed anhydride allows one to approach the equilibrium point from two directions and to establish its value with greater certainty.

Results and Discussion

By use of the infrared spectrophotometric technique described in the experimental section, the concentration of the three species comprising an equilibrium mixture can be measured and an equilibrium constant, K, determined. The results are shown in Tables 1, 2, and 3.

$$K = \frac{(\text{mixed anhydride})^2}{(\text{Acetic anhyd})\ (\text{Phenyl anhyd})}$$

Table 1

Benzoic-acetic Anhydride System

Temperature	Mixed Anhydride	Symmetrical Anhydrides	Acid and an Anhyd
	Equilibrium constant derived from mixtures with initial compositions of:		
70°C	1.07	1.04	
75°C	--	--	1.08 1.25
100°C	1.85	1.98	3.12 2.00
150°C	4.17	4.31	4.94 3.74

A plot of the ln K vs. $\frac{1}{T}$ is shown in Figure 1. From this plot, the change in enthalpy for the disproportionation reaction was determined as $\Delta H = 5.0$ Kcal.

Table 2

p-Methoxybenzoic-acetic Anhydride System

Temperature	Mixed Anhydride	Symmetrical Anhydrides
	Equilibrium constant derived from mixtures with initial compositions of:	
75°C	2.31	2.27 2.25
100°C	4.25	4.22 4.69
105°C	9.13	11.93

A plot of ln K vs. $\frac{1}{T}$ is shown in Figure 2. From this plot, the change in enthalpy for the disproportionation reaction was determined as $\Delta H = 5.8$ Kcal.

Figure 1

Plot of ln K vs. $\frac{1}{T}$ for Benzoic-Acetic Anhydride System

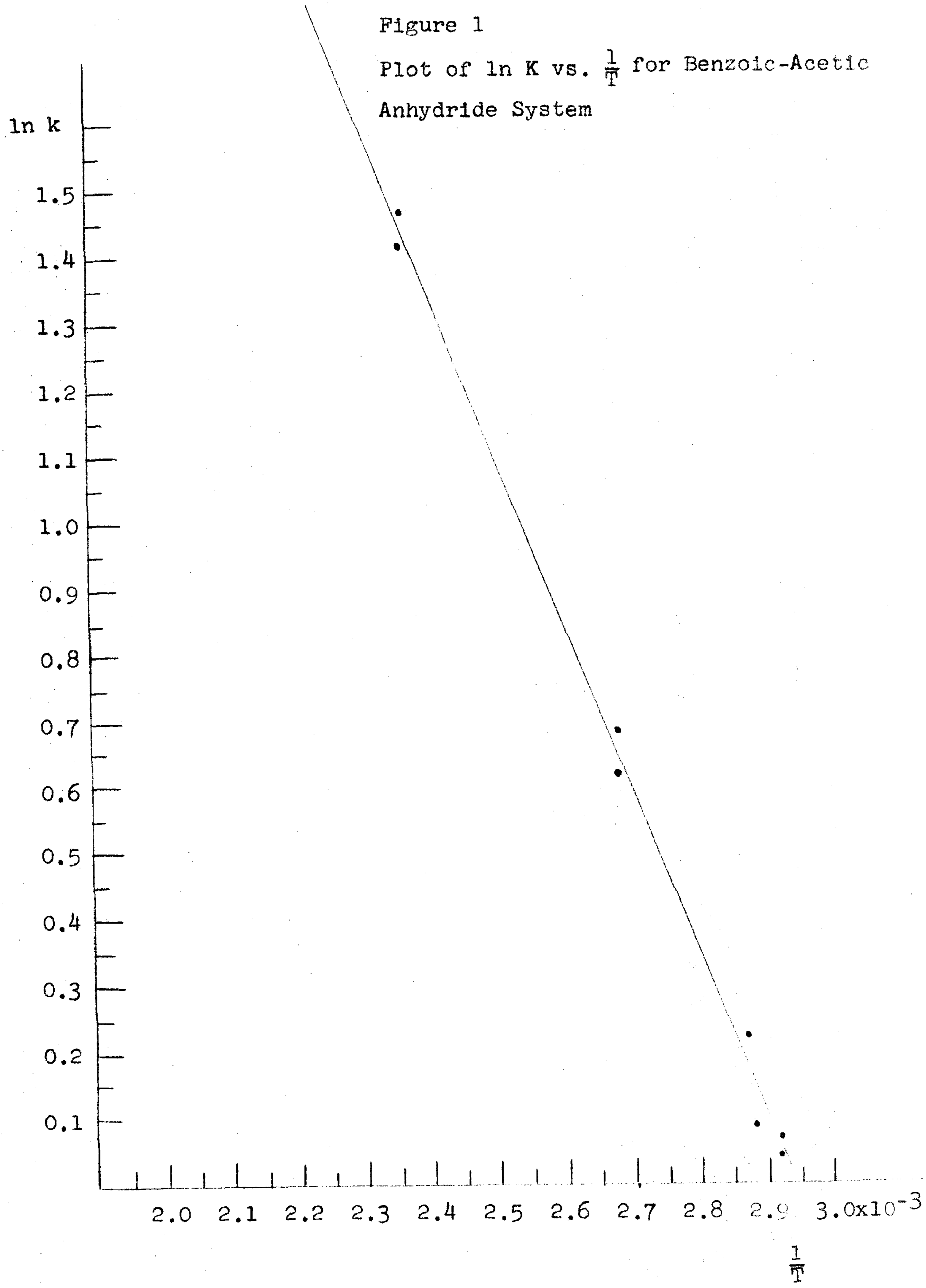


Figure 2

Plot of ln K vs. $\frac{1}{T}$ for p-Methoxybenzoic-Acetic Anhydride System

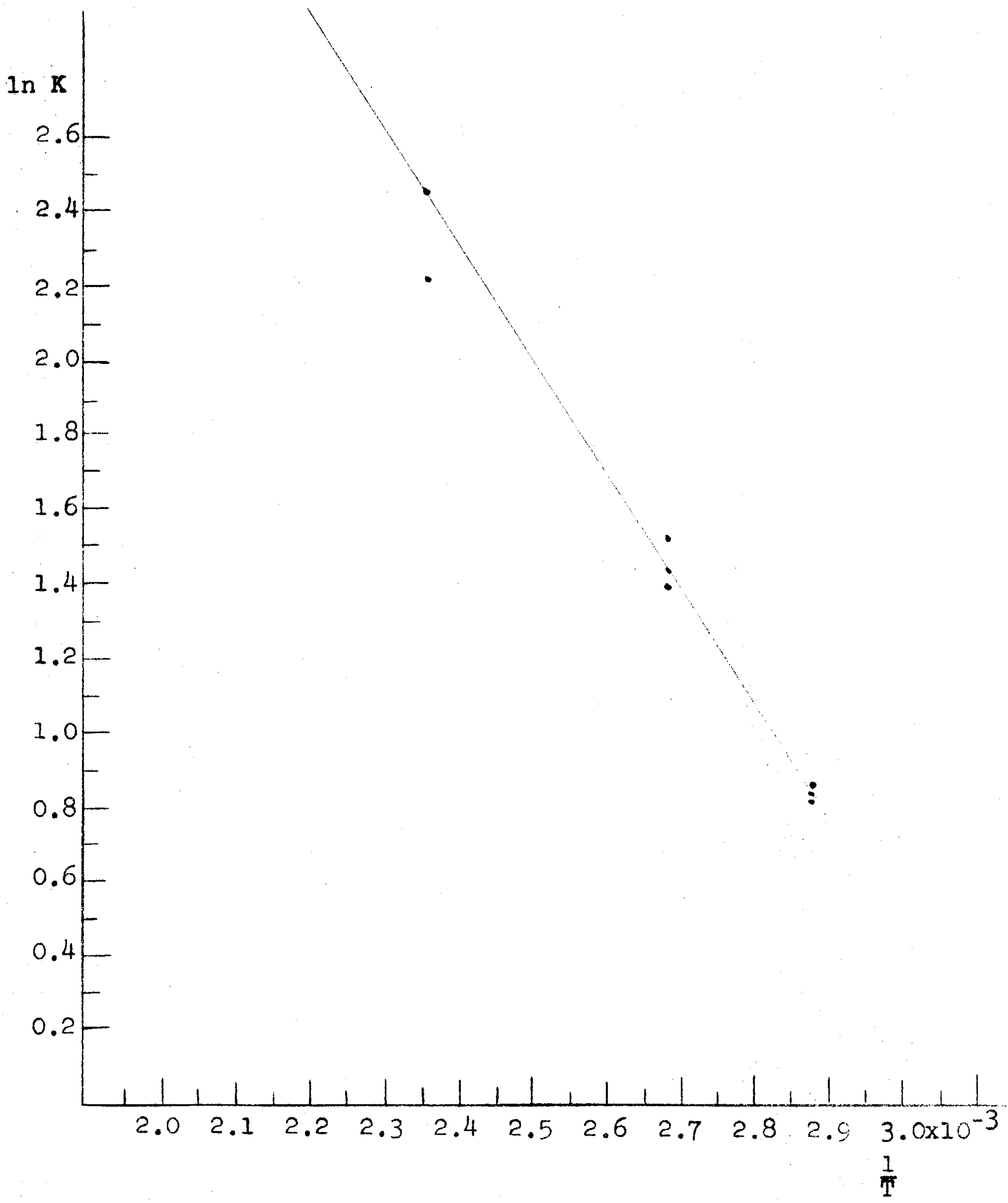


Table 3

p-Nitrobenzoic-acetic Anhydride System

Temperature	Equilibrium constant derived from mixtures with initial compositions of: Mixed Anhydride	Symmetrical Anhydrides
75°C	1.27	1.20
100°C	1.29	1.32 1.38
150°C	1.70	1.78

A plot of ln K vs. $\frac{1}{T}$ is shown in Figure 3. From this plot the change in enthalpy for the disproportionation reaction was determined as $\Delta H = 1.04$ Kcal.

The above results show that the equilibrium constants for the disproportionation reaction of each of the anhydride systems have a decided temperature dependency and hence are in marked contrast to the aliphatic mixed anhydrides.

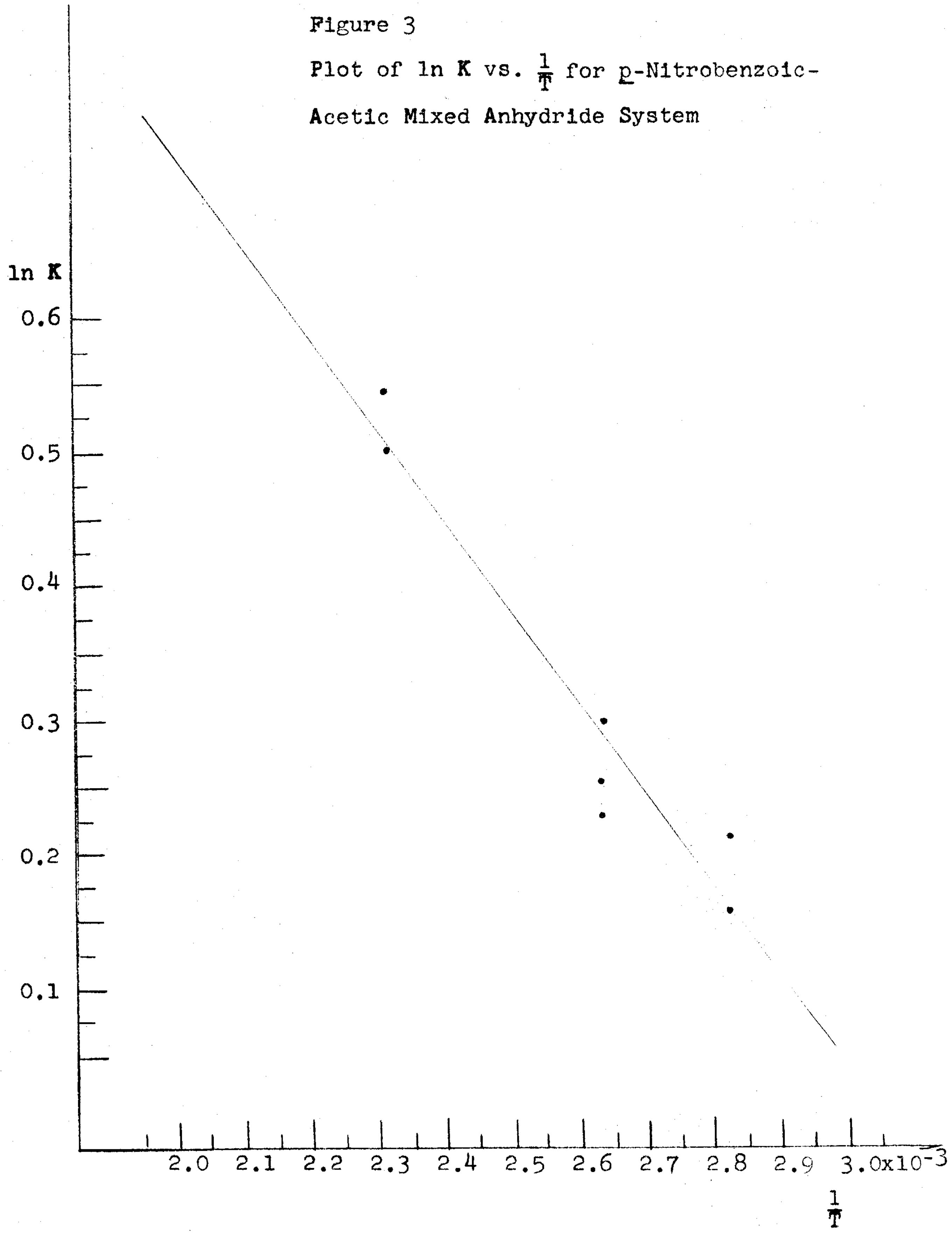
It appears at the present time that there is no simple explanation either of the differences of the substituted benzoic-acetic mixed anhydrides between one another or of the differences between the substituted benzoic-acetic mixed anhydrides and the aliphatic mixed anhydrides.

Hammett(11) has set forth the following relation which describes the change in free energy which takes place during a reaction:

$$\Delta F = -RT \ln K = \Delta E_p + \Delta E_z - RT \ln \frac{\Sigma f_P}{\Sigma f_R}$$

Figure 3

Plot of ln K vs. $\frac{1}{T}$ for p-Nitrobenzoic-Acetic Mixed Anhydride System



The term ΔE_p is the difference in potential energy between the reactants and the products. ΔE_z is the difference in zero point energies of the various reaction species present and f_p and f_r are the partition functions of the products and reactants. The term ΔE_p is the energy change which would occur if the reaction could take place so that none of the reactive species would possess the energy of motion that is associated with the various vibrations and rotations that the molecules are able to undergo. The logarithmic term and the quantity ΔE_z are concerned then, with the kinds of rotational and vibrational motion that the molecules can execute and with the various energy-level spacings of the reactants and products. The term ΔE_p is the quantity which is influenced by changes in resonance energy, electron displacements, dipole interactions, etc. Unless the kinetic terms of the Hammett equation can be calculated or shown to be insignificant, it is not possible to discuss the effect of changes in structure upon reaction rate, equilibrium constant or heat of reaction entirely in terms of changes in bond strength, resonance energy, dipole interactions, etc.

If the products and reactants of a reaction are so similar that the energy level spacings are nearly the same then,

$$\ln \frac{\sum f_P}{\sum f_R} \approx 0$$

$$\Delta E_p \approx 0$$

and $\Delta F = \Delta E_p$

Since ΔV and ΔP are small;

$$\Delta F = \Delta H$$
$$\Delta S = 0$$

Reactions between compounds which satisfy these conditions can be described by the use of the Hammett functions ρ and σ. Meta and para substituted benzene derivatives, in which the reaction takes place at a reactive center adjacent to the benzene ring, constitute the classical examples in which this type of analysis is possible. Aliphatic compounds in general do not display these properties.

Table 4 shows the thermodynamic constants of the disproportionation reaction for the three anhydride systems. It is evident that ΔS is far from being zero and ΔF is not even of the same sign as ΔH. Figure 4 shows the logarithm of the equilibrium constant for the disproportionation reaction plotted against Hammett's constant for the para nitro, para methoxy and the unsubstituted benzoic-acetic mixed anhydride. It is seen that the deviations from a linear relationship are rather large.

Table 4

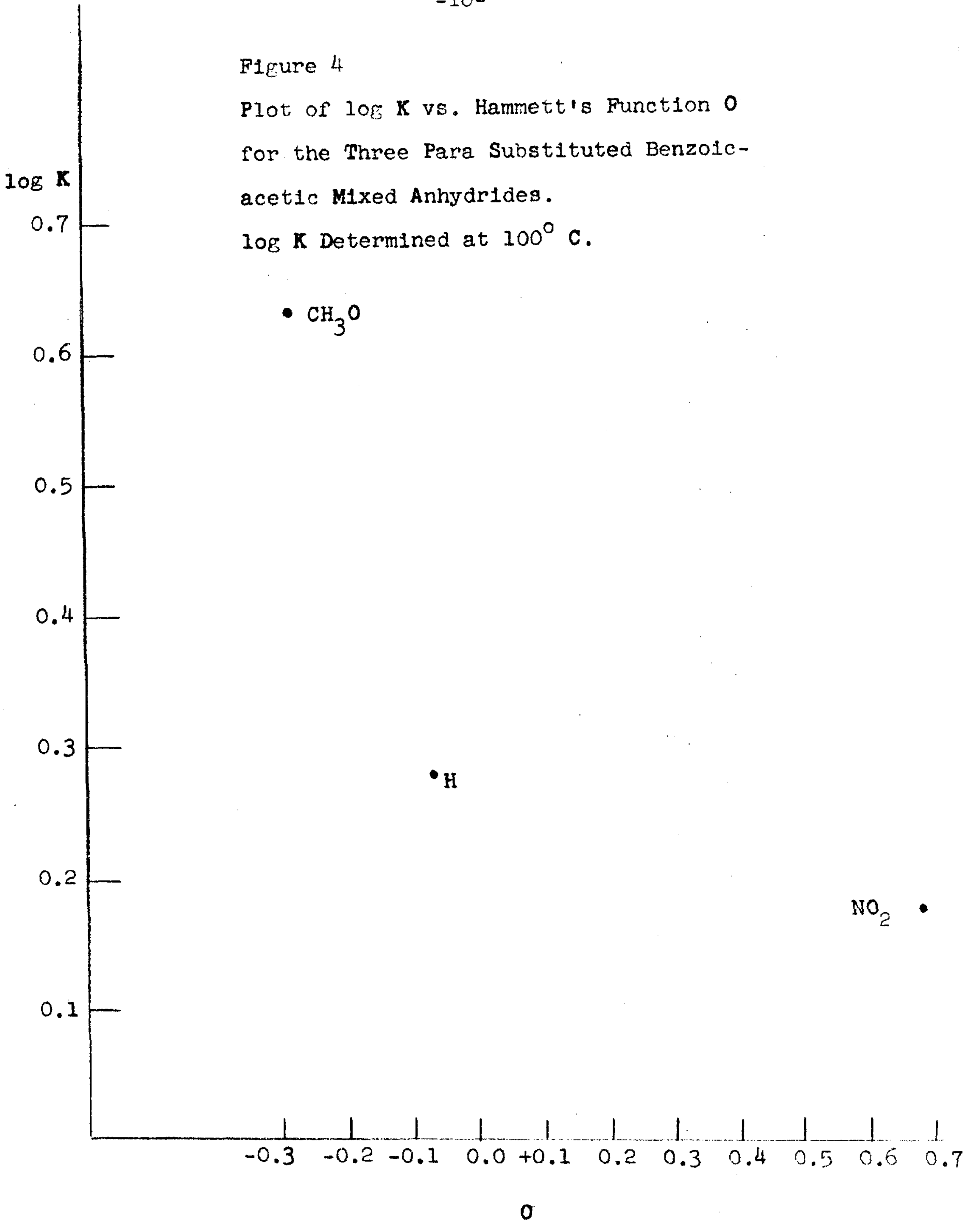
Thermodynamic Constants for the Disproportionation Reaction of the Para Substituted Benzoic-acetic Mixed Anhydrides at 100° C.

	Δ F(cal)	Δ H(cal)	Δ S cal/°C
p Nitro	-212	1,252	3.4
p Methoxy	-1.088	5,800	18.0
p Hydrogen	-480	5,000	14.7

Figure 4

Plot of log K vs. Hammett's Function O for the Three Para Substituted Benzoic-acetic Mixed Anhydrides.

log K Determined at 100° C.



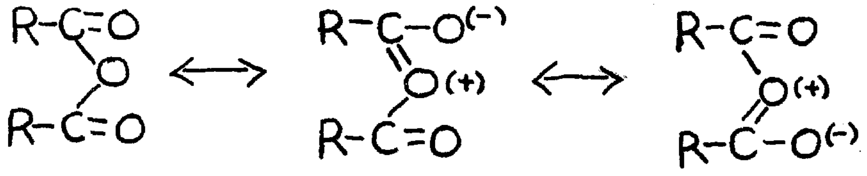
It was hoped that because the number and types of bonds in the mixed anhydride as compared to the symmetrical anhydrides are so nearly identical, this system could be analyzed in terms of changes in potential energy alone. The failure for this partly lies in the importance of the acetyl carbonyl group, which is removed from the phenyl group by a carbon and oxygen atom, as a reactive center in the disproportionation reaction.

Although it is probably not realistic to describe the differences in the thermodynamic characteristics of the mixed anhydrides in terms of changes in potential energy alone, it should be pointed out that a certain amount of correlation can be obtained by discussing these substances in terms of classical resonance structures.

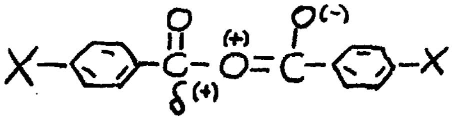
It is noted that in each case the sign of the ΔH term is positive and hence the mixed anhydride is less stable than the symmetrical anhydrides. Proceeding from the para methoxy compound to the para nitro compound the differences in ΔH become smaller.

Weiland(12) has calculated the resonance energy for acetic anhydride as 41 Kcal/mole and a value of 29 Kcal/mole has been estimated by Franklin.(13)

Wieland suggests that this resonance energy is due to the following interactions:

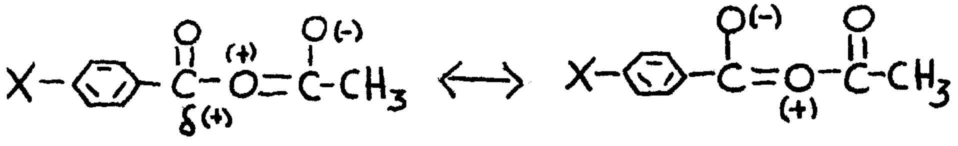


It is to be expected that substituents in the para position of the symmetrical benzoic anhydrides might well affect the importance of structures such as III.



– III

Structure III involves the creation of a charge on the central oxygen atom, and this charge is adjacent to a carbonyl carbon which already carries a partial positive charge. This situation tends to decrease the stability of III. If the para substituent X is a methoxyl group, then the partial positive charge on the carbonyl carbon is diminished and the resonance form III is of increased stability. A para nitro group exerts the opposite effect, and with an increased charge on the carbonyl carbons the contribution of III to the structure of p-nitrobenzoic anhydride is smaller. At the same time the effect of the para nitro group is to diminish the stability of only one of the corresponding structures (IV - A) in the mixed anhydride.



IV-A

IV-B

The effect of the substitution of a para nitro group is to decrease the energy difference between the symmetrical and the mixed anhydride by decreasing the resonance energy of the symmetrical anhydride to a greater extent than it decreases the resonance energy of the corresponding mixed anhydride and thus in a rough way to decrease the value of ΔH .

In the mixed anhydride the phenyl group may interact with the resonating system of the carbonyl groups so as to destroy the equivalence of the two structures corresponding to III. This would generally tend to decrease the stability of the mixed anhydrides relative to the symmetrical anhydrides.

Kinetics of the Disproportionation Reaction

Using the infrared spectrophotometric techniques described in the experimental section, it was possible to follow the disproportionation of a mixed anhydride into the symmetrical anhydrides and to examine the kinetic consequences of this reaction.

The p-nitrobenzoic-acetic mixed anhydride disproportionated at a rate that was considerably faster than either the p-methoxybenzoic-acetic or the benzoic-acetic mixed anhydrides. Because of its rather low solubility in chloroform, this was not a convenient case to study.

With p-methoxybenzoic-acetic and benzoic-acetic mixed anhydrides the disproportionation reaction displayed kinetics which could be unambiguously determined as a second order with respect to the mixed anhydride. See Figure 5A and B. Only the initial 20% of the reaction was followed, as at this point the back reaction became important and the apparent rate constant continuously decreased to zero.

Second Order Rate Constants for the Disproportionation of p-Methoxybenzoic-acetic Mixed Anhydride

A. Reaction followed by the disappearance of the mixed anhydride at 100°C

$k_2 = 1.29 \times 10^{-3}$ moles^{-1} liters min.$^{-1}$
1.38×10^{-3} " " "

B. Reaction followed by the appearance of *p*-methoxybenzoic anhydride at 100°C

$k_2 = 1.34 \times 10^{-3}$ moles^{-1} liters min.$^{-1}$
1.60×10^{-3} " " "

Second Order Rate Constants for the Disproportionation of Benzoic-acetic Mixed Anhydride

Reaction followed by the disappearance of the mixed anhydride at 100°C

$k_2 = 2.27 \times 10^{-3}$ moles^{-1} liters min.$^{-1}$

2.82×10^{-3} " " "

2.43×10^{-3} " " "

2.16×10^{-3} " " "

From the additional data presented in the experimental section, it is seen that the reaction is homogeneous, i.e., powdered glass does not affect the rate constant. The addition of sodium acetate increases the rate somewhat (p. 137).

Figure 5A

Second Order Plot for Disproportionation Reaction $(\text{Concentration})^{-1}$ vs. Time

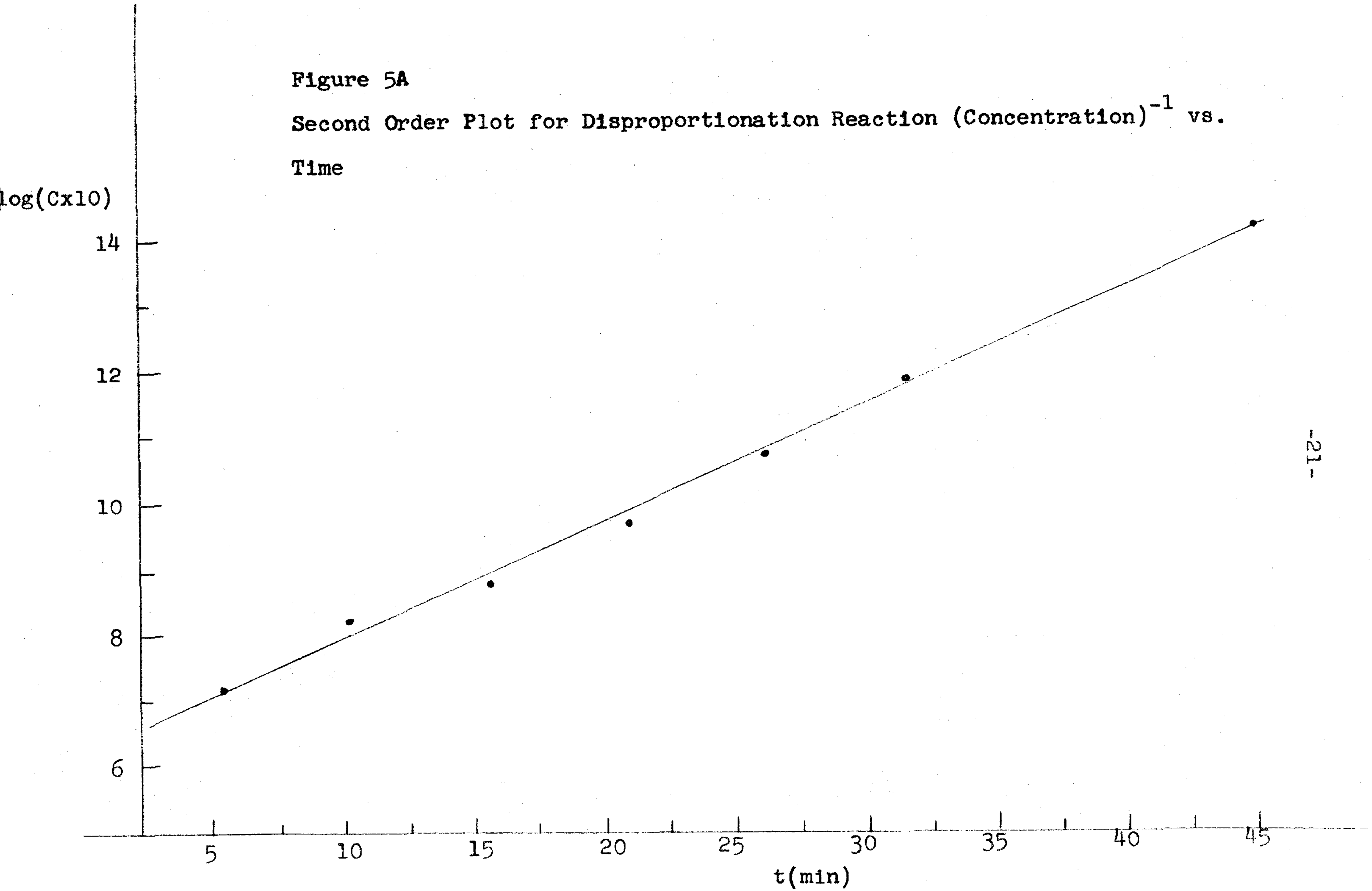
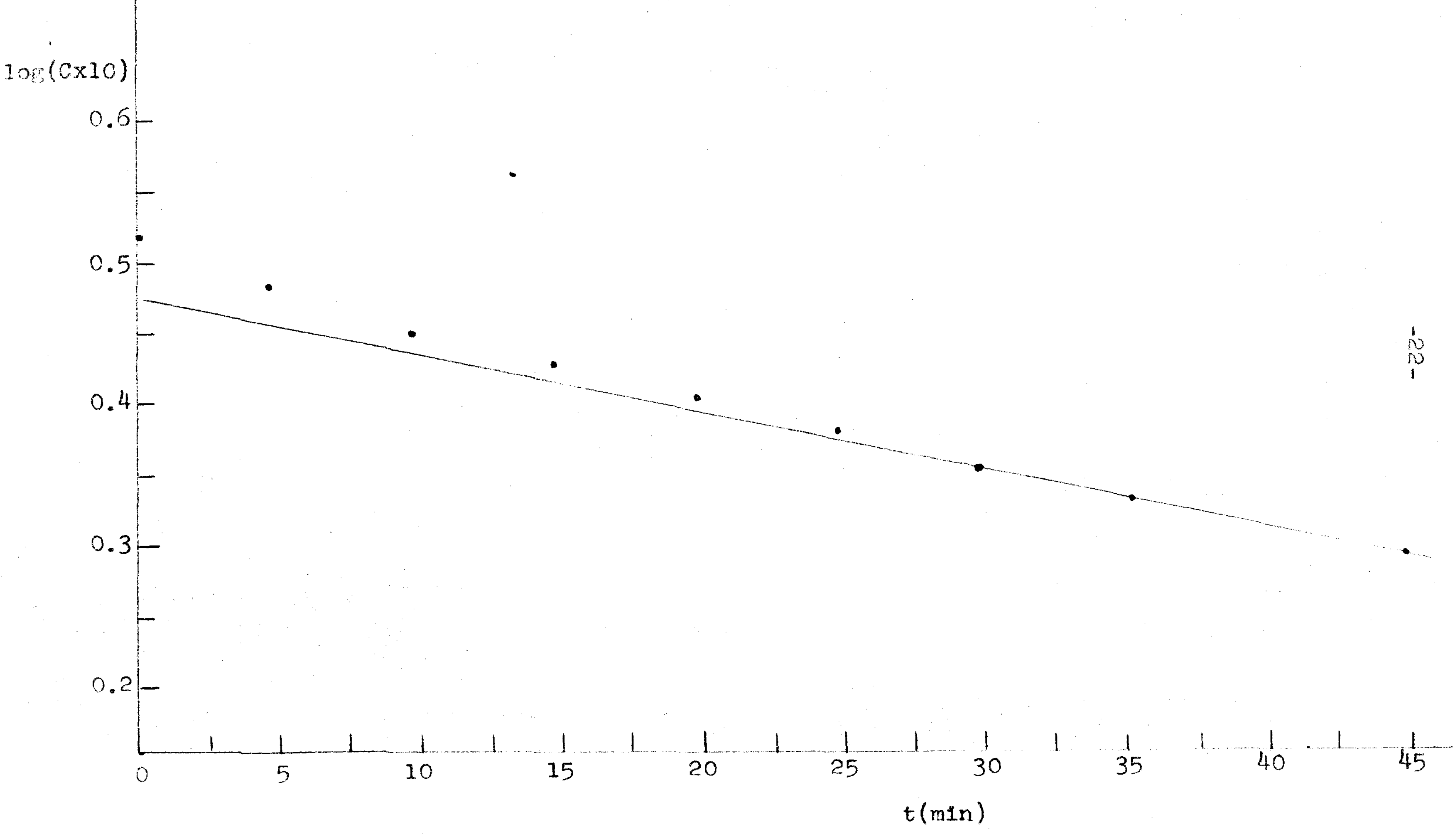


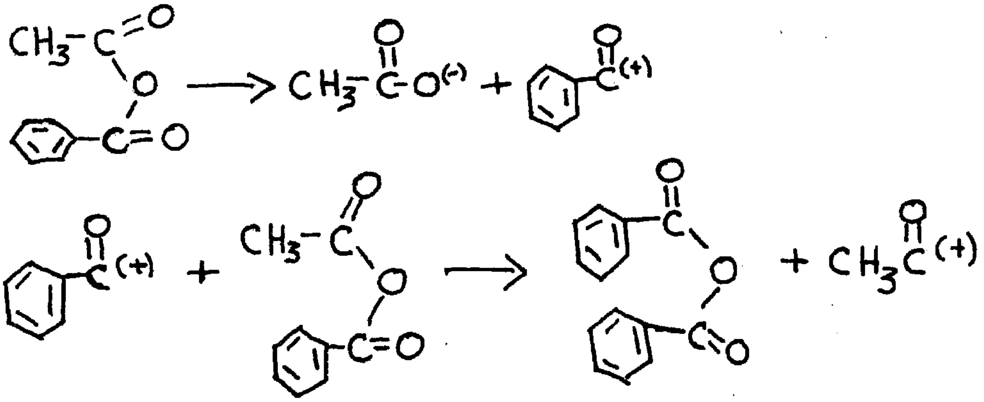
Figure 5B

First Order Plot for Disproportionation Reaction log (Cx10) vs. Time

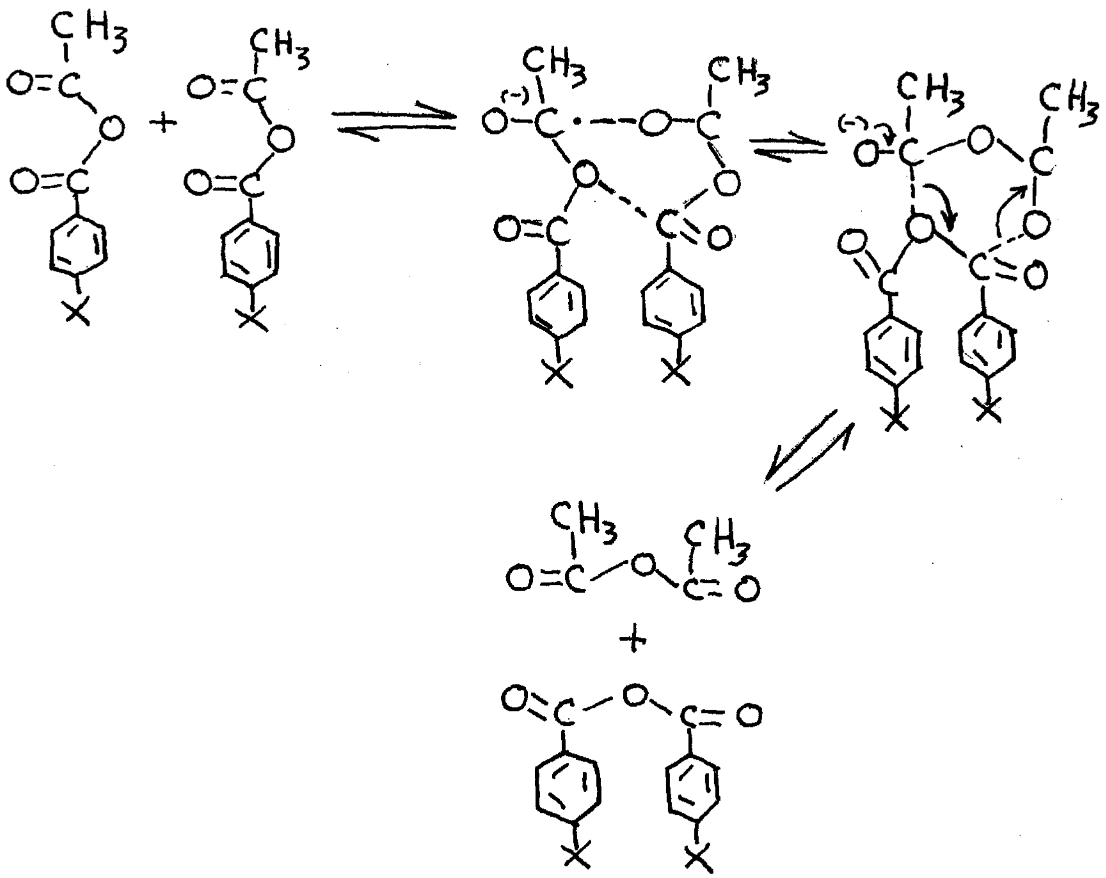


This is easily explained in terms of the work of Evans,(14) who showed that acetate ion labeled with C^{14} very rapidly exchanges with acetic anhydride. Acetic or benzoic acid also increases the rate somewhat. This is understandable in view of the work in the next section.

The evidence set forth effectively excludes from consideration of the mechanism for the disproportionation reaction any scheme which involves as a rate determining step the production of ions as suggested below.

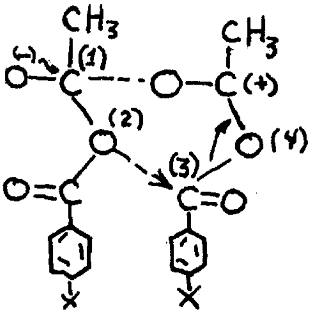


Rather it is believed that the second order reaction kinetics represent a manifestation of a true bimolecular mechanism. It is suggested that the approach of the two reacting molecules is facilitated by the dipole-dipole interaction of the carbonyl groups and that the "reactive intermediate" has the structure shown below.



The breakdown of the active intermediate is favored by a concerted shift of carbon-oxygen bonds throughout the pseudo six-membered ring.

The initial orientation of the reacting molecules is important and determines whether the combination could lead to the same or different species. Because of steric considerations, most reactions which are capable of resulting in a disproportionation probably proceed through an orientation of the activated complex in which the initial contact is formed by the carbonyl functions which have the methyl groups attached (see p. 29).



After the initial interaction of the carbonyl functions to which the methyl groups are attached, one can imagine that the facility with which the activated complex breaks down to give the disproportionated products is governed by the ease with which the carbon-oxygen bond two-three is formed and the carbon-oxygen bond three-four is broken. It is suggested that the mechanism of the disproportionation reaction is such that the rate determining step is the nucleophilic attack of the oxygen atom number two upon the carbonyl carbon atom number three. It is expected that the greater the susceptibility of this carbonyl atom to nucleophilic attack the faster will the rate of disproportionation proceed. The susceptibility of the carbonyl carbon atom to this type of attack will be profoundly affected by the nature of the para substituent X. If this substituent is a nitro group, the nucleophilic attack would be expected to proceed more readily than if the substituent is a methoxyl group.

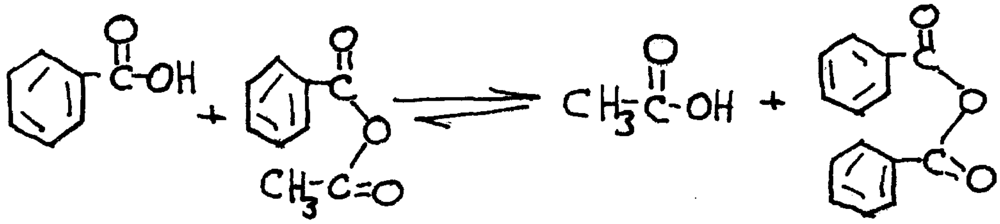
The experimental results confirm the prediction, formulated on the basis of this hypothesis, that the rate of disproportionation of the p-nitrobenzoic-acetic mixed anhydride is greater than the rate of disproportionation of p-methoxybenzoic-acetic mixed anhydride, and that the rate of disproportionation of the benzoic-acetic mixed anhydride has a value intermediate between these two.

Brown and Trotter(7) found that the rate of disproportionation of butyric-acetic mixed anhydride was one thousand times greater than the rate of disproportionation of benzoic-acetic mixed anhydride.

The Interaction of Acetic and Benzoic Acids with the Benzoic-Acetic Anhydride System.

By extending the infrared spectrophotometric techniques used to measure the anhydride systems to a five component mixture, it is possible to quantitatively measure the interaction of acetic or benzoic acids with the benzoic-acetic anhydride system. The para-methoxybenzoic or para-nitrobenzoic-acetic anhydride systems were not suitable for this study, as para-nitrobenzoic and para-methoxybenzoic acids are not soluble in chloroform.

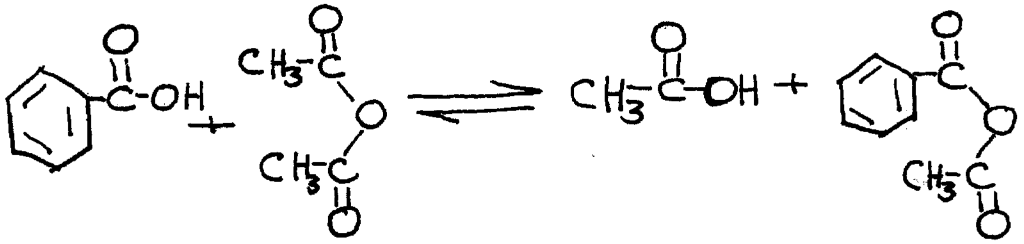
In addition to the equilibrium involving only the anhydrides, the system can be described by defining two other equilibrium constants corresponding to reactions I and II.



I

$$K_{(\text{Acid I})} = \frac{[\text{HAc}][\text{Bz}_2\text{O}]}{[{}^{\text{Bz}}_{\text{Ac}}\text{O}][\text{HBz}]}$$

II



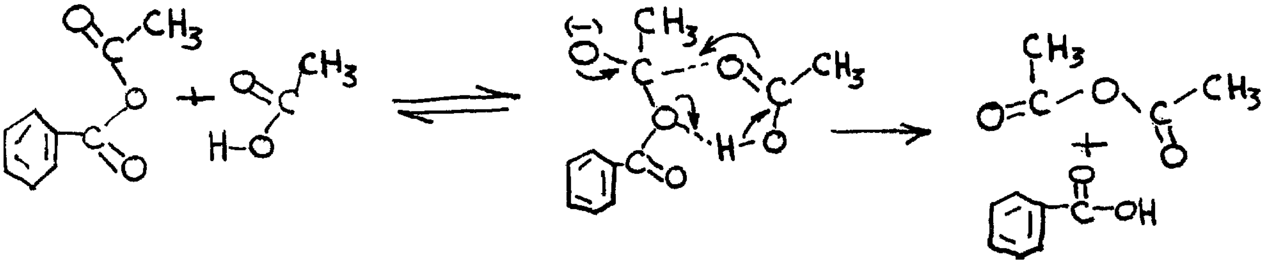
$$K(\text{Acid II}) = \frac{[HAc][{}^{Bz}_{Ac}{>}O]}{[Ac_2O][HBz]}$$

Table 5

Temperature	K (anhyd)	K (acid-I)	K (acid-II)	Composition of Initial Solution
75°C	1.25	1.58	1.97	acetic anhyd, benzoic anhyd, acetic acid
75°C	1.08	3.36	3.61	benzoic-acetic mixed anhyd, benzoic acid
100°C	2.00	1.85	2.04	benzoic-acetic mixed anhyd, benzoic acid
100°C	3.12	2.23	6.95	benzoic anhyd acetic acid
150°C	3.74	1.27	4.75	benzoic-acetic mixed anhyd, acetic acid
150°C	4.94	1.29	6.33	acetic anhyd, benzoic acid

Unfortunately the accuracy of the analytical technique does not permit, with this complicated mixture, a very accurate determination of the equilibrium constants associated with the interaction of the acids with the anhydrides. It is thus not possible to describe the details of the mechanism either in terms of a redistribution reaction or in terms of the disproportionation reaction of the para substituted benzoic-acetic mixed anhydrides. If the acid interaction followed the principles of a redistribution reaction, then the values of the first acid equilibrium constant should be one half and those for the second acid equilibrium constant should be two.

A source of ambiguity is the uncertainty over which molecular species of the acid is able to react with the anhydrides. Recent work(15) has indicated that in the concentration range in which these measurements were made, acetic acid is almost entirely converted to the dimeric form. It is possible to write a mechanism for the reaction of the monomeric acid with an anhydride which is formally quite similar to that proposed for the disproportionation reaction.



From this point of view it does not appear likely that the dimeric form of the acid would react as readily as the monomeric species. If the monomeric form of the acid is the only one which reacts, then the true acid equilibrium constants will be proportional to the square roots of the observed (or dimeric) acid concentrations. If the values for K (acid-I) and K (acid-II) are corrected by using the square roots of the observed acid concentration, no improvement in the consistency of the results is observed. To settle this point a more sensitive analytical procedure will have to be employed.

The Nucleophilic Cleavage of the Benzoic-acetic, p-Methoxybenzoic-acetic, and p-Nitrobenzoic-acetic Mixed Anhydrides.

In this section attention is directed towards the reaction of a mixed anhydride with nucleophilic agents in general and amines (p-toluidine) in particular. In the experimental section an analytical scheme is presented which enables one to determine the product composition of the reaction between a mixed anhydride and p-toluidine. From this procedure a quantity called the acetylation ratio is determined. It is defined as the amount of N-acetyl-p-toluidine found in the gross product divided by the amount of N-(p-substituted benzoyl)-p-toluidine found in the gross product.

Table 6

Acetylation Ratio

Reaction Medium	Benzoic-acetic Mixed Anhyd	p-Methoxybenzoic-acetic Mixed Anhyd	p-Nitrobenzoic-acetic Mixed Anhyd
Benzene	43.9	102.4	2.7
50 w/w Acetone-Benzene	37.7	140.7	4.7
Acetone	29.6	120.5	3.5
50 w/w Acetone-Water	42.5	93.8	3.3

It is evident that in this case the acetylation ratio is relatively independent of the polarity of the reaction solvent. This result can be expected when there are no substituents which could shield the carbonyl groups and whose steric size could be altered through changes in the amount of their solvation. Thus one would not expect the results that Gold(9) obtained with a chloroacetic-acetic mixed anhydride whose acetylation ratio was highly dependent upon the polarity of the reaction medium.

All three of the para substituted benzoic-acetic mixed anhydrides yield a preponderant of N-acetyl-p-toluidine, demonstrating the steric effectiveness of the phenyl group in hindering the attack of p-toluidine at the carbonyl function which is attached to the phenyl group.

The acetylation ratio decreases as one passes from p-methoxybenzoic-acetic mixed anhydride through benzoic-acetic mixed anhydride to the p-nitrobenzoic-acetic mixed anhydride. The marked difference between the p-nitro and p-methoxy compounds is probably due to the combination of mesomeric and inductive effects, which for the p-nitro group will increase the positive charge at the carbonyl carbon atom adjacent to the phenyl ring and for a p-methoxy group will decrease the charge. It is expected that as the charge on one of the carbonyl carbon atoms is increased by substitution in some part of the molecule, the nucleophilic attack will be increasingly favored at that position, and hence the acetylation ratio will change accordingly.

In like manner it would be expected that the rates of hydrolysis of a mixed anhydride should be affected in a similar fashion. The more the positive charge of the carbonyl carbon atom adjacent to the phenyl ring is decreased by the action of substituents in the phenyl ring the slower the rate of hydrolysis. As the positive charge on the carbonyl group is increased, it is expected that the rate of hydrolysis will increase, since the nucleophilic attack would not tend to discriminate against one of the reactive centers. This prediction has been verified, as is seen in Table 7, where apparent first order hydrolysis constants for the mixed anhydrides are given. Berliner(16) also obtained similar results with the symmetrical para substituted anhydrides.

Table 6

Hydrolysis Constants for Para Substituted Benzoic-acetic Mixed Anhydrides in 50% w/w Tetrahydrofuran-water

	Benzoic-acetic Mixed Anhyd	p-Nitrobenzoic-acetic Mixed Anhydride	p-Methoxy-benzoic-acetic Mixed Anhyd
Apparent first order rate constant k	$k = 3.77\text{x}10^{-5}\text{Sec}^{-1}$	$23.65\text{x}10^{-5}\text{Sec}^{-1}$	$1.41\text{x}10^{-5}\text{Sec}^{-1}$

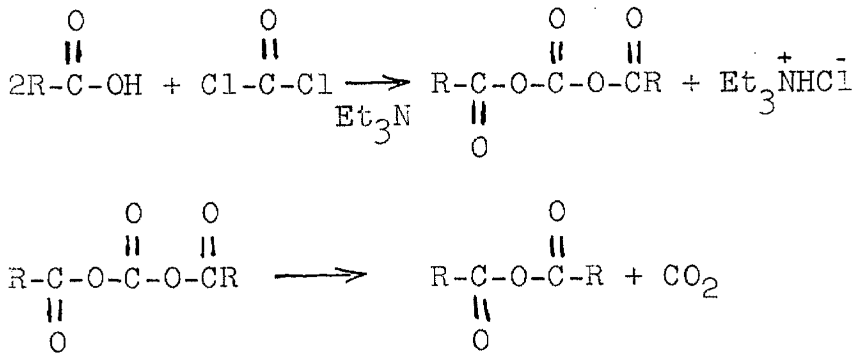
EXPERIMENTAL SECTION.

PREPARATION OF STARTING MATERIALS

Symmetrical Anhydrides

A brief survey of available methods for the preparation of symmetrical anhydrides was made. At that time it was judged that the procedure suggested by Wheland(5) was the most convenient and gave high yields consistent with purity.

This method is based upon the reaction of a carboxylic acid with phosgene in the presence of a tertiary amine and in an inert solvent to form an unstable mixed anhydride of carbonic acid. The mixed anhydride then undergoes rapid disproportionation forming the symmetrical carboxylic anhydride and carbon dioxide.



$$2R-\overset{\overset{O}{\|}}{C}-OH + Cl-\overset{\overset{O}{\|}}{C}-Cl \xrightarrow[Et_3N]{} R-\underset{\underset{O}{\|}}{C}-O-\overset{\overset{O}{\|}}{C}-O-\overset{\overset{O}{\|}}{C}R + Et_3\overset{+}{N}H\overset{-}{Cl}$$

$$R-\underset{\underset{O}{\|}}{C}-O-\overset{\overset{O}{\|}}{C}-O-\overset{\overset{O}{\|}}{C}R \longrightarrow R-\underset{\underset{O}{\|}}{C}-O-\overset{\overset{O}{\|}}{C}-R + CO_2$$

It was found necessary to carry out the reaction at -10°C where yields of 80% were obtained. At 0°C only 10% of the desired product was formed.

Procedure

In the following manner benzoic anhydride, symmetrical p-methoxybenzoic, and symmetrical p-nitrobenzoic anhydrides were synthesized:

One tenth mole of the desired acid was dissolved in 250 ml. of dry tetrahydrofuran.* The solution was placed in a 500 ml. three necked round bottomed flask equipped with a sealed mechanical stirrer and protected from the atmosphere with a calcium chloride drying tube. Twenty-four one hundredths mole of dry triethylamine was added and the mixture cooled to -10°C in an ice salt bath.

A solution of 0.11 mole of phosgene in 50 ml. tetrahydrofuran was prepared at room temperature and then cooled to -10°C. The cooled phosgene solution was slowly added by means of a dropping funnel to the stirred solution of acid and triethylamine. The process of addition requires one half hour.

The solid triethylamine hydrochloride was separated by rapid filtration and the solvent removed by distillation under reduced pressure in the presence of nitrogen. The results are shown in Table 7.

In order to detect small quantities of the parent acid

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*Tetrahydrofuran is preferred as a reaction solvent, since it dissolves larger quantities of the aromatic acids than do the other low boiling inert solvents. It may be conveniently dried by distillation over sodium wire but cannot be stored in the presence of sodium without further treatment with lithium aluminum hydride to avoid slow deterioration.

in a sample of anhydride, recourse can be made to spectrophotometric analysis in the infrared spectra. The carbonyl stretching frequencies in the neighborhood of 5.7-5.9 microns are used, since they are very intense bands. The absorption bands of the anhydride are shifted 0.13 microns toward shorter wave length relative to the corresponding bands of the acid. Using this method it is possible to detect five parts in 1000 of acid in anhydride. The final criterion of purity of a sample of anhydride was the complete absence of any absorption bands of the acid in the spectrum of the anhydride.

Table 7

Preparation of Symmetrical Anhydrides

Anhydride	M.P. after First Recrystallization	Final Yield	Final M.P.	Wave length of Characteristic Absorption	
				Acid	Anhyd.
Benzoic	43.0-44.5	71%	44.5-45.0	5.89	5.78
p-Methoxy-benzoic	87-92	53%	95.5-96.2	5.87	5.74
p-Nitro-benzoic	188-190	48%	191.5-192.0	5.90	5.79

Recrystallization Solvent

Benzoic Anhydride	2 Vol. Petroleum Ether . 1 Vol. Benzene
p-Methoxybenzoic	Carbon Tetrachloride
p-Nitrobenzoic	Purified Acetone

The acetic anhydride used in the study was purified by distillation over sodium wire and fractionated through 150 cm. of helices in a Todd column. The middle third portion was retained. The material was stored over sodium wire. Each preparation was used for two weeks only, as acetic anhydride seems to attack sodium wire slowly at room temperature.

Mixed Anhydrides (Para Substituted Benzoic-Acetic Mixed Anhydrides)

An old method of obtaining mixed anhydrides involves the reaction between the sodium salt of one acid and the acid chloride of another. This method suffers from the fact that the sodium chloride forms a finely divided precipitate and a colloidal suspension usually results. Separation of this reaction mixture usually involves lengthy filtration or contracted centrifugations, which result in lower yields. The method of choice is therefore the reaction of ketene and the desired aryl carboxylic acid. This results in the case of benzoic and *p*-methoxybenzoic acids in rapid quantitative conversions to the para substituted benzoic-acetic anhydride.

p-Nitrobenzoic acid, however, failed to react in a straight forward manner with ketene, and it was necessary to fall back upon the reaction between sodium *p*-nitrobenzoate and acetyl chloride.

Preparation of Mixed Anhydrides by Reaction of Aryl Carboxylic Acids and Ketene

One hundredth mole of the desired acid was dissolved in 200 ml. of dry tetrahydrofuran contained in a 500 ml. Erlenmeyer flask. The flask was then placed on a magnetic stirrer and mildly agitated, while a slow steady stream of ketene was passed into the solution. The reaction was followed by dipping a stirring rod into the solution and allowing the adhering solution to evaporate. As the reaction proceeded smaller quantities of solid remained on the stirring rod after the evaporation of the solvent, and at the end of the reaction only a heavy oil was produced. Near the end of the reaction the rate of addition of ketene was decreased and the solution closely observed. As soon as a slight brown color had appeared, the reaction was stopped. This brown color is due to a slight excess of ketene in the solution, which is quickly converted into dark colored condensation products.

The ketene was produced by the pyrolysis of acetone as described by Hurd.(17) The time for addition of ketene was 45 minutes.

The solvent was then removed from the reaction mixture by distillation under reduced pressure and the residue purified by fractionation or recrystallization.

Benzoic-acetic Mixed Anhydride	Yield after Fractionation 80%	B.P. 88-89°at 0.2 mm. M.P. 1.5°C n^{D}_{25} = 1.5152
p-Methoxybenzoic-acetic Mixed Anhydride	Yield after Recrystallization from Benzene 79%	M.P. 43-44°C

Synthesis of p-Nitrobenzoic-acetic Mixed Anhydride

When p-nitrobenzoic acid was treated in the above manner with ketene, only starting material could be obtained. Therefore, it was necessary to utilize the reaction between sodium p-nitrobenzoate and acetyl chloride to obtain the desired product.

The mixed anhydride is a relatively unstable substance. It reacts quickly with any moisture in its environment and upon heating is rapidly disproportionated to the symmetrical anhydrides. Therefore, in order to prepare pure samples of this anhydride, it is necessary to carry out the reaction at room temperature and in the complete absence of moisture. Sodium p-nitrobenzoic and acetyl chloride react vigorously with the evolution of heat, and hence it is necessary to introduce the acid chloride diluted with an inert solvent.

To bring about these conditions the apparatus shown in Figure 6 was designed. The reaction unit is fundamentally a Soxhlet extractor modified in such a manner that liquid is transferred from the lower chamber to the upper by means of a vapor lift using nitrogen gas.

Procedure

A 75 x 150 mm. Soxhlet extraction thimble is soaked over night in reagent grade acetyl chloride, washed with anhydrous ether and dried in the oven at 120° for 15 min. Without being cooled, it is filled with dry sodium p-nitro-

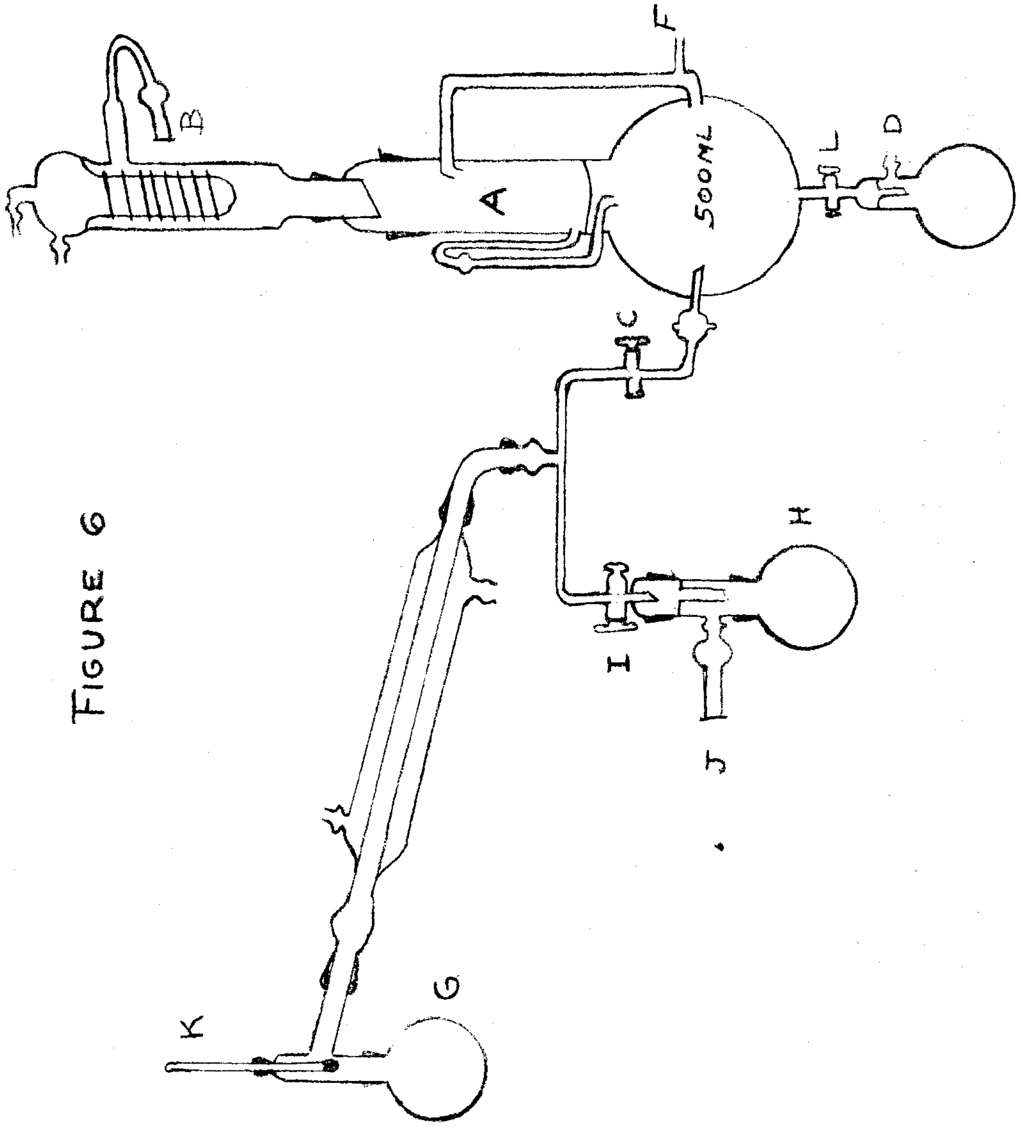

FIGURE 6

benzoate and placed in the upper chamber of the modified Soxhlet extractor shown at A.*

In the flask G was placed 500 ml. of reagent grade chloroform, which was then slowly distilled; the distillate being collected in receiver H until 100 ml. had been collected. The stop cock at C was opened and the one at I closed, and 120 ml. of chloroform was collected in the lower chamber of the reaction vessel. Stop cock C was then closed. Reagent grade acetyl chloride in the amount of 150 ml. was placed in flask G and distilled into receiver H until no hydrogen chloride gas was given off through the drying tube J and the reading of the thermometer K indicated that pure acetyl chloride was distilling. (About 20 ml. of the material collected.) Stop cock C was then opened and stop cock I closed. A stream of nitrogen was then admitted through F in such a manner that a slow steady stream of liquid contained in the lower chamber of the reaction vessel was circulated over the sodium p-nitrobenzoate contained in the extraction thimble in the upper part of the reaction vessel. In the course of 15 minutes, 30 ml. of acetyl chloride was transferred into the reaction chamber. Stop cock C was then closed and the material circulated for an

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*Before the reaction, the apparatus was thoroughly dried by placing a rubber stopper in the drying tube at B, the stop cock at C closed, and the reaction vessel evacuated through the port at D. Under reduced pressure the apparatus was flamed and then returned to atmospheric pressure by admission of dry nitrogen through port F.

additional five minutes. Stop cock L was then opened, and the liquid was transferred to the flask beneath the reaction vessel.

Seventy-five milliliters of dry chloroform was again distilled into the reaction vessel and circulated over the sodium p-nitrobenzoate for 15 minutes. This solution was then added to the main reaction mixture through stop cock L. The solvent was then removed by distillation under vacuum applied through port D.

The product was extracted twice with cold carbon tetrachloride, leaving behind a small amount of insoluble residue. An over-all yield of about 30% based upon sodium p-nitrobenzoate was obtained.

The purified material melted sharply at 86°C. Shortly after melting, a solid phase separated out of the clear melt. This material was identified as p-nitrobenzoic anhydride and presumably arose through rapid disproportionation of the mixed anhydride.

Each of the mixed anhydrides was purified until its spectrum did not change upon three consecutive purification operations. It was noted that distinct and prominent bands of the symmetrical anhydrides and of the acids were always missing from the spectra of the mixed anhydrides.

Choice and Purification of Solvent Materials

Of the various solvent materials available for use in infrared work, chloroform was found to be the most suitable.

For the p-nitro and p-methoxy systems it was a much better solvent than carbon tetrachloride, yet it did not have a strong absorption in the spectral region under investigation.

Pure chloroform has the rather distinct disadvantage of undergoing a slow photochemical conversion with the oxygen of the air to form phosgene.

The chloroform was purified by taking reagent grade material and washing it several times with 6-8 N sodium hydroxide, then several times with 6 N hydrochloric acid and finally with several portions of water. The solvent was then dried over calcium chloride and fractionated through a 300 cm. packed column under an atmosphere of nitrogen. The purified chloroform was stored in 25 ml. dark colored glass stoppered bottles at 5°C.

Only freshly prepared material was used for equilibrium studies, and once a sample was opened to the air it was discarded. With the above precautions the conversion of chloroform to phosgene was not a troublesome factor.

Demonstration of the Interconversion of the Symmetric and Mixed Anhydrides

When a sample of a mixed anhydride is dissolved in an inert solvent and allowed to stand under anhydrous conditions, its infrared spectrum slowly changes and assumes a new configuration. The greatest change occurs in the region of the C-O-C stretching frequency between eight and nine microns and is characterized by the decrease in size of some of the absorption peaks and the appearance of new peaks rather close to those which decreased. This change in configuration of the infrared absorption spectrum for benzoic-acetic mixed anhydride is illustrated in Figure 7. It is possible to identify all of these new peaks with those in the spectrum of one or the other of the symmetrical anhydrides.

In a converse manner it is observed that when the two symmetrical anhydrides are mixed in equal proportions, the initial spectrum of the mixture changes in a manner analogous to the way that the spectrum of the mixed anhydride changed. The new peaks that arise can be identified with those of the mixed anhydride. If given sufficient time, the final spectrum of the mixed anhydride and the spectrum of a mixture of equal portions of the symmetrical anhydrides become identical.

Therefore, it is judged that what is being observed is the equilibration of the three anhydride species among one another.

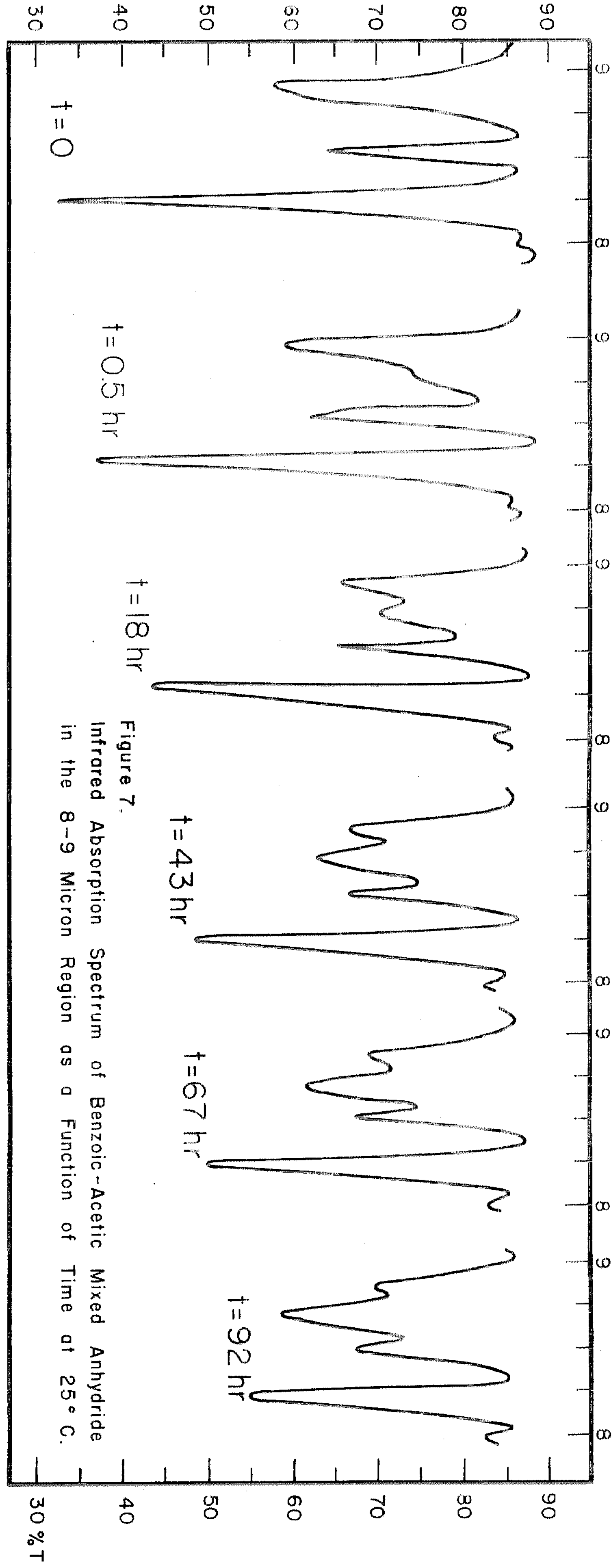


Figure 7.
Infrared Absorption Spectrum of Benzoic-Acetic Mixed Anhydride in the 8-9 Micron Region as a Function of Time at 25° C.

The pertinent spectra for the three anhydride systems studied are shown in Figures 8 to 19.

In exactly the same way it can be demonstrated that an acid and an anhydride can equilibrate to form a mixture of three anhydrides and two acids. See Figure 20.

Quantitative Measurements Made with the Perkin-Elmer Model 21 Infrared Spectrophotometer

The Perkin-Elmer instrument is designed primarily for qualitative use and is most convenient to use in this capacity. When quantitative measurements are sought it is necessary to devise a special operating procedure, which is often quite tedious in execution.

When operating in the eight and one half to nine micron region, the resolution control was set so that a slit width of 60-70 microns was obtained.

Reliance on the wave length scale was never used in setting the machine at the position of an absorption peak which was under consideration. Rather the outline of the peak was traced out each time, the machine reversed and allowed to retrace its first outline of the peak until the maximum was again reached. At this point of maximum absorption the wave length drive was stopped and the machine readied for the reading of the optical density. It was found that the position of a maximum might vary about 0.02 microns from day to day.

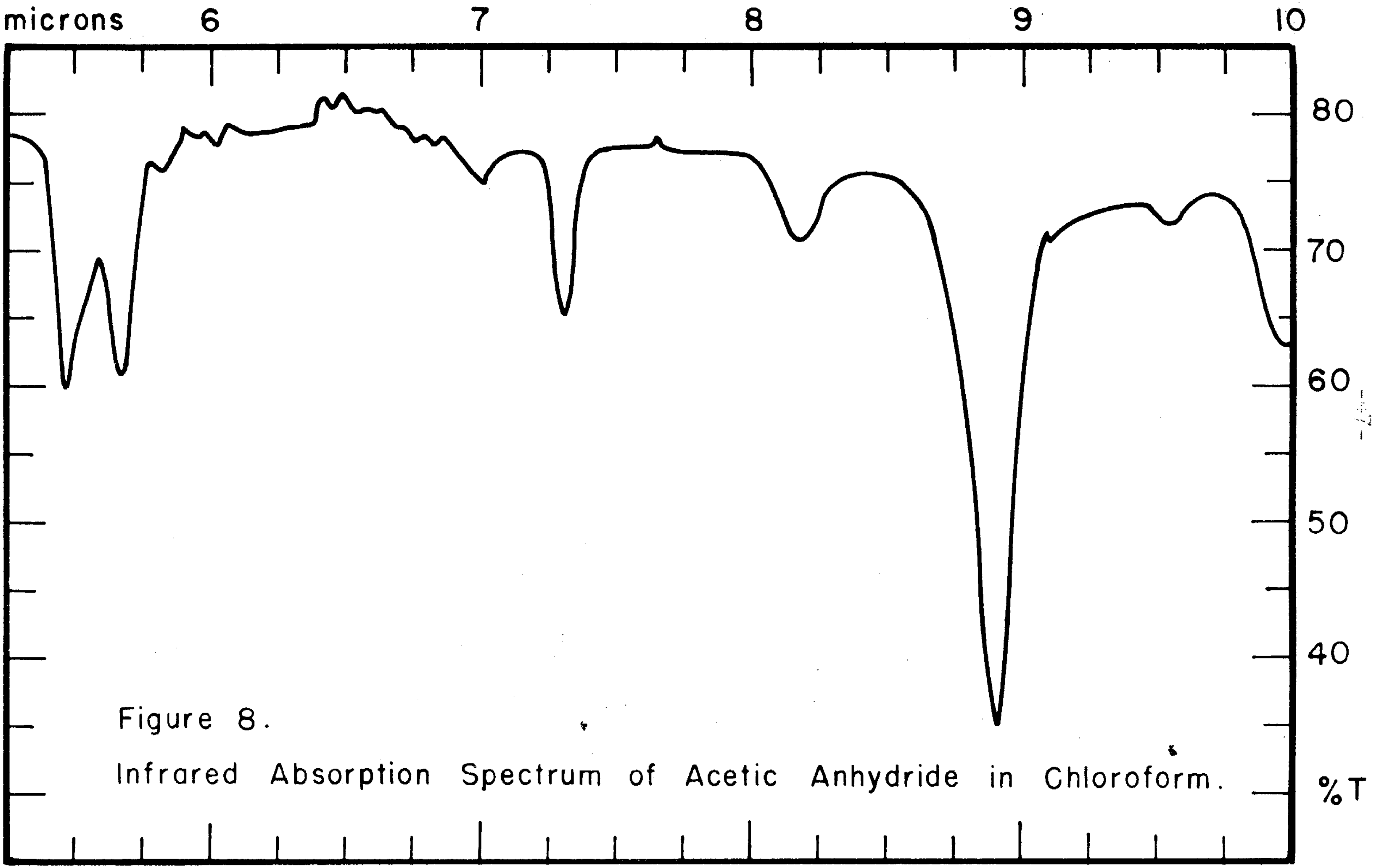


Figure 8.

Infrared Absorption Spectrum of Acetic Anhydride in Chloroform.

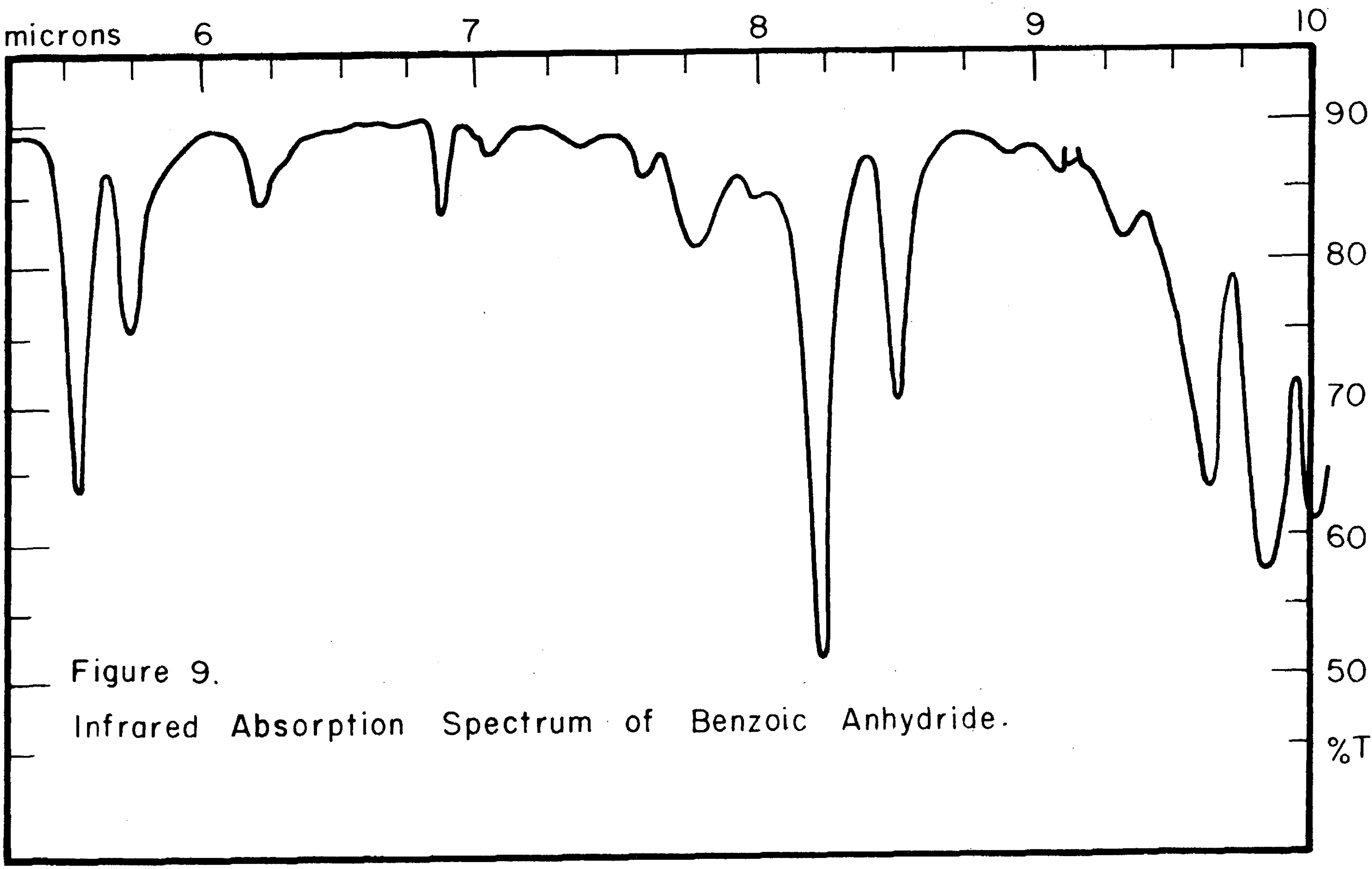


Figure 9.
Infrared Absorption Spectrum of Benzoic Anhydride.

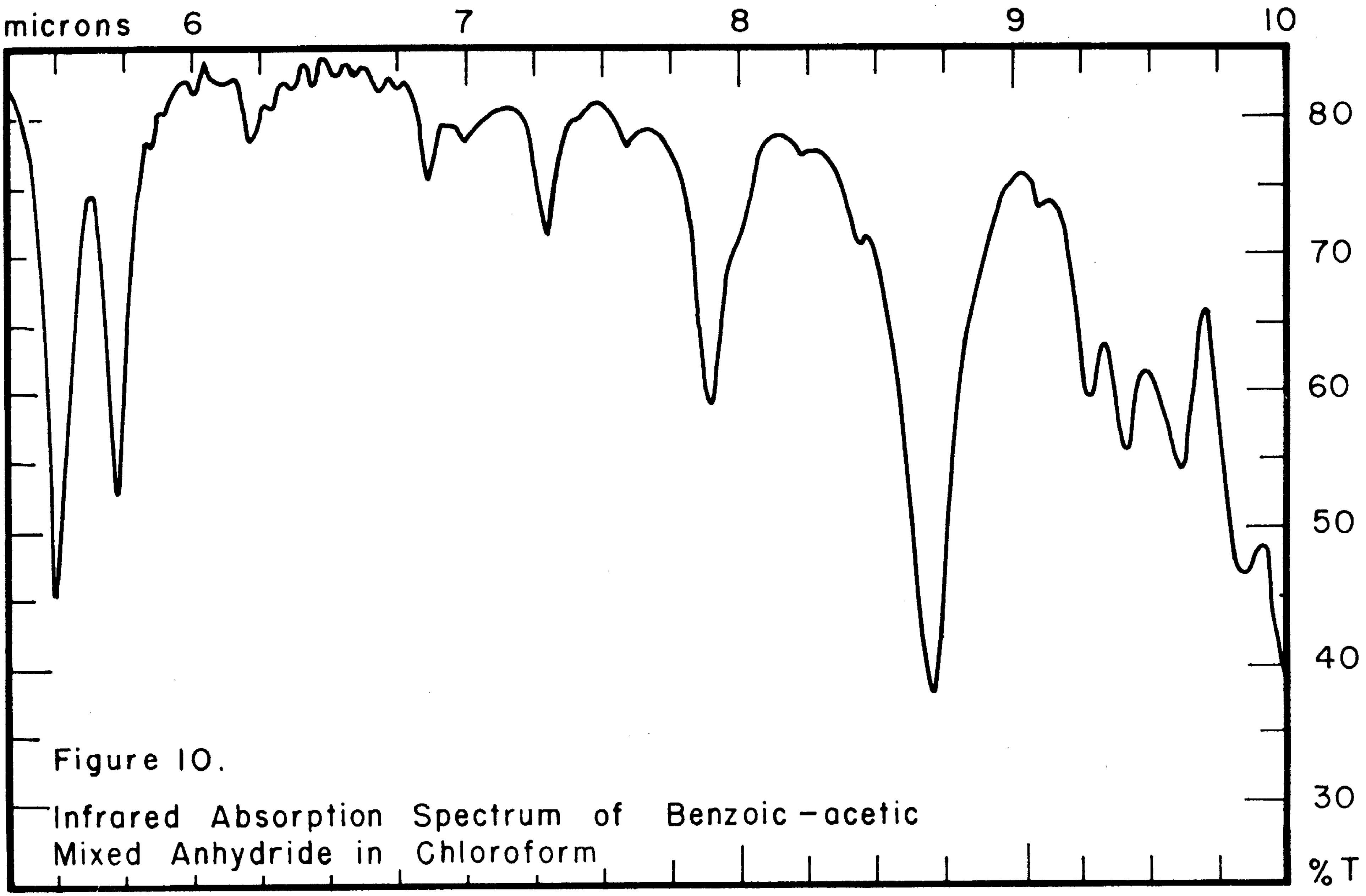


Figure 10.

Infrared Absorption Spectrum of Benzoic-acetic Mixed Anhydride in Chloroform

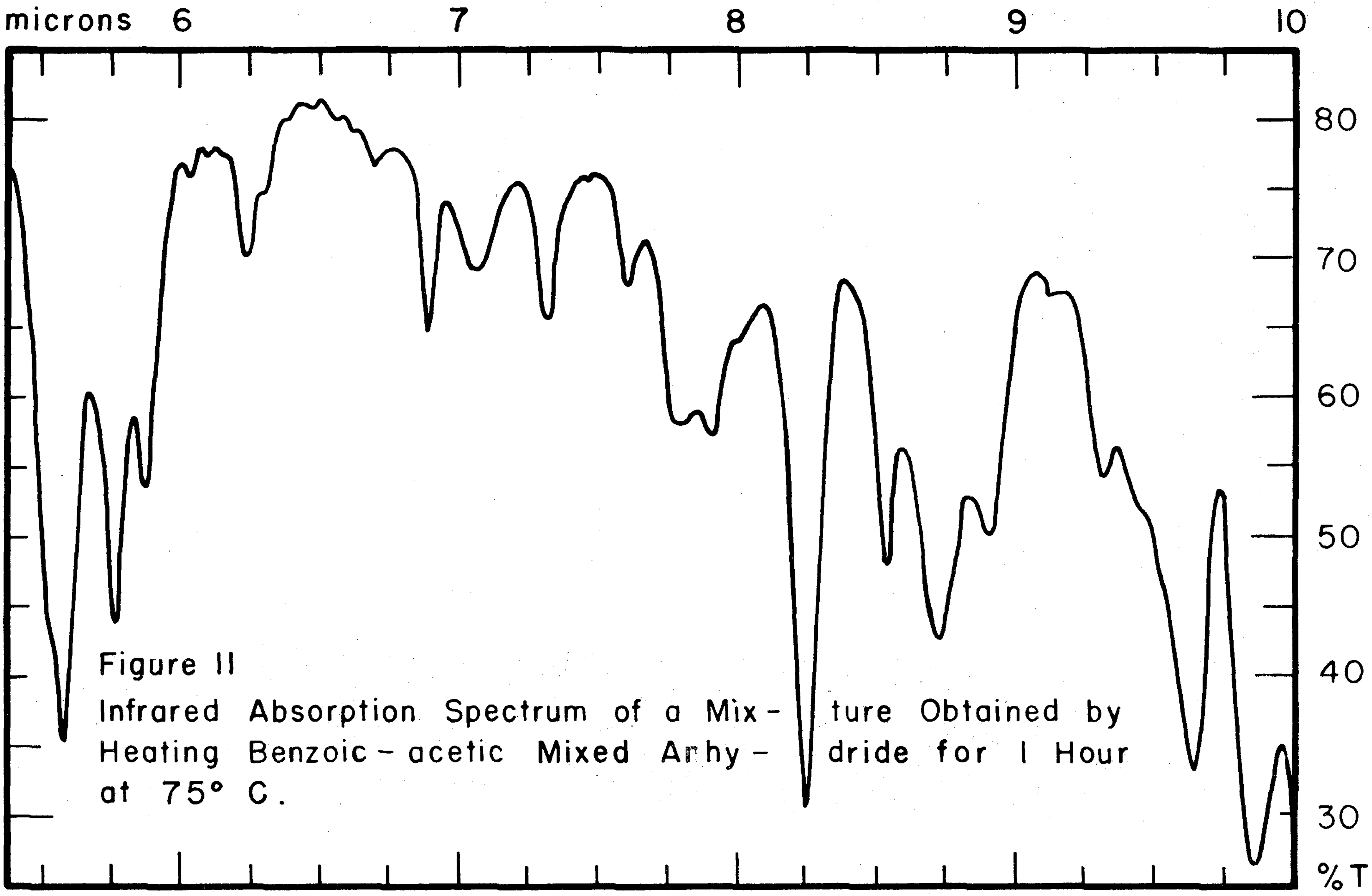


Figure II

Infrared Absorption Spectrum of a Mixture Obtained by Heating Benzoic-acetic Mixed Anhydride for 1 Hour at 75° C.

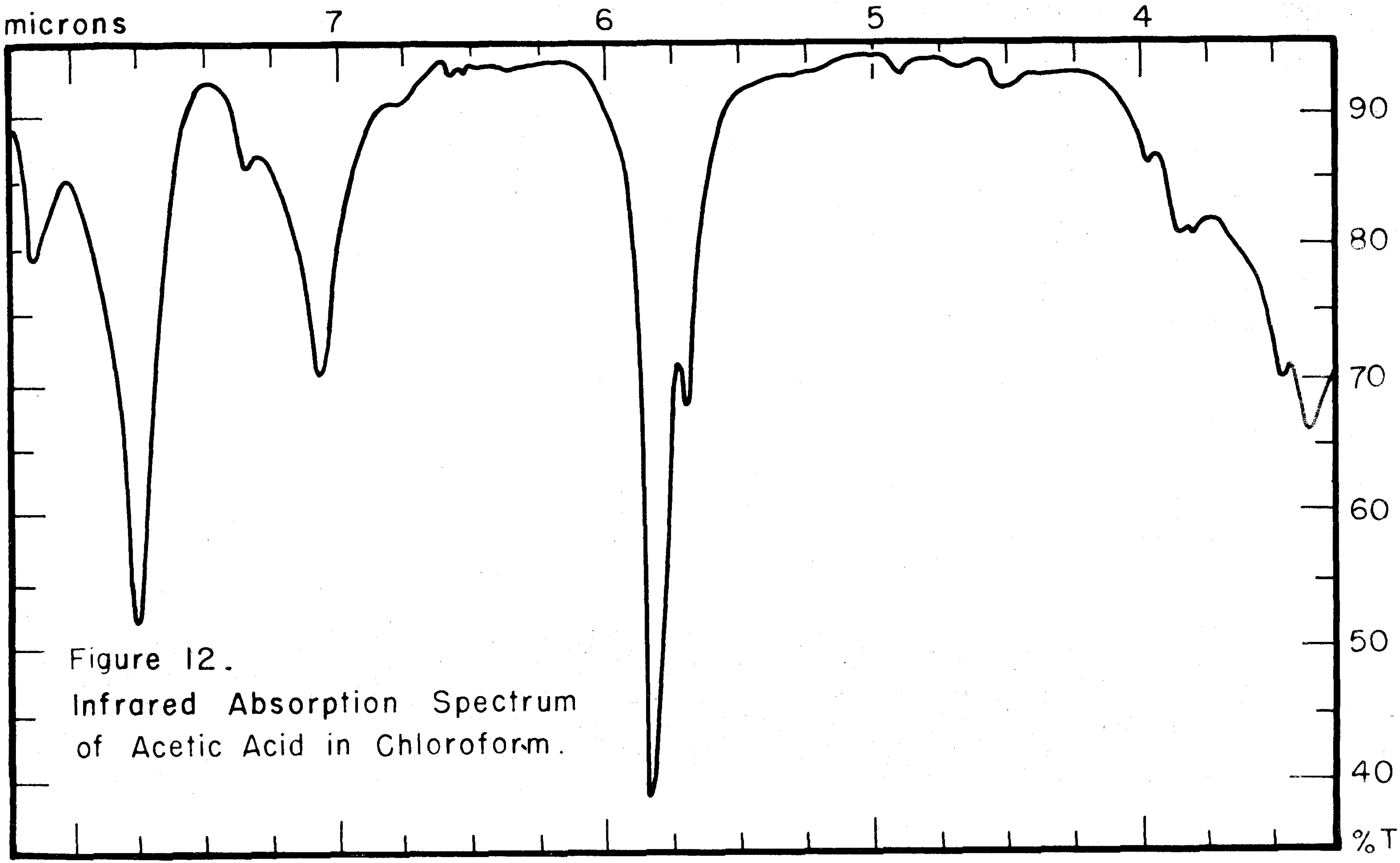


Figure 12.
Infrared Absorption Spectrum of Acetic Acid in Chloroform.

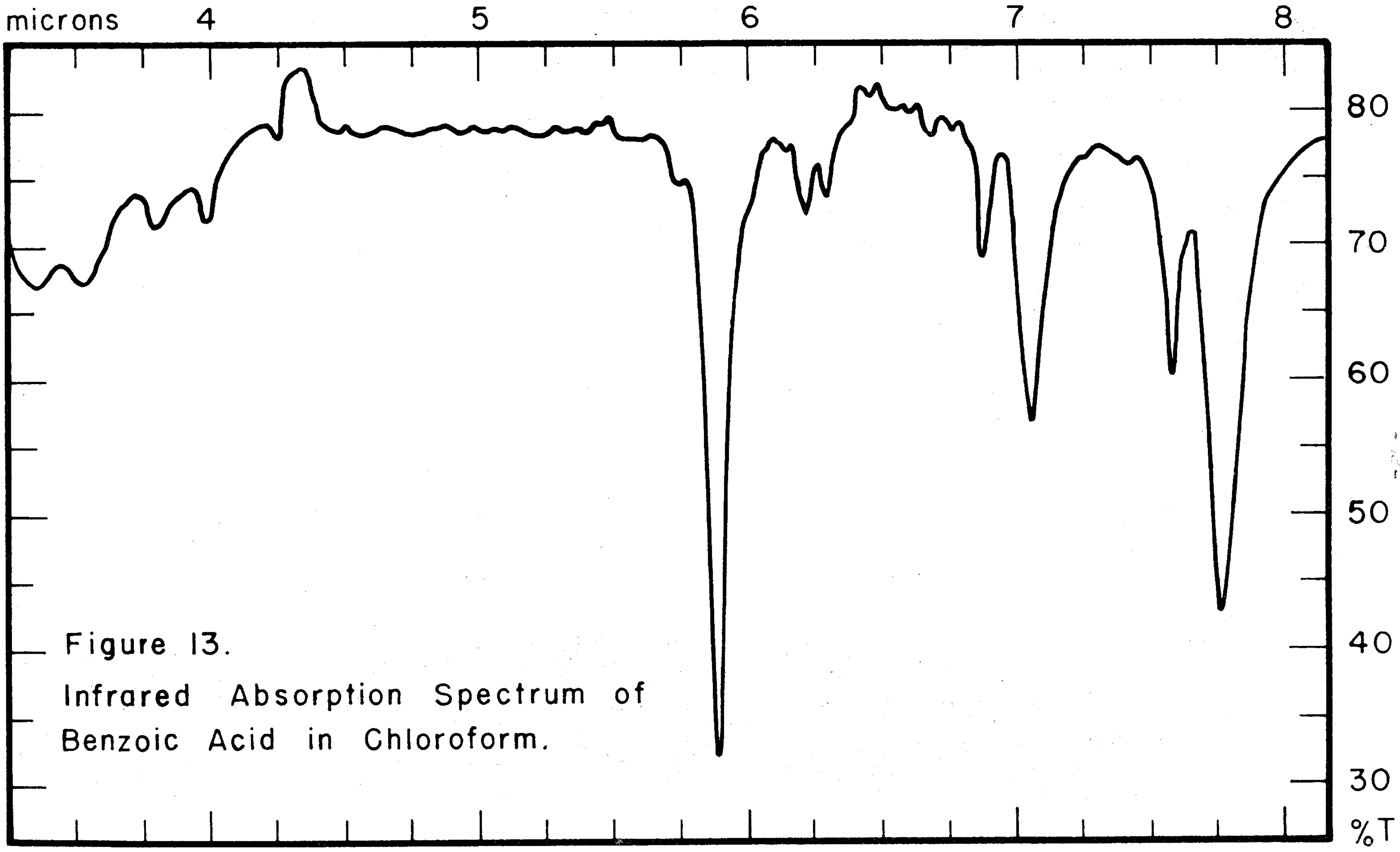


Figure 13.

Infrared Absorption Spectrum of Benzoic Acid in Chloroform.

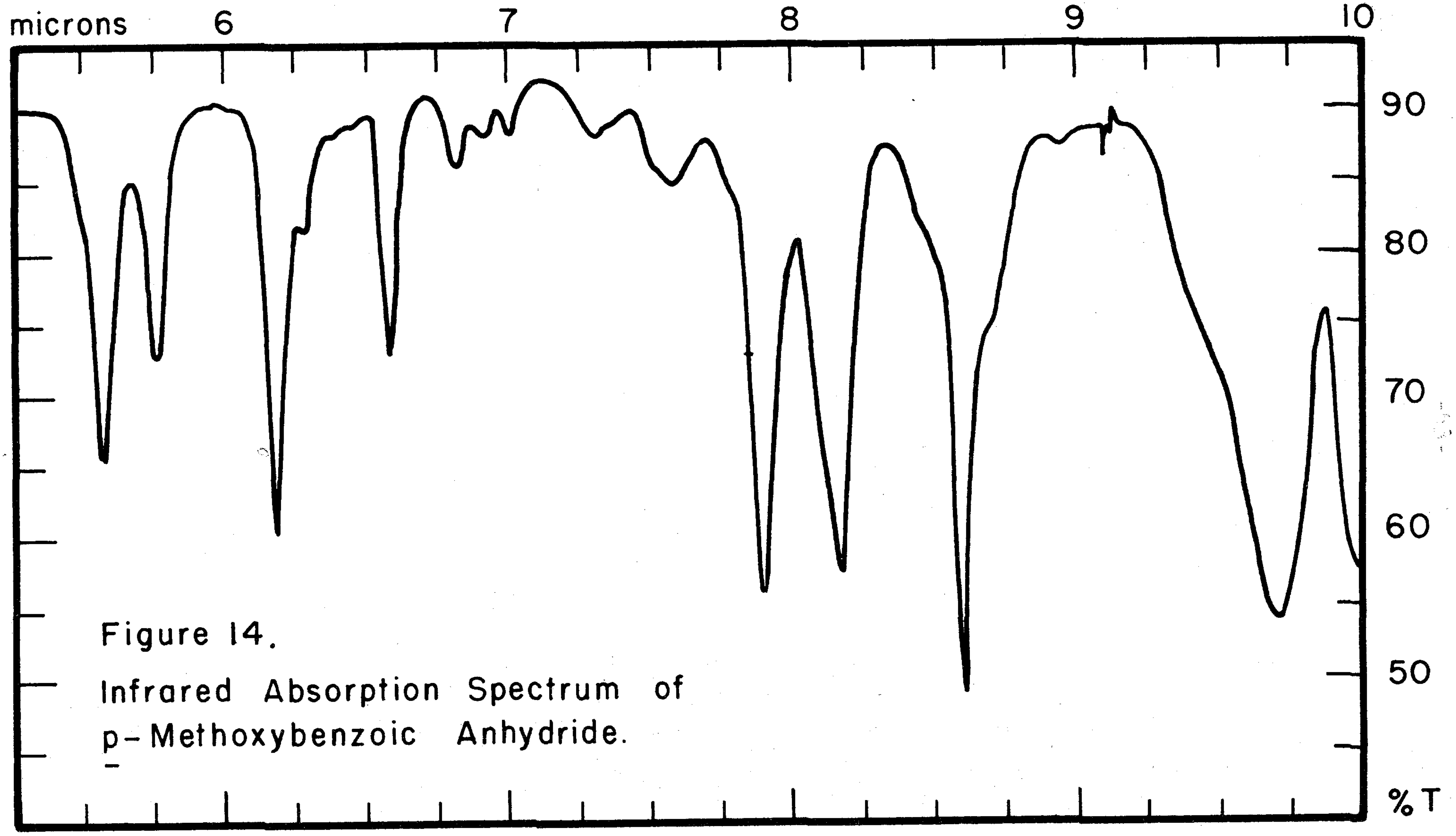


Figure 14.

Infrared Absorption Spectrum of *p*-Methoxybenzoic Anhydride.

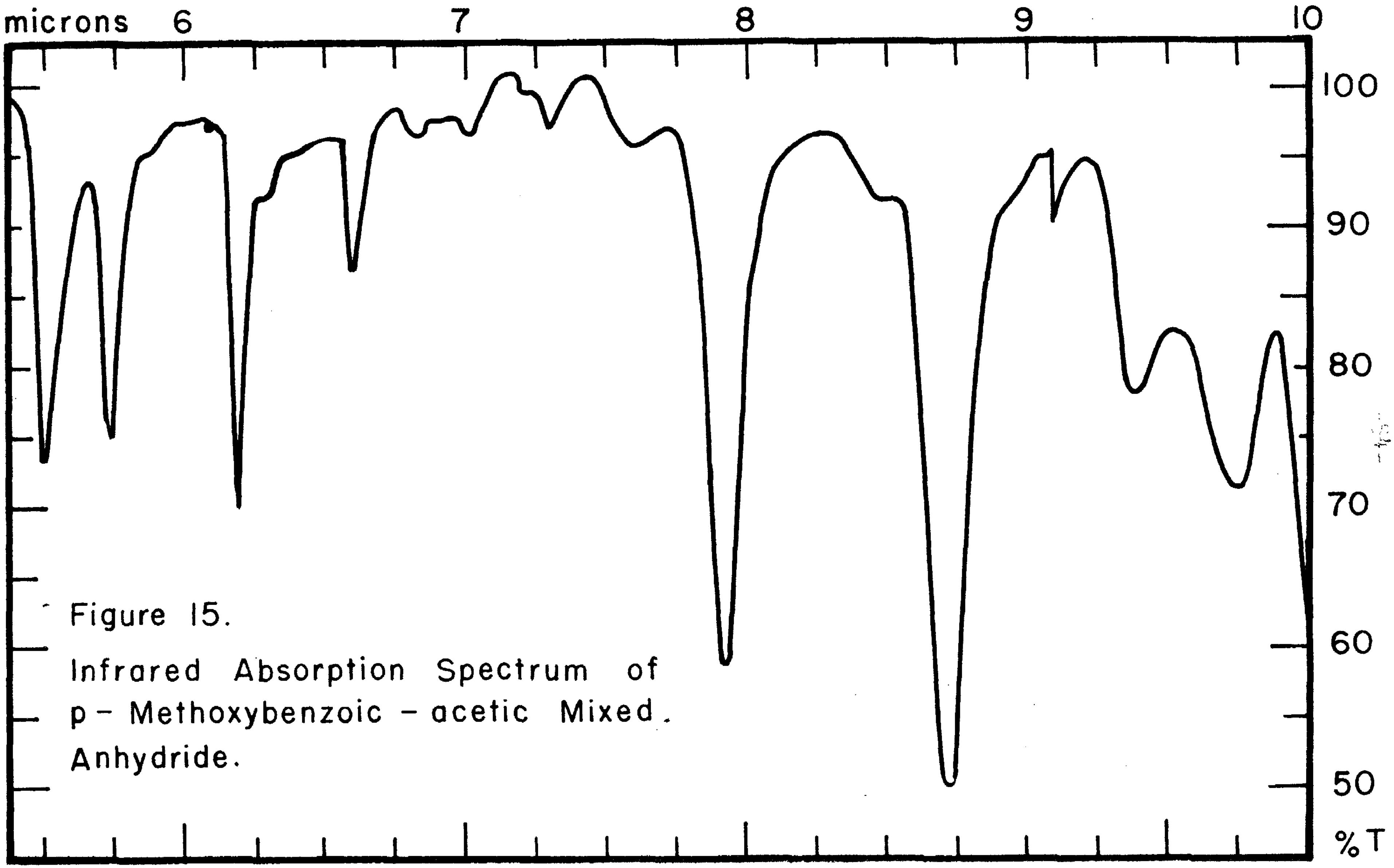


Figure 15.

Infrared Absorption Spectrum of p-Methoxybenzoic-acetic Mixed Anhydride.

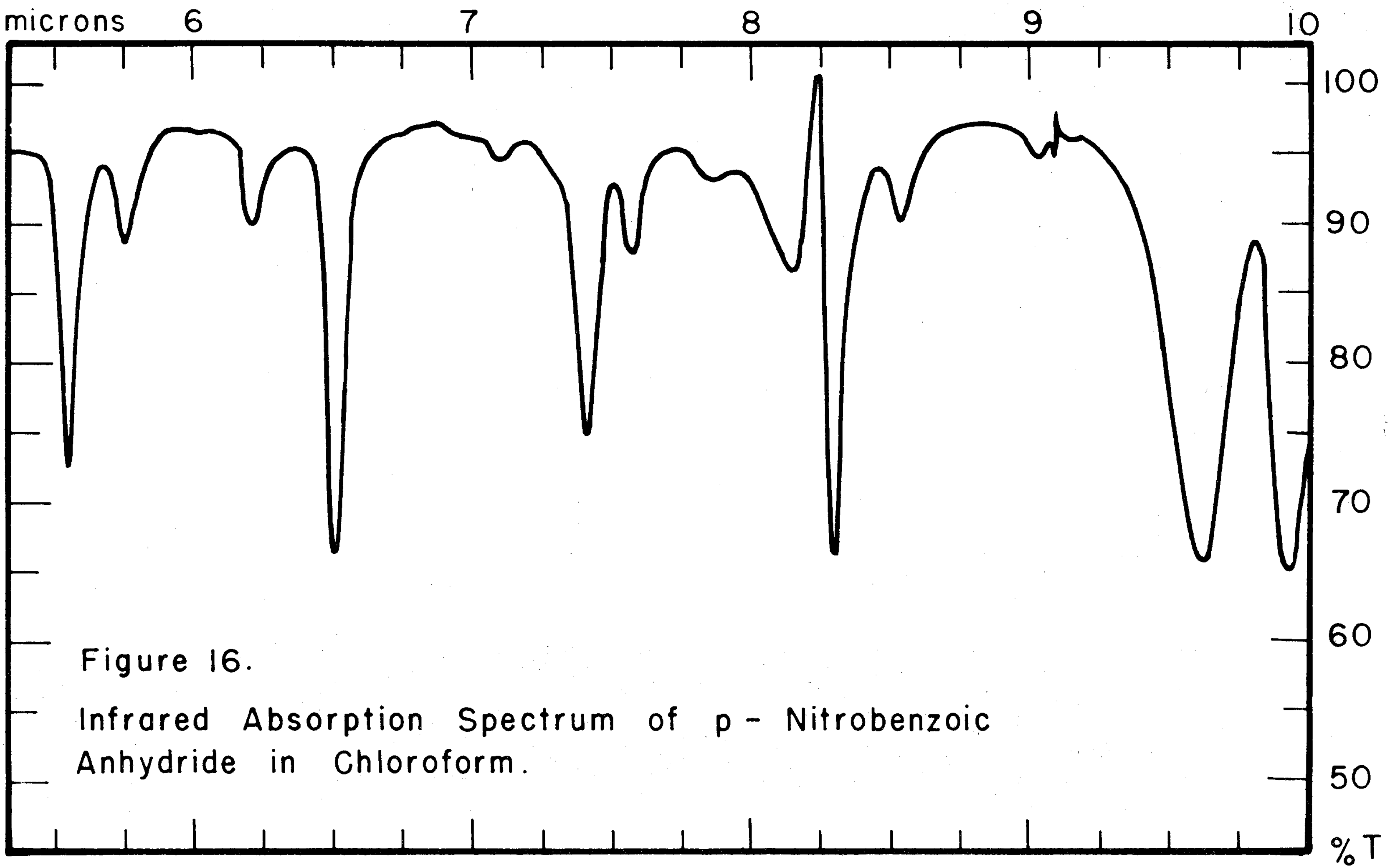


Figure 16.

Infrared Absorption Spectrum of p-Nitrobenzoic Anhydride in Chloroform.

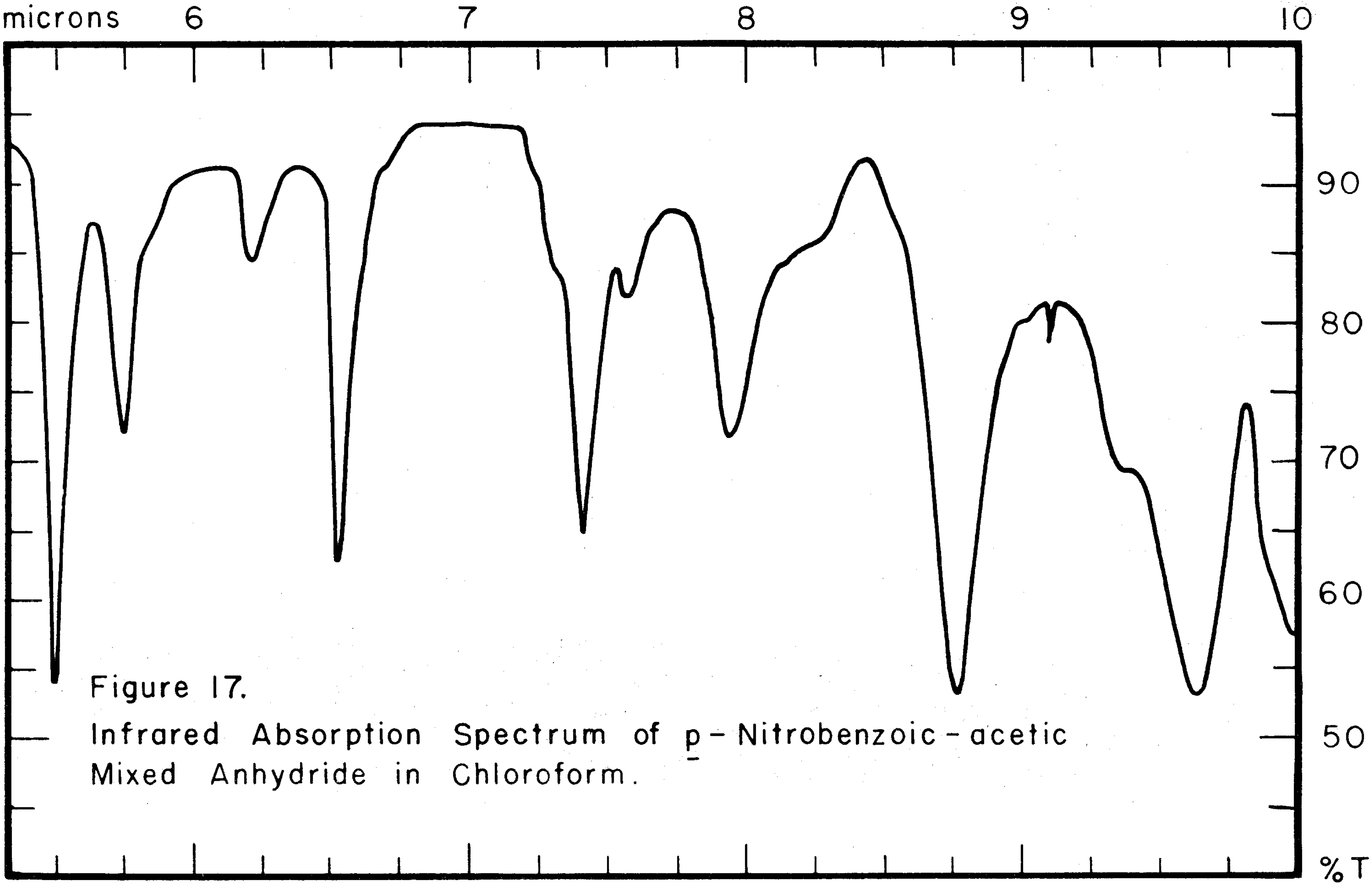


Figure 17.
Infrared Absorption Spectrum of p-Nitrobenzoic-acetic Mixed Anhydride in Chloroform.

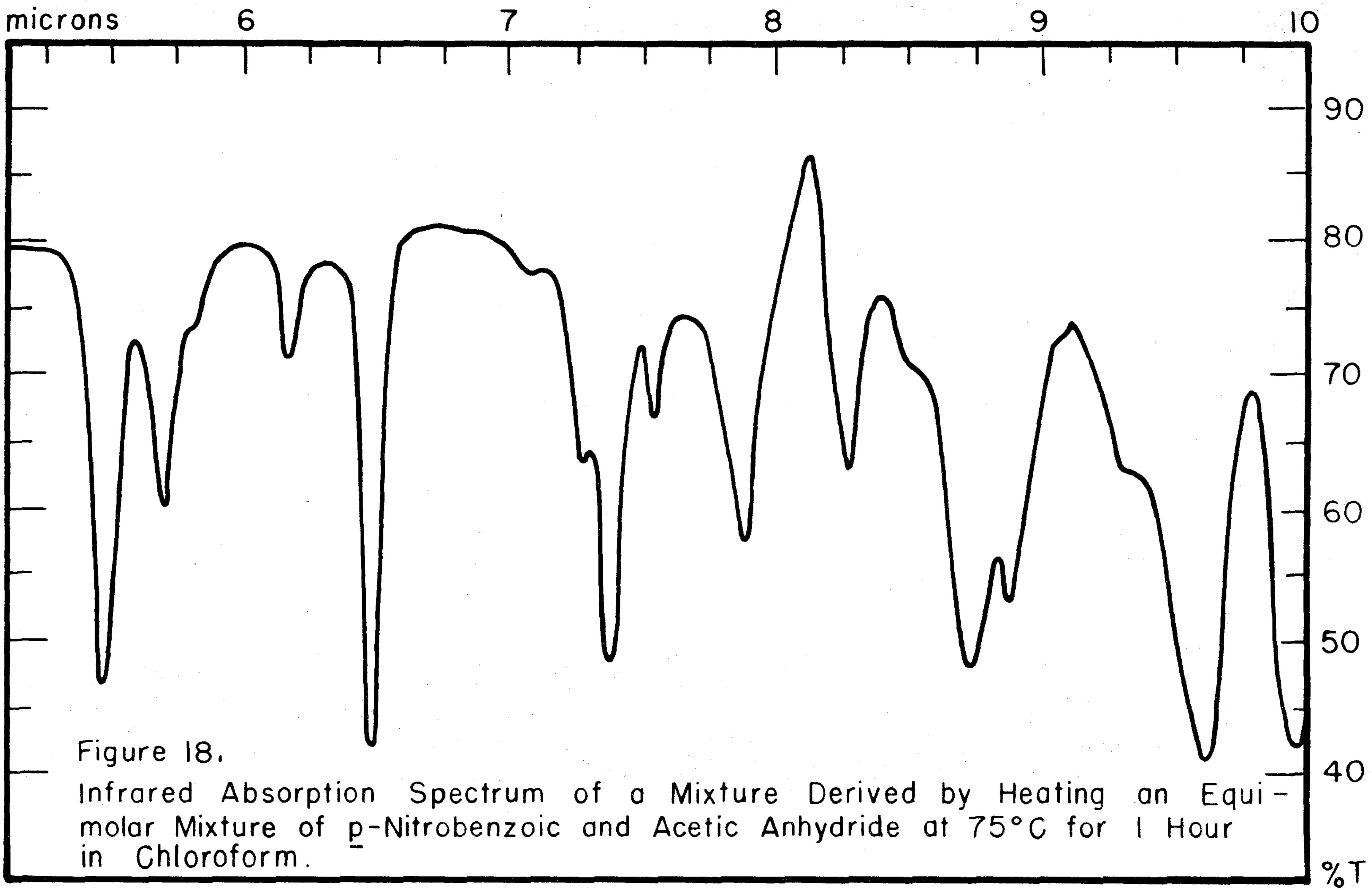


Figure 18.

Infrared Absorption Spectrum of a Mixture Derived by Heating an Equimolar Mixture of p-Nitrobenzoic and Acetic Anhydride at 75°C for 1 Hour in Chloroform.

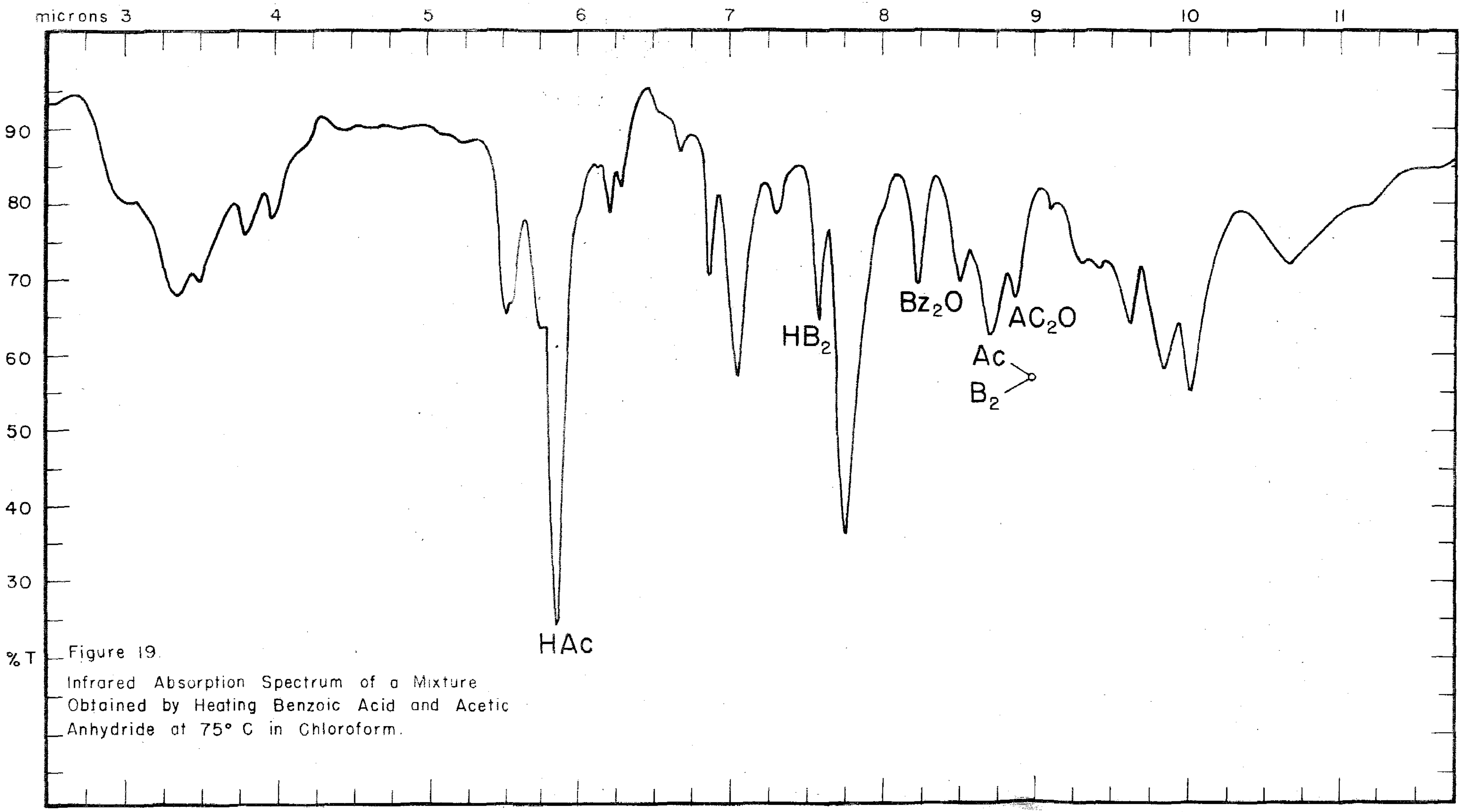
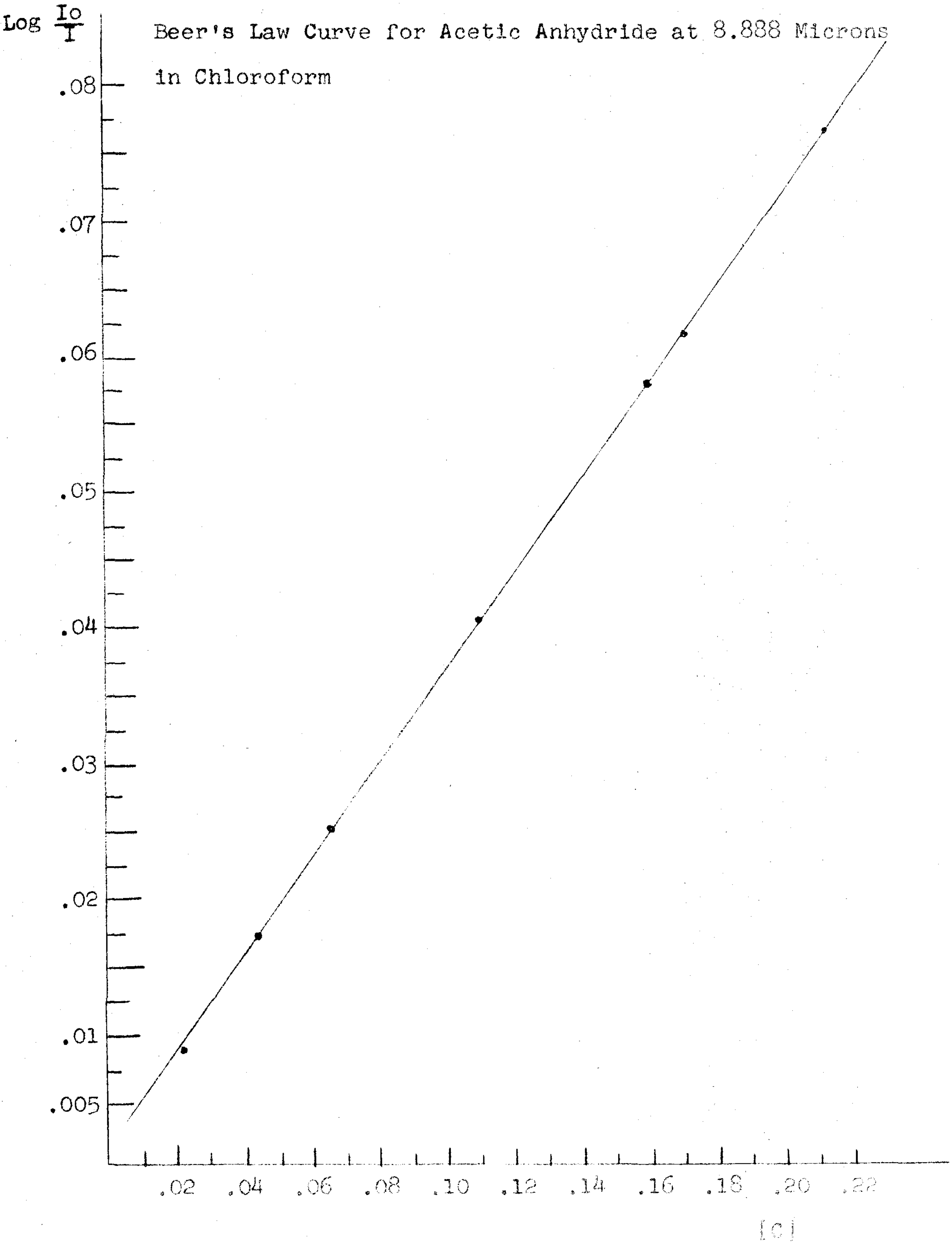


Figure 19.

Infrared Absorption Spectrum of a Mixture Obtained by Heating Benzoic Acid and Acetic Anhydride at 75° C in Chloroform.

Figure 20

Beer's Law Curve for Acetic Anhydride at 8.888 Microns in Chloroform



After the machine was adjusted so that it was reading the absorption of the desired peak, the "Gain," "Zero point," and "Balance" controls were adjusted with both reference and sample cells in place and filled with solvent (chloroform).

The "sample" light path was closed and the pen carriage was allowed to travel to the low end of the percent transmission scale. The "Gain" was then adjusted until a perceptible oscillation about an equilibrium point was just obtained. The "Gain" setting producing this effect was between five and six. The pen carriage was then moved on its drive cable until the equilibrium point of the small oscillations coincided with the zero point of the percent transmission scale. These adjustments were checked at the conclusion of each series of measurements.

After setting the "Gain" and the "Zero point" controls, the sample light path was opened and the pen allowed to travel to the high end of the percent transmission scale. The "Balance" control was then adjusted until a reading of 90-98% transmission was obtained.

If the pen carriage is displaced momentarily from its position at the high end of the percent transmission scale by slowly blocking the sample light path, and then by reopening the light path allowing the carriage to return, it is observed that the pointer does not always return to the same position at the high end of the percent transmission scale that it formerly occupied. If a number of observations of this kind are made, it is observed that five or six consecutive readings are relatively constant (varying ± 0.1 of a transmission unit) and then that a period of instability is observed when the consecutive readings vary about 0.5 to 1.0 transmission units. After this brief period of instability, a period of quiescence is again reached, which may or may not be identical with the reading of the former period.

It was planned that the initial blank reading, the sample reading, and the final blank reading would all take place during one of these quiescent periods. To try to achieve this the following procedure was used in obtaining the value of the percent transmission of an unknown sample:

The sample cell was first filled with chloroform and several readings of %T were observed. When three consecutive values were obtained which did not differ more than ± 0.1 transmission units, the average value was noted and recorded as the first blank value. The sample cell was removed and quickly filled with the sample. The cell was then placed in its holder and the light paths reopened. The pen carriage was allowed to come to rest and then slightly displaced and again allowed to come to rest. Following this, the sample reading was obtained. The sample cell then was removed and quickly flushed and filled with solvent and the second blank reading was obtained. If the second blank reading was greater than 0.4 transmission units from the first, the observations were discarded and the operations repeated.

Three sets of these observations were obtained for each quantitative measurement taken, and an average value of these observations was computed.

Another variable which must be controlled is the position of the cells in the cell holder. The cell holder is slightly larger than the flange dimensions of the absorption cells. It is necessary that the cells always occupy the same position relative to the light beam for each observation. This was brought about by making certain that the cell flanges always were flush against the side of the cell holder which is nearest the operator.

The Determination of the Various Beer's Law Curves

To determine the absorption characteristics of the various anhydride species, a given quantity of the anhydride was dissolved in chloroform. By dilution of this stock solution and by the use of the procedure which was outlined in the previous section, the optical density of the anhydride at various concentrations was obtained. In general the range of concentrations over which measurements were determined was chosen so as to cover the limits between which most of the future measurements would be made.

A typical plot of optical density vs. concentration is shown in figure 20.

It is to be noted that while a linear relationship is obtained, the curve does not pass through the origin. Therefore, a strict proportional relationship between optical density and concentration does not describe the situation which is presented by several of the anhydride species. It is therefore necessary to use the value of the intercept on the concentration axis to obtain a relationship which is useful over the concentration range of interest.

It does not seem possible at the present time to ascribe a cause for this non-proportionality. It is interesting to note that when another series of measurements were made using cells with a much wider absorption path (1.0 mm.), a linear relationship was obtained which passed through the origin. These cells permitted the use of much more dilute solutions (0.004 M). This suggests that perhaps the relationship is not strictly linear, but due to the insensitiveness of the analytical technique, this second order effect is not observed.

All the measurements in this study were made with a single set of absorption cells. The absorption path of the sample cell was 108.5 microns, and that of the blank was 105.3 microns. The data for all the Beer's law curves pertain to the use of these cells and do not strictly apply to any other set of cells.

The data obtained from these measurements and a summary of the various Beer's law constants can be found on pages 75 to 111 .

Measurements of the Equilibrium Constants

The absorption measurements were made as described previously. It was found that at 70°C approximately three days were necessary to insure that the various species present had reached a state of equilibrium. However, it was adopted as standard practice to allow four days for the equilibrium state to be established.

Measurements at high temperatures were made by sealing the samples under nitrogen in glass ampules made of 10 mm. pyrex tubing. The ampules were then placed in a small steel bomb (a war surplus bail out oxygen cylinder). The bomb was then partially filled with chloroform and inserted in a cotton seed oil bath, heated by a 200 watt immersion heater and controlled by a mercury thermoregulator to ± 0.5°C. After the period of heating, the sample tube was quickly cooled to -10°C and the values of the infrared absorption at the various wave lengths determined as soon as possible. The data from these measurements are presented on pages 112 to 128.

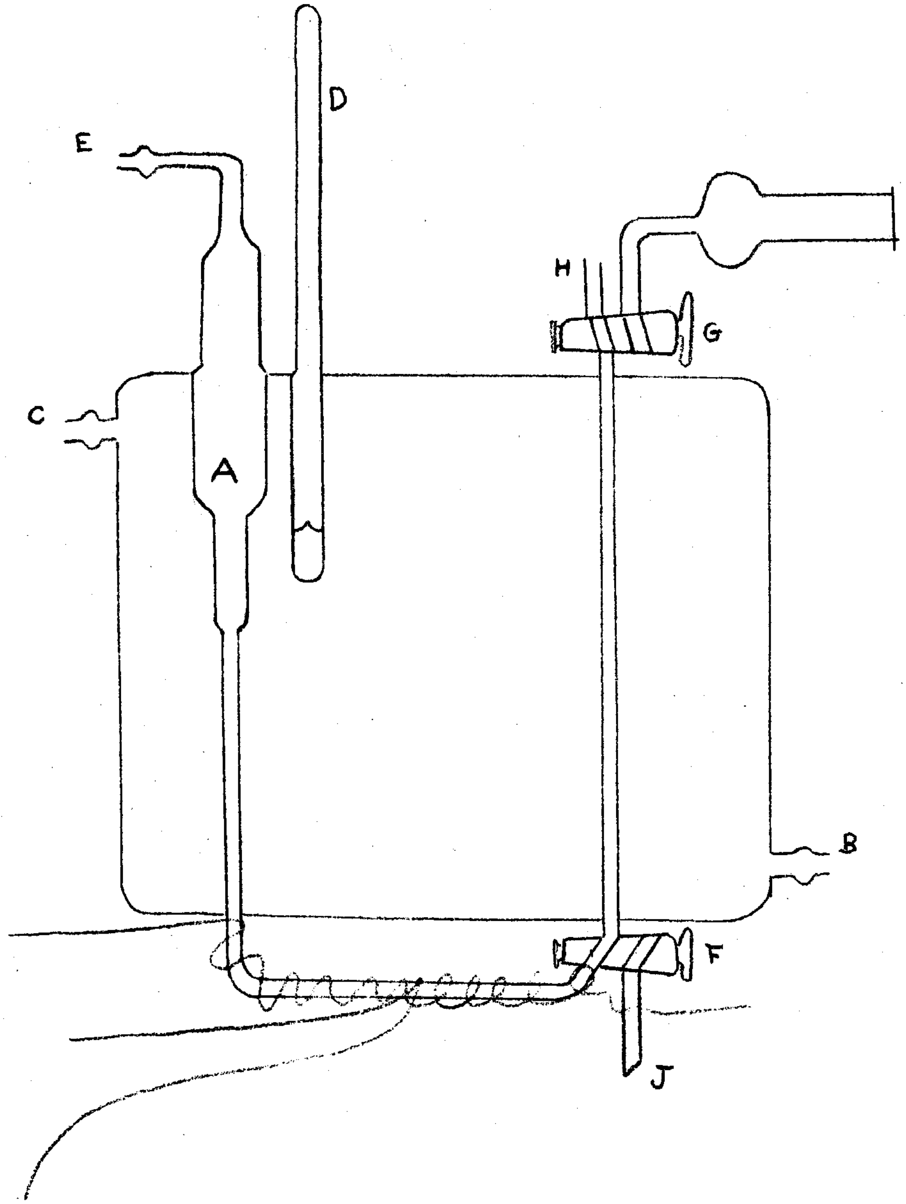
Determination of the Rate of Disproportionation

The rate of disproportionation was determined by heating a sample of the mixed anhydride at 100°C and withdrawing samples of the hot material at various times for measurement of the mixed anhydride content. Since the mixed anhydrides react rather rapidly with moisture, it is necessary to carry out the heating and sampling under anhydrous conditions. To bring this about the special apparatus shown in Figure 21 was constructed.

The chamber where the samples of mixed anhydride were heated while undergoing disproportionation is shown at A. The temperature is controlled by the flow of steam through B-C, and recorded by thermometer D. A sample is withdrawn for measurement by applying a slight positive pressure of nitrogen at E. Stop cocks F and G are adjusted such that the anhydride can flow out of exit port H. The mixed anhydride is allowed just beyond the stop cock G into H and then F is turned 90°. G is turned 180°. When the desired time has arrived to take a sample, stop cock F is turned another 90° and the mixed anhydride allowed to flow directly into a volumetric flask containing chloroform. The exit tip J is placed under the surface of the chloroform, so that the transfer of the mixed anhydride is made without it ever coming in contact with the air. After the transfer is made, the sample is quickly adjusted to volume and then quenched to -10°C.

The anhydride sample, when passing from the reaction zone to the measuring tube between stop cocks F and G, must pass outside of the steam heated zone. To avoid errors introduced by this passage, the external section is wrapped with a nichrome heating element and a thermocouple attached to the glass wall at the center of the external passage. The whole array is wrapped with asbestos tape. The temperature in the external segment is controlled by varying the passage of current through the heating element in response to the

FIGURE 21



thermocouple reading.

The volume of the tube between stop cocks F and G was found to be 0.1154 cm^3.

The samples were kept in the -10°C bath until immediately before they were placed in the sample cell of the infrared spectrophotometer.

The data obtained by this procedure are shown on pages 129 to 138.

Determination of the Nature of the Reaction of the Mixed Anhydride with Nucleophilic Agents

In order to determine the composition of the product mixture obtained from the reaction of a mixed anhydride with p-toluidine, recourse was again made to the infrared spectrophotometric analytical technique outlined in a previous section. With p-methoxybenzoic and p-nitrobenzoic-acetic mixed anhydrides, the absorption bands associated with the characteristic vibrations of the methoxyl and nitro group served as a convenient means for measuring the amount of N-(p-methoxybenzoyl)-p-toluidine and of N-(p-nitrobenzoyl)-p-toluidine. N-Benzoyl-p-toluidine was determined by the use of its carbonyl stretching absorption. The frequency of this absorption corresponded to the same absorption of the acetyl compound, but its intensity was greater. The acetyl compound was determined by the use of a relatively minor peak in its infrared spectrum. The assignment of this peak to a specific interaction does not seem possible at this time.

The generalized procedure for determining the acetylation ratio is as follows:

A 0.05 formal solution of the mixed anhydride in the organic solvent was prepared, and an equal volume of an 0.11 molar solution of p-toluidine in the same or different solvent was added. The homogeneous mixture was allowed to stand for 24 hours and then was evaporated to dryness. The solids were then treated with 10% excess of the amount of 1 N sodium carbonate necessary to convert all the free acid present to its sodium salt. The mixture evaporated to dryness at room temperature. The mixture of the sodium salts, p-toluidine, and the toluidides was then extracted with two 50 ml. portions of hot benzene. The benzene solutions were evaporated to dryness and the dry solids treated with a sufficient quantity of 6 N hydrochloric acid to neutralize all of the p-toluidine present. The heterogeneous mixture was again evaporated to dryness at room temperature. The mixture of p-toluidine hydrochloride and the toluidides was again extracted with two portion s of warm benzene. The benzene was again removed by evaporation. Finally the remaining solids were taken up in chloroform, and from this solution the value of the acetylation ratio was obtained.

This procedure removes any chance for the alteration of the acetylation ratio, which might occur through several partitions between an aqueous and organic solvent. The results of this procedure are shown on pages 149 to 154 .

The Determination of the Rate of Hydrolysis of the Mixed Anhydrides

The procedure for this determination has been well worked out.(2,18) It consisted, as modified for the present situation, of dissolving the mixed anhydride in a tetrahydrofuran-water mixture and pipetting samples of this solution at given times into an arresting solution. The arresting solution was composed of aniline and tetrahydrofuran. Its composition was adjusted so that a homogeneous phase was maintained upon the addition of the anhydride solution. The concentration of aniline in the arresting solution was at least twice that of the anhydride. The aniline reacted very quickly with the anhydride present in the aqueous solvent. Titration of the arresting solution with standard hydrochloric acid easily gave the concentration of the anhydride in the aliquot portion.

The values of the apparent first order rate constants listed on page 155 have been corrected for the amount of tetrohydrofuran used to obtain homogeneity in the initial solution.

The Estimation of the Probable Error in the Determination of an Equilibrium Constant

The probable error in the slope and intercept of a line drawn through a series of points, which deviate from the line in a random fashion, can be estimated by the following series of formulae.(19)

For $y = a + bx$
Probable errors in a and b are (19)

$$P_a = r_e\sqrt{\frac{\Sigma X_i^2}{D}} \quad ; \quad P_b = r_e\sqrt{\frac{n}{D}}$$

where

$$r_e = 0.6745\sqrt{\frac{\Sigma d_i^2}{(n-2)}} \qquad D = n\Sigma X_i^2 - (\Sigma X_i)^2$$

Using the above formulation the probable errors in the slopes and intercepts of the Beer's law functions were computed for the benzoic-acetic anhydride system and are compiled in the table:

Table 9

Probable Error in the Beer's Law Functions for the Benzoic-Acetic Anhydride System

	Wavelength					
	8.224 microns		8.888 microns		8.690 microns	
Substance	Slope	Intercept	Slope	Intercept	Slope	Intercept
Acetic Anhydride	0.360 ±0.002	0.007 ±0.004	3.49 ±0.05	0.024 ±0.002	0.662 ±0.017	0.009 ±0.005
Benzoic Anhydride	3.598 ±0.051	0.000 ±0.006	0.151 ±0.001	0.000 ±0.002	0.0912 ±0.0004	0.000 ±0.0007
Mixed Anhydride	0.290 ±0.006	0.004 ±0.002	0.663 ±0.004	0.004 ±0.003	2.292 ±0.05	0.005 ±0.007

For the final computation of the concentration of an anhydride species the following third order determinant must be solved:

$$[\text{anhyd}_{\text{I}}]a_1 + [\text{anhyd}_{\text{II}}]b_1 + [\text{anhyd}_{\text{III}}]c_1 = N_1$$

$$[\text{anhyd}_{\text{I}}]a_2 + [\text{anhyd}_{\text{II}}]b_2 + [\text{anhyd}_{\text{III}}]c_2 = N_2$$

$$[\text{anhyd}_{\text{I}}]a_3 + [\text{anhyd}_{\text{II}}]b_3 + [\text{anhyd}_{\text{III}}]c_3 = N_3$$

Where N_1, N_2, and N_3 are the corrected optical densities

The probable error in a function (f) derived from a series of quantities (S_i) whose error is Q_i is given by$^{(20)}$

$$\text{P.E. in } f = \sqrt{\sum \left(\frac{\partial f}{\partial s_i}\right)^2 Q_i^2}$$

Therefore, for the anhydride species Anhyd I the probable error would be given by:

$$\text{P.E. in anhyd}_{\text{I}} = \sqrt{\left(\frac{\text{P.E. in}\begin{vmatrix} N_1 & b_1 & c_1 \\ N_2 & b_2 & c_2 \\ N_3 & b_3 & c_3 \end{vmatrix}}{\begin{vmatrix} a_1 & b_1 & c_1 \\ a_2 & b_2 & c_2 \\ a_3 & b_3 & c_3 \end{vmatrix}}\right)^2 + \left(\frac{\begin{vmatrix} N_1 & b_1 & c_1 \\ N_2 & b_2 & c_2 \\ N_3 & b_3 & c_3 \end{vmatrix} \cdot \text{P.E. in}\begin{vmatrix} a_1 & b_1 & c_1 \\ a_2 & b_2 & c_2 \\ a_3 & b_3 & c_3 \end{vmatrix}}{\begin{vmatrix} a_1 & b_1 & c_1 \\ a_2 & b_2 & c_2 \\ a_3 & b_3 & c_3 \end{vmatrix}}\right)^2}$$

$$\text{Where P.E. in}\begin{vmatrix} N_1 & b_1 & c_1 \\ N_2 & b_2 & c_2 \\ N_3 & b_3 & c_3 \end{vmatrix} = \sqrt{\begin{array}{l} \sum [(N_1)(b_2)(dc_3)]^2 + [(N_1)(c_3)(db_3)]^2 + [(c_3)(b_2)(dN_1)]^2 \text{ ---} \\ \text{----} + [(N_1)(b_3)(dc_2)]^2 + [(N_1)(c_2)(db_3)]^2 + [(c_2)(b_3)(dN_1)]^2 \end{array}}$$

When the above calculations were carried out for the data on the top half of page 120, the following results were obtained:

(Acetic Anhydride)	= 0.0826 ± 0.0015
(Benzoic Anhydride)	= 0.0340 ± 0.0015
(Benzoic-Acetic Mixed Anhydride)	= 0.0466 ± 0.0041
Equilibrium Constant	= 4.311 ± 0.144

REFERENCES

(1) A. Behal - Ann. Chem. Phys. 19 274 (1900)

(2) M. Kilpatrick - J.A.C.S. 52 1418 (1930)

(3) A. Michael - J. Pract. Chem. 60 364 (1899)
D. S. Breslow, C. R. Hauser - J.A.C.S. 61 786 (1939)

(4) R. C. Fuson - "Advanced Organic Chemistry" - page 197 (1950) John Wiley & Sons, N.Y.

(5) T. Wieland, R. Sehring - Ann. der Chemie 569 117 (1950)

(6) J. R. Vaughn, R. Osato - J.A.C.S. 74 678 (1952)

(7) L. Brown, I. Trotter - J. Chem. Soc. 87 (1951)

(8) G. Calingaert - J.A.C.S. 61 2748 (1939)

(9) A. Emery, V. Gold - J. Chem. Soc. 1447 (1950)

(10) J. M. Tedder, J. C. Lattlow, F. Bourne - J. Chem. Soc. 2006 (1954)

(11) L. P. Hammett - Physical Organic Chemistry page 77 (1940) McGraw Hill Book Co., N. Y.

(12) G. W. Wheland - The Theory of Resonance page 73 (1944) John Wiley & Sons, N.Y.

(13) J. L. Franklin - J.A.C.S. 72 4279 (1950)

(14) E. A. Evans, J. Huston, T. Norris - J.A.C.S. 74 4985 (1954)

(15) A. M. Buswell, W. H. Rodebush, M. F. Roy - J.A.C.S. 76 2239 (1954)

(16) E. Berliner, L. H. Altashul - J.A.C.S. 74 4110 (1952)

(17) C. S. Hurd, B. K. Dull - J.A.C.S. 54 3427 (1932)

(18) K. Verkade - Red. Trav. Chim. 35 399 (1915)

(19) Margenau - Murphy "The Mathematics of Physics and Chemistry" page 502 (1943) Macmillan and Co.

(20) R. Livingston "Physico Chemical Experiments" page 22 (1948) Macmillan Co., N.Y.

Summary of Data for the Benzoic-acetic Anhydride And Benzoic-acetic Acid Systems

Principal Wave length

Benzoic-acetic mixed anhydride ---- 8.690 microns

Acetic anhydride ------------------ 8.888 microns

Benzoic anhydride ----------------- 8.224 microns

Acetic acid ----------------------- 5.823 microns

Benzoic acid ---------------------- 7.570 microns

Beer's Law Curves

A. Benzoic-acetic mixed anhydride

1. At mixed anhydride wave length (8.690 microns)

$$\text{Log}\ \frac{Io}{I} = 2.292(M\text{-}B_z) + 0.0050$$

2. At acetic anhydride wave length (8.888 microns)

$$\text{Log}\ \frac{Io}{I} = 0.6625(M\text{-}Bz) + 0.0100$$

3. At benzoic anhydride wave length (8.224 microns)

$$\text{Log}\ \frac{Io}{I} = 0.2901(M\text{-}Bz) + 0.0040$$

4. At acetic acid wave length (5.823 microns)

$$\text{Log}\ \frac{Io}{I} = 0.2818(M\text{-}Bz) + 0.0000$$

5. At benzoic acid wave length (7.570 microns)

$$\text{Log}\ \frac{Io}{I} = 0.2550(M\text{-}Bz) + 0.0000$$

B. Benzoic anhydride

1. At mixed anhydride wave length (8.690 microns)

$$\text{Log}\ \frac{Io}{I} = 0.0912(Bz_2O) + 0.000$$

2. At acetic anhydride wave length (8.888 microns)

$$\text{Log}\ \frac{Io}{I} = 0.1507(Bz_2O) + 0.000$$

3. At benzoic anhydride wave length (8.224 microns)

   $$\text{Log}\ \frac{Io}{I} = 3.598(Bz_2O) + 0.000$$

4. At acetic acid wave length (5.823 microns)

   $$\text{Log}\ \frac{Io}{I} = 0.3090(Bz_2O) + 0.000$$

5. At benzoic acid wave length (7.570 microns)

   $$\text{Log}\ \frac{Io}{I} = 0.2260(Bz_2O) + 0.000$$

C. Acetic Anhydride

1. At mixed anhydride wave length (8.690 microns)

   $$\text{Log}\ \frac{Io}{I} = 0.6623(Ac_2O) + 0.0089$$

2. At benzoic anhydride wave length (8.224 microns)

   $$\text{Log}\ \frac{Io}{I} = 0.3602(Ac_2O) + 0.0075$$

3. At acetic anhydride wave length (8.888 microns)

   $$\text{Log}\ \frac{Io}{I} = 3.491(Ac_2O) + 0.024$$

4. At acetic acid wave length (5.823 microns)

   $$\text{Log}\ \frac{Io}{I} = 0.0575(Ac_2O) + 0.000$$

5. At benzoic acid wave length (7.570 microns)

   $$\text{Log}\ \frac{Io}{I} = 0.0468(Ac_2O) + 0.000$$

D. Acetic Acid

1. At mixed anhydride wave length (8.690 microns)

$$\text{Log } \frac{I_o}{I} = 0.0191(\text{HAc}) + 0.0050$$

2. At acetic anhydride wave length (8.888 microns)

$$\text{Log } \frac{I_o}{I} = 0.0339(\text{HAc}) + 0.0030$$

3. At benzoic anhydride wave length (8.224 microns)

$$\text{Log } \frac{I_o}{I} = 0.0384(\text{HAc}) + 0.000$$

4. At acetic acid wave length (5.823 microns)

$$\text{Log } \frac{I_o}{I} = 2.140(\text{HAc}) + 0.000$$

5. At benzoic acid wave length (7.570 microns)

$$\text{Log } \frac{I_o}{I} = 0.0778(\text{HAc}) + 0.0065$$

E. Benzoic Acid

1. At mixed anhydride wave length (8.690 microns)

$$\text{Log } \frac{I_o}{I} = 0.0530(\text{HBz}) + 0.000$$

2. At acetic anhydride wave length (8.888 microns)

$$\text{Log } \frac{I_o}{I} = 0.2458(\text{HBz}) + 0.000$$

3. At benzoic anhydride wave length (8.224 microns)

$$\text{Log } \frac{I_o}{I} = 0.0404(\text{HBz}) + 0.000$$

4. At acetic acid wave length (5.823 microns)

$$\text{Log } \frac{I_o}{I} = 1.710(\text{HBz}) + 0.0200$$

5. At benzoic acid wave length (7.570 microns)

$$\text{Log } \frac{I_o}{I} = 0.7790(\text{HBz}) + 0.000$$

Simultaneous Equations Derived from the Beer's Law Data

A. No Acid Present

1. At 8.224 microns

$$3.598(Bz_2O) + 0.3602(Ac_2O) + 0.2901(M\text{-}Bz) = \text{Log}\,\frac{Io}{I} - 0.0115$$

2. At 8.888 microns

$$0.1507(Bz_2O) + 3.491(Ac_2O) + 0.6625(M\text{-}Bz) = \text{Log}\,\frac{Io}{I} - 0.034$$

3. At 8.690 microns

$$0.0912(Bz_2O) + 0.6025(Ac_2O) + 2.292(M\text{-}Bz) = \text{Log}\,\frac{Io}{I} - 0.014$$

B. Acids Present

1. At 8.690 microns

$$2.292(M\text{-}Bz) + 0.0912(Bz_2O) + 0.6025(Ac_2O) + 0.0191(HAc) + 0.0530(HBz) = \text{Log}\,\frac{Io}{I} - 0.019$$

2. At 8.888 microns

$$0.6625(M\text{-}Bz) + 0.1507(Bz_2O) + 3.491(Ac_2O) + 0.0339(HAc) + 0.2458(HBz) = \text{Log}\,\frac{Io}{I} - 0.0370$$

3. At 8.224 microns

$$0.2901(M\text{-}Bz) + 3.598(Bz_2O) + 0.3602(Ac_2O) + 0.0384(HAc) + 0.0404(HBz) = \text{Log}\,\frac{Io}{I} - 0.0115$$

4. At 5.823 microns

$$0.2818(M\text{-}Bz) + 0.3090(Bz_2O) + 0.3602(Ac_2O) + 2.140(HAc) + 1.710(HBz) = \text{Log}\,\frac{Io}{I} - 0.0200$$

5. At 7.570 microns

$$0.2550(M\text{-}Bz) + 0.2260(Bz_2O) + 0.0468(Ac_2O) + 0.0778(HAc) + 0.7790(HBz) = \text{Log}\,\frac{Io}{I} - 0.0065$$

Beer's Law Curve For Acetic Anhydride At 8.888 Microns

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.2122	92.6	16.1	92.8	17.367	5.7934	0.762932
	92.6	15.9	92.4	17.189		
	92.4	15.9	92.2	17.226		
0.1700	97.0	23.6	97.0	24.329	4.1109	0.613939
	97.0	23.6	97.1	24.317		
	96.9	23.6	97.1	24.329		
0.1591	90.6	23.9	90.8	26.350	3.8021	0.580021
	90.9	24.0	91.3	26.344		
	91.7	24.1	91.5	26.310		
0.1061	93.6	37.4	93.7	39.935	2.5095	0.399585
	93.5	37.5	94.0	40.000		
	93.9	37.4	94.0	39.808		
0.0636	91.4	51.1	91.2	55.969	1.7844	0.251456
	91.0	51.1	91.1	56.123		
	91.2	51.1	91.2	56.030		
0.0424	91.5	61.7	91.4	67.468	1.4808	0.170202
	91.2	61.6	91.3	67.506		
	91.2	61.7	91.3	67.616		
0.0212	91.6	74.5	91.6	81.331	1.2298	0.089838
	91.1	74.1	91.0	81.383		
	91.2	74.0	91.0	81.229		

$$\text{Log}\ \frac{Io}{I} = 3.491(Ac_2O) + 0.024$$

Beer's Law Curve For Acetic Anhydride At 8.224 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.6166	92.7	55.0	93.0	59.235	1.6850	0.22660
	93.3	55.2	93.0	59.259		
	93.0	55.1	93.0	59.247		
0.4144	93.0	64.9	93.0	69.784	1.4304	0.155460
	93.1	65.0	93.4	70.270		
	93.2	64.9	93.1	69.672		
0.3083	92.7	70.8	92.5	76.457	1.3099	0.117237
	92.6	70.8	92.7	76.416		
	92.2	70.2	92.2	76.138		
0.2122	93.0	77.0	93.2	82.706	1.2066	0.081566
	93.1	77.4	93.4	83.002		
	93.3	77.3	93.4	82.806		
0.1061	90.7	82.3	91.0	90.588	1.1044	0.043126
	91.0	82.3	91.1	90.389		
	91.0	83.3	91.9	90.642		

$$\text{Log } \frac{Io}{I} = 0.3602(Ac_2O) + 0.0075$$

Beer's Law Curve For Acetic Anhydride At 8.690 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.4244	91.8	48.7	92.0	52.992	1.8882	0.276046
	91.7	48.6	91.8	52.970		
	91.7	48.5	91.6	52.918		
0.3183	91.3	55.0	90.7	60.439	1.6615	0.220400
	90.8	54.5	91.2	59.890		
	91.3	54.9	91.0	60.230		
0.2122	92.0	65.7	92.3	71.296	1.4069	0.148260
	92.0	65.0	91.4	70.883		
	91.3	64.8	91.1	71.052		
0.1061	93.9	78.3	93.5	83.564	1.1937	0.076892
	93.0	78.0	93.0	83.870		
	92.4	77.5	92.4	83.874		
0.06375	90.4	80.7	90.7	89.122	1.1222	0.050068
	90.2	80.3	90.1	89.073		
	91.0	81.1	91.0	89.120		

$$\text{Log } \frac{Io}{I} = 0.6623(Ac_2O) + 0.0089$$

Beer's Law Curve For Acetic Anhydride At 5.823 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
1.6958	90.2	71.6	90.5	79.247	1.2609	0.100685
	90.3	71.5	89.9	79.356		
	90.0	71.3	89.8	79.310		
1.2718	90.5	76.5	90.3	84.623	1.1183	0.073065
	90.3	76.2	90.3	84.385		
	90.4	76.4	90.5	84.466		
1.0599	91.3	79.3	91.2	86.904	1.1510	0.061080
	91.4	79.3	91.2	86.856		
	91.8	79.8	91.9	86.880		
0.8479	90.2	80.5	90.5	89.196	1.1216	0.049638
	90.0	80.2	90.2	89.101		
	90.1	80.3	90.0	89.172		

$$\text{Log}\ \frac{Io}{I} = 0.0575(Ac_2O) + 0.000$$

Beer's Law Curve For Acetic Anhydride At 7.570 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
1.6958	93.5	78.0	93.5	83.422	1.1982	0.078532
	93.6	78.1	93.5	83.484		
	93.3	78.0	93.4	83.556		
1.2718	94.8	82.7	94.6	87.382	1.1447	0.058796
	94.7	82.8	94.5	87.480		
	93.7	81.9	94.0	87.266		
1.0599	95.9	85.5	95.7	89.248	1.1208	0.049508
	94.3	94.3	94.6	89.253		
	95.0	84.7	94.8	89.251		
0.8479	95.0	86.6	95.0	91.157	1.0949	0.039371
	95.0	86.8	95.3	91.224		
	95.2	86.9	95.2	91.281		

$$\text{Log}\ \frac{Io}{I} = 0.0468(Ac_2O) + 0.0000$$

Beer's Law Curve For Benzoic Anhydride At 8.224 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.1210	97.5	35.6	97.5	36.512	2.7385	0.43751
	97.4	35.6	97.6	36.512		
	96.9	35.4	96.9	36.532		
0.1061	93.8	38.8	93.8	41.364	2.4124	0.382448
	94.1	39.0	94.1	41.445		
	93.5	39.0	93.7	41.655		
0.0975	97.5	43.3	97.5	44.410	2.2513	0.352437
	97.4	43.3	97.6	44.410		
	98.0	43.5	97.8	44.433		
0.08488	93.2	46.5	93.1	49.919	2.0097	0.303034
	93.0	46.2	93.1	49.650		
	92.9	46.2	93.0	49.704		
0.06366	92.4	54.8	92.8	59.179	1.6921	0.228530
	92.0	54.5	92.5	59.078		
	92.6	54.6	92.4	59.027		
0.04240	92.8	65.0	92.7	70.080	1.4307	0.155510
	92.7	64.9	93.2	69.822		
	93.0	64.9	93.0	69.784		
0.00408	92.0	89.1	92.5	96.585	1.0339	0.014478
	92.3	89.5	92.4	96.913		
	92.0	89.0	92.2	96.663		

$$\text{Log } \frac{Io}{I} = 3.598(Bz_2O) + 0.000$$

Beer's Law Curve For Benzoic Anhydride At 8.888 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.3183	95.5	85.4	95.7	89.330	1.1207	0.049493
	95.7	85.3	95.6	89.179		
	94.5	84.4	94.8	89.170		
0.19098	92.5	86.1	92.0	93.333	1.0691	0.029020
	91.8	86.0	91.7	93.732		
	92.0	86.0	91.9	93.529		
0.15612	92.5	87.5	92.5	94.594	1.0576	0.024322
	92.7	87.5	92.5	94.492		
	92.6	87.5	92.4	94.594		
0.10610	93.5	90.2	94.0	96.223	1.0392	0.016702
	94.0	90.4	94.0	96.170		
	94.0	90.5	94.0	96.276		
0.0795	92.2	89.5	92.3	97.018	1.0297	0.011864
	92.1	89.5	92.2	97.124		
	92.2	89.6	92.2	97.180		

$$\text{Log}\ \frac{Io}{I} = 0.1507(Bz_2O) + 0.0000$$

Beer's Law Curve For Benzoic Anhydride At 8.690 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.1061	92.6	90.7	92.8	97.842	1.0222	0.009536
	92.5	90.5	92.7	97.732		
	92.5	90.6	92.6	97.893		
0.09549	92.0	90.4	92.2	98.154	1.0197	0.008471
	92.1	90.4	92.0	98.100		
	92.2	90.3	92.2	97.939		
0.08488	92.5	91.0	92.8	98.219	1.0173	0.007449
	92.6	91.0	92.5	98.325		
	92.5	91.1	92.8	98.327		

$$\text{Log}\ \frac{Io}{I} = 0.0912(Bz) + 0.000$$

Beer's Law Curve For Benzoic Anhydride At 5.823 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.7250	96.0	57.4	96.4	59.667	1.6752	0.224062
	95.8	57.3	96.0	59.749		
	95.9	57.3	96.2	59.656		
0.4350	95.5	70.0	96.0	73.107	1.3673	0.135866
	95.5	69.5	95.5	72.774		
	94.5	69.4	94.3	73.516		
0.3625	94.6	73.0	94.8	77.085	1.2982	0.113340
	94.9	73.1	95.1	76.906		
	94.7	73.0	94.7	77.085		
0.2900	96.5	77.9	96.5	80.725	1.2309	0.090225
	95.5	78.0	95.7	81.589		
	95.4	77.9	96.0	81.400		
0.18125	95.0	84.0	96.0	87.958	1.1362	0.055446
	95.5	84.0	95.4	88.004		
	95.5	84.1	95.5	88.062		

$$\text{Log}\ \frac{Io}{I} = 0.3090(\text{Bz}_2\text{O}) + 0.000$$

Beer's Law Curve For Benzoic Anhydride At 7.570 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log Ave. $\frac{Io}{I}$
0.7250	95.2	64.7	95.2	67.962	1.4707	0.167523
	95.1	64.6	95.1	67.928		
	95.0	64.7	95.0	68.105		
0.4350	94.4	75.1	94.2	79.639	1.2557	0.098885
	94.1	75.1	94.3	79.723		
	94.4	75.0	94.2	79.533		
0.3625	93.7	77.4	93.7	82.604	1.2117	0.083392
	93.9	77.5	94.0	82.490		
	94.0	77.4	93.7	82.472		
0.2900	93.8	80.5	94.0	85.729	1.1683	0.067551
	93.9	80.6	94.0	85.790		
	94.2	80.4	94.4	85.259		
0.18125	93.5	85.2	93.7	91.025	1.0984	0.040760
	93.4	85.2	93.7	91.074		
	93.7	85.2	93.5	91.025		
0.1450	93.7	86.9	93.9	92.643	1.0674	0.032380
	93.6	87.0	94.0	92.750		
	94.1	86.9	93.5	92.643		

$$\mathrm{Log}\ \frac{Io}{I} = 0.2261(Bz_2O) + 0.000$$

Beer's Law Curve For Benzoic-acetic Mixed Anhydride at 8.690 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.29095	98.6	21.3	98.7	21.591	4.6264	0.665246
	98.0	21.2	98.0	21.632		
	98.0	21.2	98.1	21.621		
0.2645	91.3	21.8	91.4	23.864	4.1867	0.621870
	91.3	21.8	91.3	23.877		
	91.2	21.8	91.1	23.916		
0.23276	98.4	25.6	98.3	26.029	3.8454	0.584944
	98.2	25.5	98.1	25.980		
	98.0	25.5	98.1	26.007		
0.1980	91.5	31.4	90.6	34.696	2.8841	0.460015
	90.8	31.5	90.5	34.749		
	90.1	31.2	90.4	34.570		
0.13225	92.3	45.3	92.4	49.052	2.0391	0.309441
	92.0	45.1	92.2	48.968		
	91.8	45.0	91.9	48.992		
0.0990	91.2	53.5	91.6	58.469	1.7093	0.232818
	91.1	53.4	91.4	58.520		
	91.2	53.4	91.3	58.520		
0.0661	91.1	62.0	91.4	67.945	1.4723	0.168003
	91.3	62.0	91.2	67.945		
	91.4	62.0	91.3	67.870		
0.02695	90.5	77.0	91.0	84.848	1.1817	0.072549
	91.5	76.8	91.0	84.164		
	90.7	77.0	90.8	84.848		

$$\text{Log}\ \frac{Io}{I} = 2.292(M) + 0.005$$

Beer's Law Curve For Benzoic-acetic Mixed Anhydride At 8.224 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.5745	91.5	60.9	91.5	66.557	1.5013	0.176467
	91.4	60.9	91.5	66.592		
	91.5	61.0	91.5	66.666		
0.4220	91.2	67.7	91.5	74.110	1.3505	0.130495
	91.5	67.7	91.5	73.989		
	91.5	67.7	91.4	74.029		
0.3591	91.5	69.5	91.5	75.956	1.3164	0.119392
	94.1	71.4	93.9	75.957		
	91.4	69.4	91.5	75.888		
0.2680	92.2	75.6	92.0	82.084	1.2163	0.085038
	91.5	75.5	92.0	82.288		
	92.0	75.5	92.0	82.065		
0.1325	93.5	84.5	93.1	90.568	1.1063	0.043877
	92.6	83.8	92.7	90.447		
	92.7	83.8	92.6	90.447		

$$\text{Log } \frac{Io}{I} = 0.2901(M) + 0.0040$$

Beer's Law Curve For Benzoic-acetic Mixed Anhydride At 8.888 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.5745	93.5	43.5	93.5	46.524	2.1476	0.331950
	93.5	43.6	93.6	46.606		
	93.6	43.6	93.7	46.556		
0.4220	94.2	54.0	94.3	57.355	1.7444	0.24165
	94.0	54.0	94.2	57.285		
	94.5	54.1	94.2	57.339		
0.2680	93.6	64.2	93.9	68.480	1.4553	0.16295
	93.0	64.3	93.2	69.065		
	93.0	63.7	93.0	68.494		
0.1325	93.3	77.1	93.6	82.504	1.2136	0.084076
	93.9	77.8	94.0	82.810		
	93.5	77.2	93.7	82.478		
0.06625	93.5	84.6	93.8	90.336	1.1102	0.045398
	93.8	84.5	94.1	89.941		
	93.9	84.5	94.0	89.941		

$$\text{Log } \frac{Io}{I} = 0.6625(M) + 0.0100$$

Beer's Law Curve For Benzoic-acetic Mixed Anhydride At 5.823 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.7835	98.5	59.3	98.5	60.203	1.6637	0.221072
	98.6	59.2	98.6	60.040		
	98.5	59.3	98.6	60.172		
0.66125	97.0	63.6	97.0	65.567	1.5246	0.183154
	96.9	63.7	97.2	65.636		
	97.2	63.7	97.1	65.568		
0.5290	95.3	68.5	95.6	71.765	1.3946	0.144446
	95.4	68.5	95.5	71.765		
	95.5	68.4	95.6	71.585		
0.39675	98.0	74.5	98.3	75.904	1.3084	0.116742
	97.9	74.5	98.0	76.059		
	97.8	74.6	97.7	76.317		
0.2645	95.5	81.5	95.4	85.385	1.1724	0.069078
	95.4	81.5	95.2	85.519		
	95.2	80.9	95.2	84.978		

$$\text{Log}\ \frac{Io}{I} = 0.2818(M) + 0.000$$

Beer's Law Curve For Benzoic-acetic Mixed Anhydride At 7.570 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.66125	97.1	65.0	97.2	66.906	1.4944	0.174466
	97.0	65.0	97.3	66.906		
	97.3	65.1	97.2	66.940		
0.5290	96.5	70.0	96.7	72.463	1.3819	0.140378
	97.0	70.0	97.0	72.164		
	96.5	70.0	96.7	72.463		
0.2645	97.0	82.1	97.3	84.508	1.1846	0.073572
	97.2	82.1	97.1	84.508		
	97.4	82.0	97.3	84.232		
0.19838	97.1	86.6	97.2	89.140	1.1227	0.050263
	97.2	86.6	97.2	89.094		
	97.4	86.7	97.5	88.968		
0.13225	97.6	90.7	96.4	93.989	1.0701	0.029421
	96.3	90.1	96.2	93.610		
	96.0	89.9	95.8	93.743		

$$\text{Log}\ \frac{Io}{I} = 0.2550(M) + 0.000$$

Beer's Law Curve For Acetic Acid At 8.690 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
1.424	98.2	85.4	98.1	87.009	1.1491	0.060358
	98.2	85.4	97.9	87.098		
	98.0	85.5	98.3	87.111		
1.187	97.5	87.6	97.3	89.938	1.1115	0.045905
	97.3	87.5	97.2	89.974		
	97.7	88.0	97.9	89.979		
0.7120	97.0	89.7	96.8	92.569	1.0783	0.03274
	96.7	89.9	96.9	92.871		
	96.1	89.1	96.0	92.764		

$$\text{Log}\ \frac{Io}{I} = 0.0191(\text{HAc}) + 0.005$$

Beer's Law Curve For Acetic Acid At 8.888 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
1.424	98.5	78.4	98.4	79.634	1.2528	0.097880
	98.4	78.4	98.2	79.755		
	98.0	78.5	98.1	80.061		
1.187	98.6	83.1	98.3	84.408	1.1844	0.073498
	98.3	82.9	98.0	84.462		
	98.0	82.8	98.2	84.403		
0.7120	95.5	85.1	95.2	89.250	1.1213	0.049724
	95.5	85.2	95.6	89.167		
	95.5	85.2	95.6	89.167		
0.3560	95.3	89.2	95.5	93.501	1.0678	0.028488
	95.5	89.4	95.7	93.514		
	95.6	89.5	95.2	93.913		

$$\text{Log}\ \frac{Io}{I} = 0.0339(\text{HAc}) + 0.003$$

Beer's Law Curve For Acetic Acid At 8.224 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
1.424	94.6	72.9	94.2	77.224	1.2890	0.11025
	94.0	73.1	94.2	77.683		
	93.9	73.0	93.7	77.825		
0.1187	93.4	82.6	93.6	88.342	1.1326	0.054078
	93.9	82.8	93.7	88.272		
	94.0	83.1	94.3	88.263		
0.3560	93.6	88.3	93.9	94.186	1.0621	0.026162
	93.7	88.2	93.8	94.080		
	93.8	88.4	93.9	94.192		

$$\text{Log } \frac{Io}{I} = 0.0384(\text{HAc}) + 0.000$$

Beer's Law Curve For Acetic Acid At 5.823 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.3560	94.5	19.4	94.5	20.529	4.8732	0.687818
	94.0	19.3	94.0	20.531		
	94.1	19.3	94.2	20.499		
0.2848	95.4	23.7	95.2	24.868	4.0268	0.604958
	95.0	23.6	95.1	24.829		
	94.8	23.5	94.7	24.802		
0.2541	92.0	25.9	91.5	28.383	3.5360	0.54851
	91.8	25.9	91.9	28.198		
	91.7	25.9	91.6	28.259		
0.2136	93.4	31.6	93.0	33.905	2.9509	0.469955
	93.0	31.5	93.2	33.834		
	93.2	31.6	93.1	33.923		
0.1825	96.2	38.3	96.5	39.750	2.5157	0.406590
	96.5	38.4	96.8	39.730		
	96.0	38.2	96.1	39.770		
0.1762	93.0	37.5	92.6	40.409	2.4742	0.393436
	92.8	37.5	92.5	40.474		
	93.1	37.6	93.2	40.365		
0.1460	95.2	45.1	95.6	47.274	2.1159	0.325499
	95.7	45.2	95.5	47.280		
	94.8	44.9	95.0	47.312		
0.1250	93.2	48.8	93.1	52.388	1.9100	0.281030
	93.7	49.0	93.5	52.322		
	93.5	48.9	93.3	52.355		
0.0701	93.5	63.0	93.5	67.379	1.4835	0.171290
	93.3	62.7	92.9	67.346		
	92.9	62.7	92.9	67.491		
0.0350	92.5	75.9	92.5	82.054	1.2202	0.086432
	92.2	75.5	92.0	81.976		
	92.0	75.4	92.3	81.823		

$$\text{Log}\ \frac{Io}{I} = 2.140(\text{HAc}) + 0.000$$

(Concentration Limit 0.24 molar)

Beer's Law Curve For Acetic Acid At 7.570 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
1.5150	95.0	70.0	95.4	73.529	1.3642	0.134874
	95.6	70.1	95.5	73.364		
	95.8	69.9	95.7	73.002		
1.1120	93.0	74.1	93.4	79.506	1.2579	0.109646
	96.2	76.8	96.5	79.709		
	96.5	76.5	96.5	79.274		
0.727	94.4	80.7	94.5	85.442	1.1644	0.066102
	93.4	80.2	93.4	85.867		
	93.4	79.9	93.6	85.454		
0.539	95.9	85.0	95.9	88.633	1.1281	0.052348
	96.0	85.1	96.3	88.507		
	95.8	85.0	95.7	88.772		
0.359	96.5	88.7	96.8	91.774	1.0871	0.036270
	96.2	88.5	96.1	92.043		
	96.0	88.4	95.9	92.131		

$$\text{Log}\ \frac{Io}{I} = 0.0778(\text{HAc}) + 0.0065$$

Beer's Law Curve For Benzoic Acid At 8.690 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.7366	95.2	86.8	94.8	91.368	1.0956	0.039650
	95.0	86.9	95.1	91.425		
	95.3	86.7	95.0	91.023		
0.58928	96.8	90.5	97.1	93.347	1.0715	0.030190
	97.1	90.7	97.3	93.321		
	97.2	90.8	97.4	93.319		
0.3183	97.5	93.8	97.5	96.205	1.0391	0.01661
	97.8	93.9	97.7	96.061		
	97.0	93.7	97.3	96.448		

$$\text{Log}\ \frac{Io}{I} = 0.0530(\text{HBz}) + 0.000$$

Beer's Law Curve For Benzoic Acid At 8.888 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.7366	97.2	64.8	96.9	66.769	1.4966	0.175004
	97.0	64.9	97.1	66.872		
	96.9	64.7	96.8	66.804		
0.58928	96.2	69.5	96.3	76.207	1.3844	0.141268
	96.5	69.6	96.2	76.236		
	96.0	69.4	96.1	72.254		
0.43196	96.2	75.1	96.5	77.944	1.2791	1.06904
	96.5	75.5	96.1	78.400		
	96.0	75.2	96.3	78.211		
0.3183	96.0	81.3	96.0	84.687	1.1852	0.073792
	96.5	81.1	96.3	84.128		
	96.3	81.4	96.8	84.308		

$$\text{Log}\ \frac{Io}{I} = 0.2458(\text{HBz}) + 0.000$$

Beer's Law Curve For Benzoic Anhydride At 8.224 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.7366	97.1 97.5 97.5	90.6 90.7 90.6	97.4 97.2 97.3	93.161 93.168 93.018	1.0737	0.03088
0.58928	96.8 97.0 97.3	91.7 92.0 91.9	97.0 97.5 96.9	94.633 94.601 94.644	1.0567	0.023947
0.3183	95.7 96.0 96.2	92.9 93.3 93.2	95.8 96.3 95.9	97.023 97.035 97.032	1.0306	0.013032

$$\text{Log} \frac{Io}{I} = 0.0404(\text{HBz}) + 0.000$$

Beer's Law Curve For Benzoic Acid At 5.823 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.3183	96.1	42.3	96.1	44.016	2.2837	0.358643
	96.3	42.2	96.5	43.798		
	96.7	42.1	96.6	43.559		
0.1912	94.5	57.2	94.2	60.625	1.6559	0.219034
	95.4	57.3	95.2	60.125		
	94.8	57.3	94.9	60.411		
0.1592	92.8	60.6	92.7	65.336	1.5377	0.186766
	93.1	60.5	93.3	64.914		
	93.3	60.5	93.3	64.844		
0.1274	93.5	66.0	93.5	70.588	1.4169	0.15308
	93.7	66.0	93.5	70.512		
	93.5	66.0	93.4	70.626		
0.1061	92.7	68.5	92.5	73.974	1.3537	0.131524
	92.7	68.5	93.1	73.735		
	92.7	68.5	92.7	73.894		
0.0795	92.1	72.9	92.2	79.110	1.2602	0.10034
	92.2	73.0	92.3	79.132		
	91.9	73.1	92.0	79.499		
0.0637	92.0	75.4	92.0	81.956	1.2203	0.08468
	92.2	75.8	92.3	82.168		
	92.3	75.5	92.5	81.709		

$$\text{Log } \frac{Io}{I} = 1.710(\text{HBz}) + 0.020$$

Beer's Law Curve For Benzoic Acid At 7.570 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.3183	96.1	53.5	96.2	55.642	1.7885	0.25249
	95.3	53.6	95.3	56.243		
	95.4	53.5	95.5	56.050		
0.25464	95.3	60.1	95.2	63.097	1.5850	0.20003
	93.4	59.0	93.6	63.101		
	93.6	59.1	93.8	63.073		
0.1912	92.8	65.5	93.0	70.505	1.4197	0.152197
	93.0	65.5	93.2	70.354		
	92.9	65.3	92.5	70.442		
0.15915	92.4	68.4	92.0	74.186	1.3443	0.128464
	91.6	68.1	91.4	74.426		
	91.4	68.1	91.3	74.548		
0.1273	94.3	74.0	94.5	78.389	1.2755	0.105680
	94.7	74.1	94.3	78.412		
	94.3	73.8	94.0	78.385		
0.0795	94.6	81.6	94.8	86.166	1.1602	0.064534
	94.8	81.7	94.9	86.136		
	94.6	81.6	94.6	86.257		
0.0637	95.1	84.2	95.1	88.538	1.1278	0.051462
	94.5	83.9	94.5	88.783		
	95.0	84.2	94.9	88.678		

$$\text{Log}\ \frac{Io}{I} = 0.7790(\text{HBz}) + 0.000$$

Summary Of Data For The Para-Methoxybenzoic-Acetic Anhydride System

Principal Wave length

A. *p*-Methoxybenzoic-acetic mixed anhydride -- 8.711 microns

B. Acetic anhydride -------------------- 8.888 microns

C. *p*-Methoxybenzoic anhydride --------- 8.1770 microns

Beer's Law Curves

A. *p*-Methoxybenzoic-acetic mixed anhydride

1. At mixed anhydride wave length (8.711 microns)

$$\text{Log}\ \frac{\text{Io}}{\text{I}} = 4.220(\text{M-pMeOBz}) + 0.015$$

2. At acetic anhydride wave length (8.888 microns)

$$\text{Log}\ \frac{\text{Io}}{\text{I}} = 0.9541(\text{M-pMeOBz}) + 0.0042$$

3. At p-methoxybenzoic anhydride wave length (8.1770 microns)

$$\text{Log}\ \frac{\text{Io}}{\text{I}} = 0.1732(\text{M-p-MeOBz}) + 0.00225$$

B. *p*-Methoxybenzoic anhydride

1. At mixed anhydride wave length (8.711 microns)

$$\text{Log}\ \frac{\text{Io}}{\text{I}} = 0.3945\ (\text{p-MeOBz}) + 0.000$$

2. At acetic anhydride wave length (8.888 microns)

$$\text{Log}\ \frac{\text{Io}}{\text{I}} = 0.1885(\text{p-MeOBz}) + 0.000$$

3. At *p*-methoxybenzoic anhydride wave length (8.1770 microns)

$$\text{Log}\ \frac{\text{Io}}{\text{I}} = 4.350(\text{p-MeOBz}) + 0.0135$$

C. Acetic anhydride

1. At mixed anhydride wave length (8.711 microns)

   $\text{Log}\ \frac{Io}{I} = 0.857(Ac) + 0.003$

2. At acetic anhydride wave length (8.888 microns)

   $\text{Log}\ \frac{Io}{I} = 3.491(Ac) + 0.024$

3. p-Methoxybenzoic anhydride wave length (8.1770 microns)

   $\text{Log}\ \frac{Io}{I} = 0.5045(Ac) + 0.002$

Simultaneous Equations Derived From The Above Data

At 8.711 microns

$4.220(M\text{-}p\text{-}MeOBz) + 0.3945(pMeOBz) + 0.857(Ac) =$

$\text{Log}\ \frac{Io}{I} - 0.018$

At 8.888 microns

$0.954(M\text{-}p\text{-}MeOBz) + 0.1885(p\text{-}MeOBz) + 3.491(Ac) =$

$\text{Log}\ \frac{Io}{I} - 0.028$

At 8.1770 microns

$0.1732(M\text{-}p\text{-}MeOBz) + 4.35(p\text{-}MeOBz) + 0.5045(Ac) =$

$\text{Log}\ \frac{Io}{I} - 0.018$

Beer's Law Curve For *p*-Methoxybenzoic Anhydride At 8.7110 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.0941	97.4	88.9	97.7	91.132	1.0987	0.040880
	97.6	88.9	97.8	90.992		
	97.8	89.0	98.0	90.909		
0.07528	97.2	90.1	97.3	92.647	1.0795	0.03220
	97.3	90.1	97.2	92.647		
	97.4	90.0	97.0	92.592		
0.05646	97.1	91.8	97.1	94.541	1.0582	0.024572
	97.2	91.9	97.4	94.450		
	97.1	91.7	96.9	94.487		
0.04705	98.5	93.9	98.5	95.329	1.0488	0.020696
	98.5	93.9	98.5	95.329		
	98.5	93.9	98.4	95.378		

$$\text{Log } \frac{Io}{I} = 0.3945(\text{p-MeoBz}) + 0.000$$

Beer's Law Curve For *p*-Methoxybenzoic Anhydride At 8.888 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.1414	97.6	91.8	97.5	94.105	1.0608	0.025638
	97.6	91.8	97.6	94.057		
	97.5	91.7	97.4	94.099		
0.10605	97.5	93.0	97.3	95.482	1.0474	0.020014
	97.0	92.7	97.2	95.468		
	97.0	92.5	96.8	95.459		
0.0707	97.4	94.5	97.5	96.972	1.0317	0.013554
	97.5	94.6	97.6	96.975		
	97.6	94.5	97.4	96.923		

$$\text{Log } \frac{Io}{I} = 0.1888(\text{p-MeoBz}) + 0.000$$

Beer's Law Curve For p-Methoxybenzoic Anhydride At 8.177 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.0707	97.4	46.6	97.2	47.893	2.0913	0.320210
	96.9	46.3	96.7	47.830		
	96.7	46.2	96.5	47.826		
0.0565	94.6	52.5	94.7	55.467	1.8043	0.256312
	94.6	52.5	94.9	55.408		
	94.7	52.6	95.0	55.455		
0.0424	95.6	60.6	95.7	63.355	1.5803	0.198768
	95.6	60.6	95.7	63.355		
	95.8	60.5	95.9	63.119		
0.03535	95.7	65.0	95.5	67.991	1.4704	0.167436
	95.4	64.8	95.5	67.888		
	95.0	64.9	95.5	68.136		
0.0283	95.5	69.8	95.2	73.203	1.3653	0.135196
	95.0	69.7	95.2	73.291		
	95.1	69.5	94.7	73.234		
0.01415	94.8	80.1	95.0	84.404	1.1844	0.073498
	94.3	79.6	94.3	84.411		
	94.5	79.9	94.7	84.460		
0.007075	95.7	86.8	95.5	90.794	1.1019	0.042141
	95.4	86.7	95.7	90.737		
	95.8	87.0	96.0	90.719		

$$\text{Log}\ \frac{Io}{I} = 4.350(\text{p-MeoBz}) + 0.0135$$

Beer's Law Curve For p-Methoxybenzoic-Acetic Mixed Anhydride at 8.7110 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{I_o}{I}$	Log ave. $\frac{I_o}{I}$
0.13980	97.7	24.5	97.7	25.076	3.9836	0.600166
	98.0	24.6	97.6	25.153		
	97.5	24.5	97.9	25.076		
0.11140	98.3	32.5	98.5	33.028	3.0329	0.481856
	98.5	32.4	98.4	32.910		
	98.3	32.3	98.2	32.875		
0.09320	98.0	38.4	98.0	89.183	2.5583	0.407951
	97.9	38.1	97.8	38.937		
	97.9	38.3	97.8	39.141		
0.05592	97.7	53.8	97.7	56.090	1.7843	0.251465
	97.8	54.9	97.8	56.134		
	98.1	54.9	98.3	55.906		
0.04464	95.7	58.9	95.9	61.482	1.6255	0.210985
	96.0	59.0	95.8	61.522		
	95.8	58.9	95.6	61.546		
0.02796	98.4	72.6	98.1	73.893	1.3531	0.131335
	97.6	72.3	98.0	73.926		
	98.3	72.6	98.2	73.893		
0.011160	98.0	85.7	98.2	87.461	1.1414	0.057434
	98.1	85.9	97.9	87.653		
	97.9	86.0	98.2	87.710		

$$\text{Log } \frac{I_o}{I} = 4.220(\text{M-p-MeoBz}) + 0.015$$

Beer's Law Curve For p-Methoxybenzoic-Acetic Mixed Anhydride At 8.888 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.12140	97.9 97.5 97.6	73.8 73.6 73.7	97.6 97.6 97.6	75.498 75.448 75.512	1.3265	0.122705
0.09712	98.1 97.5 97.7	77.9 77.7 77.6	97.8 97.7 97.4	79.530 79.610 79.548	1.2600	0.10037
0.07284	97.9 97.8 97.7	82.6 82.6 82.7	97.7 97.9 97.7	84.458 83.414 84.646	1.1861	0.074117
0.04856	98.1 98.1 98.0	87.0 87.1 87.0	98.1 98.2 98.3	88.685 88.741 88.639	1.1275	0.052115
0.03642	98.3 98.0 98.0	89.7 89.4 89.4	98.2 97.9 98.0	91.297 91.271 91.224	1.0957	0.039697

$$\text{Log}\ \frac{Io}{I} = 0.9541(\text{M-p-MeoBz}) + 0.0042$$

Beer's Law Curve For p-Methoxybenzoic-Acetic Mixed Anhydride At 8.177 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.01398	98.0 98.1 97.8	87.1 87.2 87.1	97.9 98.1 98.0	88.922 88.888 88.968	1.1245	0.050960
0.01118	97.8 97.5 97.4	88.9 88.7 88.5	97.6 97.5 97.2	90.992 90.974 90.955	1.0992	0.041078
0.00839	97.0 97.5 97.0	90.1 90.5 90.1	96.8 97.2 97.3	92.982 92.963 92.743	1.0764	0.031974
0.00699	96.5 96.5 96.4	90.9 90.9 91.0	96.5 96.5 96.3	94.196 94.196 94.447	1.0606	0.025556

$$\text{Log}\ \frac{Io}{I} = 0.1732(\text{M-p-MeoBz}) + 0.00225$$

Beer's Law Curve For Acetic Anhydride At 8.7110 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.4242	97.3	40.3	97.4	41.294	2.4224	0.384242
	97.4	40.1	97.4	41.170		
	97.2	40.2	97.1	41.379		
0.3610	97.4	46.7	97.3	47.971	2.0838	0.318858
	97.3	46.8	97.2	48.123		
	97.3	46.6	97.0	47.967		
0.2760	97.4	55.5	97.3	57.010	1.7572	0.244820
	97.3	55.3	97.3	56.834		
	97.3	55.4	97.5	56.878		
0.2122	96.8	63.3	96.8	65.392	1.5306	0.184864
	96.6	63.1	96.5	65.354		
	96.5	63.0	96.3	65.352		
0.1488	96.7	71.5	96.5	74.016	1.3495	0.130138
	96.5	71.5	96.5	74.093		
	96.4	71.4	96.1	74.181		
0.10561	96.4	77.6	96.4	80.597	1.2418	0.094050
	96.3	77.5	96.4	80.435		
	96.2	77.4	96.0	80.541		
0.06375	96.5	84.5	96.6	87.519	1.1424	0.057822
	96.6	84.6	96.6	87.577		
	96.6	84.6	96.8	87.487		
0.04244	96.4	88.0	96.3	91.333	1.0960	0.03981
	96.4	88.0	96.3	91.333		
	96.5	87.9	96.6	91.040		

$$\text{Log } \frac{Io}{I} = 0.8570(Ac) + 0.003$$

Beer's Law Curve For Acetic Anhydride At 8.177 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.4244	98.4	59.3	98.4	60.264	1.6571	0.219346
	98.3	59.2	98.4	60.193		
	98.5	59.3	98.4	60.233		
0.2548	98.3	72.1	98.2	73.384	1.3617	0.133764
	98.2	72.1	98.2	73.421		
	98.0	72.0	97.9	73.506		
0.2122	98.0	75.8	98.0	77.346	1.2903	0.110692
	98.0	75.8	97.6	77.505		
	97.6	75.7	97.4	77.641		
0.1488	98.4	82.0	98.4	83.333	1.1997	0.079072
	98.4	82.0	98.4	83.333		
	98.5	82.1	98.4	83.392		
0.1061	97.7	85.8	97.4	87.954	1.1396	0.056748
	98.3	86.2	98.2	87.735		
	98.3	86.1	98.4	87.545		
0.06375	98.4	90.8	98.4	92.276	1.0771	0.03226
	98.5	91.0	98.5	92.385		
	98.5	90.9	98.7	92.190		

$$\text{Log}\ \frac{Io}{I} = 0.5045(Ac) + 0.002$$

Summary Of Data For The Para-Nitrobenzoic-acetic Anhydride System

Principal Wave Length

A. p-Nitrobenzoic-acetic mixed anhydride --- 7.897 microns

B. Acetic anhydride ------------------------ 8.888 microns

C. p-Nitrobenzoic anhydride ---------------- 6.486 microns

Beer's Law Curves

A. p-Nitrobenzoic-acetic mixed anhydride

1. At mixed anhydride wave length (7.897 microns)

   $$\mathrm{Log}\ \frac{I_o}{I} = 3.341(\text{M-p-Nitro Bz}) + 0.000$$

2. At acetic anhydride wave length (8.888 microns)

   $$\mathrm{Log}\ \frac{I_o}{I} = 1.601(\text{M-p-Nitro Bz}) + 0.0036$$

3. At p-Nitrobenzoic anhydride wave length (6.486 microns)

   $$\mathrm{Log}\ \frac{I_o}{I} = 5.30(\text{M-p-Nitro Bz}) + 0.004$$

B. p-Nitrobenzoic anhydride

1. At mixed anhydride wave length (7.897 microns)

   $$\mathrm{Log}\ \frac{I_o}{I} = 0.559\ (\text{p-Nitro Bz}) + 0.0025$$

2. At acetic anhydride wave length (8.888 microns)

   $$\mathrm{Log}\ \frac{I_o}{I} = 0.181\ (\text{p-Nitro Bz}) + 0.000$$

3. At p-nitrobenzoic anhydride wave length (6.486 microns)

   $$\mathrm{Log}\ \frac{I_o}{I} = 11.09(\text{p-Nitro Bz}) + 0.000$$

C. Acetic Anhydride

1. At mixed anhydride wave length (7.897 microns)

$$\text{Log } \frac{Io}{I} = 0.2535(Ac) + 0.000$$

2. At acetic anhydride wave length (8.888 microns)

$$\text{Log } \frac{Io}{I} = 3.491(Ac) + 0.024$$

3. At p-nitrobenzoic anhydride wave length (6.486 microns)

$$\text{Log } \frac{Io}{I} = 0.587(Ac) + 0.002$$

Simultaneous Equations Derived From The Above Data

At 7.897 microns

$$3.341(\text{M-p-Nitro Bz}) + 0.559(\text{p-Nitro Bz}) + 0.2535(Ac) = \text{Log } \frac{Io}{I} - 0.0025$$

At 8.888 microns

$$1.601(\text{M-p-Nitro Bz}) + 0.181(\text{p-Nitro Bz}) + 3.491(Ac) = \text{Log } \frac{Io}{I} - 0.028$$

At 6.486 microns

$$5.30(\text{M-p-Nitro Bz}) + 11.09(\text{p-Nitro Bz}) + 0.587(Ac) = \text{Log } \frac{Io}{I} - 0.006$$

Beer's Law Curve For p-Nitrobenzoic Anhydride At 7.897 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.0381	98.9	93.7	98.5	94.934	1.0535	0.022635
	98.7	93.6	98.6	94.880		
	98.6	93.7	98.8	94.934		
0.03422	98.1	93.4	97.8	95.354	1.0492	0.020846
	98.4	93.5	98.3	95.068		
	97.8	93.3	97.6	95.496		
0.02285	98.9	95.6	98.9	96.663	1.0336	0.014352
	98.7	95.5	98.5	96.855		
	98.8	95.6	98.9	96.713		
0.01123	98.9	97.0	98.9	98.078	1.0194	0.008346
	98.8	97.0	98.9	98.128		
	98.9	97.0	98.9	98.078		

$$\text{Log } \frac{Io}{I} = 0.5590(\text{p-Nitro Bz}) + 0.0025$$

Beer's Law Curve For p-Nitrobenzoic Anhydride At 8.888 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.0960	98.6	94.3	98.8	95.542	1.0468	0.019866
	98.9	94.4	98.7	95.546		
	98.9	94.3	98.8	95.493		
0.0480	98.6	96.5	98.7	97.820	1.0221	0.009493
	98.5	96.4	98.6	97.818		
	98.4	96.3	98.4	97.865		
0.0384	98.4	96.7	98.6	98.172	1.0168	0.007234
	98.6	96.7	98.3	98.222		
	98.4	96.7	98.3	98.322		

$$\text{Log } \frac{Io}{I} = 0.1810(\text{p-Nitro Bz}) + 0.000$$

Beer's Law Curve For p-Nitrobenzoic Anhydride At 6.486 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.03060	98.2	45.1	98.1	45.950	2.1915	0.340740
	98.5	45.2	98.4	45.911		
	98.1	45.1	98.3	45.926		
0.02295	98.5	54.9	98.7	55.679	1.7952	0.254110
	98.9	55.0	98.6	55.724		
	98.3	54.7	98.1	55.702		
0.01530	97.7	66.2	97.6	67.793	1.4755	0.168940
	97.7	66.2	97.5	67.827		
	97.5	66.1	97.8	67.690		
0.00922	97.5	77.0	97.3	79.055	1.2641	0.101784
	97.4	77.0	97.3	79.096		
	97.3	76.9	97.0	79.155		
0.00765	97.5	80.4	97.5	82.461	1.2142	0.084288
	97.7	80.4	97.6	82.334		
	97.8	80.5	97.9	82.268		
0.00614	97.2	83.3	97.1	85.743	1.1668	0.066996
	97.1	83.3	97.2	85.743		
	97.3	83.4	97.5	85.626		
0.00307	97.1	89.9	97.1	92.584	1.0808	0.033748
	97.2	89.9	97.1	92.537		
	97.3	90.0	97.4	92.449		

$$\text{Log } \frac{Io}{I} = 11.09(\text{p-Nitro Bz}) + 0.0000$$

Beer's Law Curve For p-Nitrobenzoic-acetic Mixed Anhydride At 7.897 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.0783	90.8	49.8	91.1	54.755	1.8309	0.262466
	91.0	49.8	91.2	54.665		
	91.2	49.8	90.9	54.426		
0.06264	91.2	56.3	91.4	61.664	1.6223	0.212531
	91.3	56.2	91.0	61.656		
	91.3	56.3	91.5	61.597		
0.0426	89.9	65.3	90.0	72.595	1.3819	0.140168
	90.1	65.2	89.9	72.444		
	90.1	65.2	90.0	72.044		
0.3915	92.3	68.7	92.1	74.511	1.3429	0.128047
	92.1	68.7	92.5	74.431		
	92.5	68.9	92.6	74.446		
0.02555	90.4	74.1	90.2	82.059	1.2172	0.085362
	90.2	74.4	90.4	82.392		
	90.4	74.1	90.3	82.014		
0.01704	90.4	78.8	90.3	87.216	1.1449	0.058763
	90.3	78.8	90.1	87.361		
	90.1	78.7	89.9	87.444		
0.00852	98.0	91.5	97.9	93.415	1.0710	0.02979
	98.1	91.5	97.9	93.319		
	97.9	91.6	98.3	93.374		

$$\text{Log}\ \frac{Io}{I} = 3.341(\text{M-p-Nitro Bz}) + 0.000$$

Beer's Law Curve For *p*-Nitrobenzoic-acetic Mixed Anhydride At 8.888 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.0475	97.8	81.2	97.7	83.069	1.2027	0.080159
	97.6	81.1	97.5	83.136		
	97.6	81.1	97.3	83.222		
0.0380	97.4	83.8	97.0	86.213	1.1585	0.063895
	97.0	83.8	97.0	86.391		
	97.1	83.8	97.0	86.347		
0.0285	97.1	86.8	97.1	89.392	1.1206	0.049454
	97.2	86.5	97.0	89.083		
	97.1	86.6	97.0	89.232		
0.0190	97.1	89.8	97.1	92.481	1.0815	0.034030
	97.1	89.7	97.0	92.426		
	97.0	89.8	97.2	92.481		
0.0095	97.1	92.9	97.1	95.674	1.0442	0.018784
	97.1	93.0	97.0	95.826		
	97.0	93.0	97.2	95.777		

$$\text{Log}\ \frac{Io}{I} = 1.601(\text{M-p-Nitro Bz}) + 0.0036$$

Beer's Law Curve For *p*-Nitrobenzoic-acetic Anhydride At 6.486 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.0480	97.1	52.6	97.1	54.170	1.8475	0.266585
	97.3	52.7	97.3	54.162		
	97.5	52.8	97.5	54.153		
0.0384	96.5	58.6	96.6	60.693	1.6481	0.216986
	96.4	58.6	96.8	60.662		
	96.7	58.7	96.8	60.671		
0.0288	98.2	67.4	98.2	68.635	1.4562	0.16220
	98.2	67.4	98.1	68.670		
	98.0	67.3	97.9	68.708		
0.0192	98.2	75.9	98.1	77.330	1.2932	0.111666
	98.1	75.9	98.1	77.370		
	98.0	75.8	98.2	77.268		
0.0096	98.1	85.9	98.0	87.608	1.1416	0.057134
	98.0	85.9	98.0	87.653		
	98.2	85.9	98.1	87.519		

$$\text{Log}\ \frac{Io}{I} = 5.300(\text{M-p-Nitro Bz}) + 0.004$$

Beer's Law Curve For Acetic Anhydride At 7.897 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.5244	98.8	73.0	98.5	73.924	1.3535	0.13146
	98.6	72.9	98.7	73.897		
	98.7	72.9	98.8	73.822		
0.3486	98.2	80.1	98.5	81.443	1.2268	0.088500
	98.8	80.5	98.8	81.477		
	98.1	80.1	98.2	81.609		
0.2622	98.8	84.9	98.9	85.887	1.1632	0.065654
	98.8	84.9	98.5	86.061		
	98.6	84.8	98.7	85.960		
0.1748	98.5	88.7	98.5	90.050	1.1116	0.045956
	98.4	88.6	98.7	89.903		
	98.7	88.7	98.6	89.913		
0.1049	98.8	92.8	98.8	93.927	1.0645	0.027145
	98.7	92.7	98.7	93.920		
	98.7	92.8	98.8	93.974		

$$\text{Log } \frac{Io}{I} = 0.2535(Ac) + 0.0000$$

Beer's Law Curve For Acetic Anhydride At 6.486 Microns In Chloroform

Concentration moles per liter	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
0.5244	98.1	91.0	98.1	92.762	1.0778	0.032540
	98.1	91.1	98.2	92.817		
	98.2	91.0	98.0	92.762		
0.4370	98.1	92.0	98.1	93.781	1.0661	0.027800
	98.1	92.0	98.0	93.829		
	98.1	92.1	98.3	93.788		
0.3486	97.9	93.0	97.9	94.994	1.0526	0.022225
	97.9	93.0	97.9	94.994		
	97.9	93.0	97.9	94.994		
0.31464	97.8	93.4	97.7	95.549	1.0467	0.019824
	97.8	93.3	97.6	95.496		
	97.6	93.3	97.7	95.545		

$$\text{Log } \frac{Io}{I} = 0.5870(Ac) + 0.002$$

Equilibrium Mixture Derived From Acetic Anhydride And Benzoic Acid In Chloroform At 150°C

Wave length (microns)	Blank	Sample	Blank	%T	Average $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
8.690	96.8	45.4	96.9	46.876	2.1333	0.32905
	97.0	45.5	97.2	46.858		
	97.3	45.6	97.2	46.889		
8.888	96.0	44.8	96.1	46.642	2.1433	0.33108
	96.2	44.9	96.2	46.673		
	96.5	45.0	96.4	46.656		
8.224	96.5	58.5	96.7	60.559	1.6518	0.217958
	96.7	58.6	96.9	60.537		
	96.9	58.7	97.1	60.515		
5.823	96.0	63.0	96.2	65.556	1.5256	0.183438
	96.3	63.1	96.2	65.558		
	96.2	63.1	96.4	65.524		
7.570	95.9	84.4	96.2	87.870	1.1386	0.056368
	96.0	84.3	95.9	87.858		
	95.9	84.2	95.8	87.845		

Concentration benzoic-acetic mixed anhydride -- 0.1213350

Concentration benzoic anhydride --------------- 0.0433680

Concentration acetic anhydride ---------------- 0.0686636

Concentration benzoic acid -------------------- 0.0121030

Concentration acetic acid --------------------- 0.0437800

Equilibrium Constant (anhydride)--- 4.94

Equilibrium Constant (acid-I) ----- 1.29

Equilibrium Constant (acid-II) ---- 6.33

Equilibrium Mixture Derived From Acetic Anhydride, Benzoic Anhydride And Acetic Acid In Chloroform At 75°C

Wave length (microns)	Blank	Sample	Blank	%T	Average $\frac{I_o}{I}$	Log ave. $\frac{I_o}{I}$
8.690	97.6	67.0	97.8	68.5772	1.45843	0.163881
	97.5	66.9	97.6	68.5802		
	97.8	67.0	97.7	68.5421		
8.888	97.8	63.8	97.9	65.2018	1.53375	0.185755
	97.8	63.7	97.6	65.1995		
	97.6	63.6	97.5	65.1973		
8.224	97.4	49.6	97.3	50.9501	1.96277	0.292874
	97.5	49.7	97.6	50.9482		
	97.8	49.8	97.7	50.9462		
5.823	97.8	57.1	98.0	58.3248	1.71528	0.234338
	98.1	57.2	98.2	58.2781		
	98.0	57.1	97.9	58.2950		
7.570	97.9	84.7	97.9	86.5168	1.15557	0.062601
	97.8	84.6	97.7	86.5473		
	97.7	84.6	97.8	86.5473		

Concentration benzoic-acetic mixed anhydride -- 0.056873

Concentration benzoic anhydride --------------- 0.0721210

Concentration acetic anhydride ---------------- 0.038472

Concentration benzoic acid ------------------- 0.0418791

Concentration acetic acid -------------------- 0.0520808

Equilibrium Constant (anhydride) -- 1.25

Equilibrium Constant (acid-I) ----- 1.58

Equilibrium Constant (acid-II) ---- 1.97

Equilibrium Mixture Derived From Benzoic-acetic Mixed Anhydride And Benzoic Acid In Chloroform At 75°C

Wave length (microns)	Blank	Sample	Blank	%T	Average $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
8.690	95.7	56.5	95.8	59.0078	1.69436	0.229013
	95.8	56.6	95.9	59.0505		
	95.9	56.6	96.0	58.9890		
8.888	96.1	46.4	96.3	48.2077	2.07711	0.317462
	96.5	46.5	96.5	48.1865		
	96.4	46.5	96.4	48.2365		
8.224	96.7	50.1	96.5	51.8633	1.92962	0.285470
	96.5	50.1	96.6	51.8902		
	96.6	50.1	96.4	51.9170		
5.823	96.3	48.7	96.2	50.5474	1.97671	0.295834
	96.1	48.6	96.0	50.5986		
	96.2	48.7	96.4	50.5711		
7.570	96.4	83.1	96.5	86.1586	1.16092	0.064794
	96.5	83.2	96.6	86.1729		
	96.6	83.2	96.7	86.0838		

Concentration benzoic-acetic mixed anhydride -- 0.0752055

Concentration benzoic anhydride ---------------- 0.0738982

Concentration acetic anhydride ----------------- 0.0708808

Concentration benzoic acid -------------------- 0.0250481

Concentration acetic acid --------------------- 0.0857286

Equilibrium Constant (anhydride) - 1.08

Equilibrium Constant (acid-I) ---- 3.36

Equilibrium Constant (acid-II) --- 3.61

Equilibrium Mixture Derived From Benzoic-acetic Mixed Anhydride And Benzoic Acid At 100°C In Chloroform

Wave length (microns)	Blank	Sample	Blank	%T	Average $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
8.690	97.3	56.7	97.2	58.303	1.7153	0.234338
	97.5	56.8	97.3	58.316		
	97.2	56.7	97.4	58.273		
8.888	96.8	53.4	96.9	55.136	1.8149	0.258859
	96.7	53.2	96.5	55.072		
	96.4	53.1	96.4	55.082		
8.224	96.3	51.4	96.2	53.402	1.8709	0.272047
	96.1	51.4	96.1	53.485		
	96.1	51.4	96.2	53.458		
5.823	96.5	44.7	96.4	46.345	2.1561	0.333670
	96.4	44.7	96.4	46.369		
	96.5	44.8	96.5	46.424		
7.570	95.8	80.4	96.0	83.837	1.1922	0.076352
	96.0	80.5	95.9	83.897		
	95.9	80.4	95.8	83.881		

Concentration benzoic-acetic mixed anhydride -- 0.0829080

Concentration benzoic anhydride --------------- 0.0663150

Concentration acetic anhydride ---------------- 0.0517563

Concentration benzoic acid -------------------- 0.0408189

Concentration acetic acid --------------------- 0.0946918

Equilibrium Constant (anhydride) -- 2.00

Equilibrium Constant (acid-I) ----- 1.86

Equilibrium Constant (acid-II) ---- 2.04

Equilibrium Mixture Derived From Benzoic Anhydride And Acetic Acid At 100°C In Chloroform

Wave length (microns)	Blank	Sample	Blank	%T	Average $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$
8.690	96.7 96.8 96.9	45.2 45.2 45.2	96.8 96.9 96.7	46.718 46.670 46.743	2.1409	0.330590
8.888	95.9 95.9 95.8	47.2 47.1 47.1	96.0 95.8 95.8	49.192 49.139 49.164	2.0339	0.308329
8.224	95.4 95.6 95.6	42.2 42.2 42.2	95.6 95.5 95.7	44.188 44.165 44.119	2.2651	0.355079
5.823	95.7 95.8 96.0	43.5 43.6 43.7	95.7 96.1 96.0	45.454 45.393 45.520	2.2001	0.342439
7.570	96.1 96.3 96.5	79.3 79.6 79.6	96.2 96.5 96.5	82.475 82.572 82.487	1.2119	0.083464

Concentration benzoic-acetic mixed anhydride -- 0.122560

Concentration benzoic anhydride --------------- 0.082450

Concentration acetic anhydride ---------------- 0.058464

Concentration benzoic acid -------------------- 0.029669

Concentration acetic acid --------------------- 0.098309

Equilibrium Constant (anhydride) -- 3.12

Equilibrium Constant (acid-I) ----- 2.23

Equilibrium Constant (acid-II) ---- 6.95

Equilibrium Mixture Derived From Benzoic-acetic Mixed Anhydride And Acetic Acid At 150°C In Chloroform

Wave length (microns)	Blank	Sample	Blank	%T	Average $\frac{I_o}{I}$	Log ave. $\frac{I_o}{I}$
8.690	97.8	65.4	97.8	66.871	1.4956	0.174814
	97.7	65.2	97.4	66.837		
	97.4	65.1	97.3	66.872		
8.888	97.5	63.3	97.4	64.959	1.5403	0.187604
	97.3	63.2	97.5	64.887		
	97.5	63.3	97.5	64.923		
8.224	97.4	71.2	97.3	73.138	1.3675	0.13583
	97.1	71.1	97.3	73.148		
	97.3	71.1	97.0	73.185		
5.823	97.0	56.3	96.9	58.071	1.7230	0.23629
	96.9	56.2	96.7	58.057		
	96.7	56.1	96.8	57.984		
7.570	97.3	86.9	97.2	89.357	1.1191	0.048860
	97.2	86.9	97.2	89.403		
	97.3	86.9	97.3	89.311		

Concentration benzoic-acetic mixed anhydride -- 0.06296500

Concentration benzoic anhydride --------------- 0.02781174

Concentration acetic anhydride ---------------- 0.03813330

Concentration benzoic acid -------------------- 0.02406000

Concentration acetic acid --------------------- 0.07184750

Equilibrium Constant (anhydride) -- 3.74

Equilibrium Constant (acid I) ----- 1.27

Equilibrium Constant (acid II) ---- 4.75

Equilibrium Mixture Derived From Benzoic And Acetic Anhydrides At 70° In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{Io}{I}$
8.224	96.8	73.9	96.7	76.382	76.395	0.116907
	96.7	73.9	96.8	76.382		
	96.7	73.9	96.7	76.421		
8.888	97.7	59.7	97.8	61.074	61.078	0.214102
	97.5	59.6	97.7	61.056		
	97.6	59.6	97.5	61.096		
8.690	96.5	57.5	96.4	59.512	59.564	0.224998
	96.4	57.4	96.3	59.574		
	96.3	57.4	96.3	59.605		

Concentration of benzoic-acetic mixed anhydride - 0.0818490
Concentration of benzoic anhydride -------------- 0.0018287
Concentration of acetic anhydride --------------- 0.0352680

Equilibrium Constant = 1.04

Equilibrium Mixture Derived From Benzoic-Acetic Mixed Anhydride At 70° C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{Io}{I}$
8.224	95.4	66.6	95.5	69.774	69.772	0.156340
	95.6	66.8	95.7	69.837		
	95.4	66.5	95.4	69.706		
8.888	94.8	58.2	95.0	61.327	61.362	0.212182
	94.7	58.1	94.6	61.384		
	94.5	58.0	94.5	61.375		
8.690	95.8	52.7	96.0	54.953	54.955	0.259974
	96.1	52.8	96.1	54.942		
	96.0	52.8	96.1	54.971		

Concentration of benzoic-acetic mixed anhydride -- 0.0972660
Concentration of benzoic anhydride --------------- 0.0281644
Concentration of acetic anhydride ---------------- 0.0313660

Equilibrium Constant = 1.07

Equilibrium Mixture Derived From Benzoic And Acetic Anhydrides At 100°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{I_o}{I}$
8.224	94.8 95.0 95.2	68.1 68.3 68.3	94.9 95.2 95.1	71.797 71.819 71.781	71.799	0.143733
8.888	95.4 95.5 95.6	67.0 67.0 67.1	95.5 95.5 95.5	70.193 70.157 70.225	70.192	0.153660
8.690	96.0 96.0 96.0	55.9 55.9 55.9	96.1 95.9 96.0	58.198 58.259 58.229	58.229	0.234845

Concentration of benzoic-acetic mixed anhydride - 0.0911480
Concentration of benzoic anhydride -------------- 0.0265109
Concentration of acetic anhydride --------------- 0.0157940

Equilibrium Constant = 1.98

Equilibrium Mixture Derived From Benzoic-Acetic Mixed Anhydride At 100°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{I_o}{I}$
8.224	96.0 96.3 96.3	38.3 38.4 38.3	96.2 96.4 96.3	39.854 39.853 39.771	39.826	0.399836
8.888	97.8 97.6 97.8	35.8 35.8 35.8	97.8 97.7 97.7	36.605 36.661 36.624	36.630	0.4361600
8.690	96.5 96.5 96.6	43.0 43.1 43.1	96.4 96.6 96.7	44.582 44.640 44.593	44.605	0.350620

Concentration of benzoic-acetic mixed anhydride - 0.120114
Concentration of benzoic anhydride -------------- 0.088116
Concentration of acetic anhydride --------------- 0.088350

Equilibrium Constant = 1.85

Equilibrium Mixture Derived From Benzoic And Acetic Anhydrides At 150°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{I_o}{I}$
	95.6	63.4	95.7	66.283		
8.224	95.4	63.3	95.6	66.282	66.294	0.178516
	95.5	63.3	95.4	66.317		
	94.8	52.5	94.7	55.408		
8.888	94.7	52.5	94.9	55.379	55.391	0.256552
	95.0	52.6	94.9	55.397		
	96.7	56.3	96.8	58.191		
8.690	96.9	56.4	96.9	58.204	58.210	0.234995
	96.8	56.4	96.9	58.234		

Concentration of benzoic-acetic mixed anhydride - 0.0826050
Concentration of benzoic anhydride -------------- 0.0339594
Concentration of acetic anhydride --------------- 0.0466070

Equilibrium Constant = 4.31

Equilibrium Mixture Derived From Benzoic-Acetic Mixed Anhydride At 150°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{I_o}{I}$
	96.4	57.5	96.3	59.678		
8.224	96.3	57.5	96.3	59.709	59.709	0.223935
	96.3	57.5	96.2	59.740		
	95.7	51.6	95.8	53.890		
8.888	96.1	51.9	96.2	53.978	53.946	0.268041
	96.3	52.0	96.4	53.969		
	95.4	52.0	95.4	54.507		
8.690	95.5	52.1	95.5	54.554	54.529	0.263352
	95.6	52.1	95.5	54.526		

Concentration of benzoic-acetic mixed anhydride - 0.094565
Concentration of benzoic anhydride -------------- 0.045713
Concentration of acetic anhydride --------------- 0.046949

Equilibrium Constant = 4.17

Equilibrium Mixture Derived From *p*-Methoxybenzoic And Acetic Anhydrides At 75°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{Io}{I}$
8.711	97.0	62.6	97.1	64.502	64.537	0.190162
	97.2	62.7	97.1	64.539		
	97.1	62.7	97.1	64.572		
8.888	98.1	70.8	98.2	72.134	72.155	0.141729
	98.0	70.7	97.9	72.179		
	97.9	70.6	97.8	72.151		
8.177	97.5	72.0	97.6	73.808	73.720	0.132068
	97.7	72.1	97.8	73.759		
	97.7	72.0	97.7	73.694		

Concentration of *p*-methoxybenzoic-acetic mixed anhyd - 0.0342403
Concentration of *p*-methoxybenzoic anhydride ---------- 0.0223059
Concentration of acetic anhydride -------------------- 0.0220160

Equilibrium Constant = 2.26

Equilibrium Mixture Derived From *p*-Methoxybenzoic Acetic Mixed Anhydride At 75°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{Io}{I}$
8.711	95.8	46.8	95.9	48.826	48.790	0.311666
	95.9	46.7	95.7	48.747		
	95.7	46.7	95.7	48.798		
8.888	94.9	57.0	95.0	60.031	60.003	0.221805
	95.2	57.1	95.1	60.010		
	95.0	57.0	95.1	59.968		
8.177	96.0	58.2	96.2	60.561	60.554	0.217854
	96.2	58.3	96.3	60.571		
	96.2	58.3	96.4	60.539		

Concentration of *p*-methoxybenzoic-acetic mixed anhyd - 0.058300
Concentration of *p*-methoxybenzoic anhydride ---------- 0.039276
Concentration of acetic anhydride -------------------- 0.037472

Equilibrium Constant = 2.31

Equilibrium Mixture Derived From p-Methoxybenzoic And Acetic Anhydrides At 75°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{Io}{I}$
8.711	97.2	33.5	97.3	34.465	34.456	0.462730
	97.5	33.6	97.5	34.461		
	97.6	33.6	97.5	34.443		
8.888	97.9	35.0	97.8	35.769	35.787	0.446275
	97.8	35.0	97.8	35.787		
	97.7	35.0	97.8	35.805		
8.177	96.0	57.8	96.1	60.176	60.179	0.220452
	96.1	57.8	96.1	60.145		
	96.2	57.9	96.1	60.218		

Concentration of p-methoxybenzoic-acetic mixed anhyd - 0.083015
Concentration of p-methoxybenzoic anhydride ---------- 0.032177
Concentration of acetic anhydride -------------------- 0.095341

Equilibrium Constant = 2.25

Equilibrium Mixture Derived From p-Methoxybenzoic And Acetic Anhydrides At 100°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{Io}{I}$
8.711	98.0	57.3	98.1	58.439	58.477	0.233000
	98.2	57.4	98.1	58.481		
	98.1	57.4	98.1	58.511		
8.888	97.5	66.8	97.6	68.477	68.465	0.164530
	97.4	66.7	97.5	68.445		
	97.9	67.0	97.8	68.472		
8.177	96.0	73.7	96.2	76.690	76.683	0.115280
	96.3	73.9	96.4	76.699		
	96.4	73.9	96.4	76.659		

Concentration of p-methoxybenzoic-acetic mixed anhyd - 0.0440581
Concentration of p-methoxybenzoic anhydride ---------- 0.0175770
Concentration of acetic anhydride -------------------- 0.0261400

Equilibrium Constant = 4.22

Equilibrium Mixture Derived From p-Methoxybenzoic And Acetic Anhydrides At 100°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{Io}{I}$
8.711	94.8	60.6	94.9	63.890	63.872	0.194678
	95.0	60.7	95.1	63.861		
	95.2	60.8	95.2	63.865		
8.888	95.8	65.1	95.9	67.918	67.940	0.167850
	96.0	65.2	96.0	67.916		
	96.1	65.3	96.0	67.985		
8.177	97.0	81.2	97.0	83.711	83.751	0.076892
	97.0	81.3	97.1	83.771		
	97.1	81.3	97.0	83.771		

Concentration of p-methoxybenzoic-acetic mixed anhyd - 0.0349620
Concentration of p-methoxybenzoic anhydride ---------- 0.0086781
Concentration of acetic anhydride -------------------- 0.0300000

Equilibrium Constant = 4.69

Equilibrium Mixture Derived From p-Methoxybenzoic-Acetic Mixed Anhydride At 100°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{Io}{I}$
8.711	96.4	56.3	96.3	58.432	58.451	0.233200
	95.8	56.0	95.8	58.455		
	95.9	56.1	96.0	58.467		
8.888	94.8	66.9	94.8	70.569	70.590	0.151246
	94.9	67.0	94.9	70.600		
	94.9	67.0	94.9	70.600		
8.177	95.3	71.0	95.2	74.540	74.566	0.127430
	94.9	70.8	95.0	74.565		
	95.0	70.9	95.1	74.592		

Concentration of p-methoxybenzoic-acetic mixed anhyd - 0.044280
Concentration of p-methoxybenzoic anhydride ---------- 0.020830
Concentration of acetic anhydride -------------------- 0.022085

Equilibrium Constant = 4.26

Equilibrium Mixture Derived From p-Methoxybenzoic And Acetic Anhydrides At 150°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{I_o}{I}$
8.711	98.0	72.7	98.2	74.108	74.329	0.128819
	98.4	72.9	98.3	74.123		
	97.9	72.6	97.9	74.157		
8.888	96.3	74.4	96.2	77.298	77.319	0.111699
	96.4	74.6	96.5	77.345		
	96.5	74.6	96.5	77.305		
8.177	95.8	69.1	95.6	72.280	72.205	0.141574
	95.9	69.2	95.9	72.158		
	96.1	69.4	96.2	72.178		

Concentration of p-methoxybenzoic-acetic mixed anhyd - 0.022783
Concentration of p-methoxybenzoic anhydride ---------- 0.025524
Concentration of acetic anhydride -------------------- 0.017042

Equilibrium Constant = 11.93

Equilibrium Mixture Derived From p-Methoxybenzoic-Acetic Mixed Anhydride At 150°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{I_o}{I}$
8.711	97.5	61.5	97.4	63.044	63.043	0.200356
	97.3	61.3	97.2	63.033		
	97.4	61.4	97.3	63.071		
8.888	98.0	75.5	97.9	77.080	77.072	0.113032
	97.5	75.1	97.5	77.025		
	97.7	75.3	97.6	77.112		
8.177	96.4	78.6	96.4	81.535	81.548	0.088560
	96.7	78.8	96.6	81.531		
	96.4	78.6	96.3	81.577		

Concentration of p-methoxybenzoic-acetic mixed anhyd - 0.039340
Concentration of p-methoxybenzoic anhydride ---------- 0.013158
Concentration of acetic anhydride -------------------- 0.012894

Equilibrium Constant = 9.13

Equilibrium Mixture Derived From p-Nitrobenzoic And Acetic Anhydrides At 75°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{I_o}{I}$
7.897	96.8	65.5	96.9	67.630	67.549	0.170380
	96.7	65.4	96.7	67.422		
	96.7	65.4	96.8	67.596		
8.888	98.4	18.8	98.4	19.105	19.076	0.720168
	98.5	18.8	98.6	19.076		
	98.7	18.8	98.7	19.047		
6.486	97.8	42.2	97.9	43.127	43.156	0.364948
	96.9	41.8	96.8	43.159		
	96.8	41.8	96.8	43.181		

Concentration of p-nitrobenzoic-acetic mixed anhyd - 0.0354960
Concentration of p-nitrobenzoic anhydride ---------- 0.0057885
Concentration of acetic anhydride ----------------- 0.1816900

Equilibrium Constant ≃ 1.20

Equilibrium Mixture Derived From p-Nitrobenzoic-Acetic Mixed Anhydride At 75°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{I_o}{I}$
7.897	95.2	76.3	95.3	80.104	80.119	0.096244
	95.1	76.2	95.0	80.168		
	95.1	76.2	95.2	80.084		
8.888	97.4	70.8	97.6	72.615	72.665	0.138651
	97.2	70.7	97.3	72.699		
	97.5	70.9	97.6	72.680		
6.486	98.1	41.6	97.9	42.448	42.438	0.372026
	97.5	41.4	97.7	42.418		
	98.0	41.6	98.0	42.448		

Concentration of p-nitrobenzoic-acetic mixed anhyd - 0.0230540
Concentration of p-nitrobenzoic anhydride ---------- 0.0209410
Concentration of acetic anhydride ----------------- 0.0200378

Equilibrium Constant ≃ 1.27

Equilibrium Mixture Derived From p-Nitrobenzoic And Acetic Anhydrides At 100°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{Io}{I}$
7.897	97.1	85.3	97.2	87.802	87.815	0.0564060
	96.8	85.0	96.8	87.809		
	96.9	85.2	97.1	87.835		
8.888	95.8	78.8	95.9	82.211	82.214	0.0845058
	96.0	79.0	96.2	82.206		
	96.1	79.1	96.3	82.224		
6.486	97.8	58.0	97.8	59.304	59.338	0.226652
	97.8	58.1	98.0	59.346		
	97.8	58.0	97.6	59.365		

Concentration of p-nitrobenzoic-acetic mixed anhyd - 0.0129194
Concentration of p-nitrobenzoic anhydride ---------- 0.0130697
Concentration of acetic anhydride ------------------ 0.0098700

Equilibrium Constant = 1.29

Equilibrium Mixture Derived From p-Nitrobenzoic-Acetic Mixed Anhydride At 100°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{Io}{I}$
7.897	95.6	64.9	95.7	67.851	67.898	0.168113
	95.7	65.0	95.7	67.920		
	95.5	64.9	95.6	67.922		
8.888	95.6	47.5	95.6	60.146	60.234	0.220136
	95.4	57.4	95.6	60.421		
	95.4	57.4	95.5	60.136		
6.486	94.8	21.3	94.9	22.456	22.417	0.649470
	95.0	21.3	95.2	22.397		
	95.1	21.3	95.1	22.397		

Concentration of p-nitrobenzoic-acetic mixed anhyd - 0.040816
Concentration of p-nitrobenzoic anhydride ---------- 0.036704
Concentration of acetic anhydride ------------------ 0.034427

Equilibrium Constant = 1.32

Equilibrium Mixture Derived From p-Nitrobenzoic And Acetic Anhydrides At 100°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{Io}{I}$
7.897	97.6	79.9	97.5	81.906	81.920	0.086612
	97.4	79.8	97.4	81.930		
	97.7	80.0	97.6	81.925		
8.888	95.6	69.6	95.4	72.879	72.869	0.137446
	95.8	69.8	95.7	72.898		
	95.5	69.7	95.9	72.831		
6.486	94.8	47.6	94.7	50.237	50.227	0.299048
	94.3	47.4	94.5	50.211		
	96.0	48.2	95.9	50.234		

Concentration of p-nitrobenzoic-acetic mixed anhyd - 0.0210147

Concentration of p-nitrobenzoic anhydride ---------- 0.0152745

Concentration of acetic anhydride ------------------ 0.0209212

Equilibrium Constant = 1.38

Equilibrium Mixture Derived From p-Nitrobenzoic And Acetic Anhydrides At 150°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{I_o}{I}$
7.897	96.8	71.6	96.8	73.966	73.979	0.130884
	96.8	71.6	96.7	74.005		
	96.8	71.6	96.8	73.966		
8.888	95.7	61.2	95.5	64.016	64.041	0.193512
	95.6	61.3	95.7	64.087		
	95.7	61.3	95.8	64.020		
6.486	95.2	36.0	95.3	37.795	37.749	0.423080
	95.3	36.0	95.3	37.775		
	95.4	35.7	95.5	37.777		

Concentration of p-nitrobenzoic-acetic mixed anhyd - 0.033568
Concentration of p-nitrobenzoic anhydride ---------- 0.020507
Concentration of acetic anhydride ------------------ 0.030951

Equilibrium Constant = 1.77

Equilibrium Mixture Derived From p-Nitrobenzoic-Acetic Mixed Anhydride At 150°C In Chloroform

Wave Length	Blank	Sample	Blank	%T	Average %T	Log ave. $\frac{I_o}{I}$
7.897	95.4	79.9	95.3	83.796	83.841	0.078748
	95.7	80.3	95.8	83.864		
	95.8	80.3	95.7	83.864		
8.888	96.4	74.4	96.4	77.178	77.135	0.112742
	96.5	74.4	96.4	77.138		
	96.3	74.2	96.2	77.090		
6.486	97.3	51.1	97.5	52.464	52.449	0.281076
	97.7	51.3	97.6	52.534		
	97.8	51.3	97.7	52.348		

Concentration of p-nitrobenzoic-acetic mixed anhyd - 0.019224
Concentration of p-nitrobenzoic anhydride ---------- 0.014840
Concentration of acetic anhydride ------------------ 0.014690

Equilibrium Constant = 1.70

Rate Of Disproportionation Of p-Methoxybenzoic-acetic Mixed Anhydride At 100°C Reaction Followed By Measurement Of p-Methoxybenzoic Anhydride

Time (min.)	Blank	Sample	Blank	%T	Ave $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$	Concentration^{-1} (liters.mole^{-1})
0	98.2	91.8	98.3	93.435	1.0703	0.029503	8.8261
	98.0	91.6	98.1	93.421			
	97.9	91.6	98.2	93.421			
5	98.3	89.5	98.6	90.909	1.1015	0.041995	9.0334
	98.6	89.4	98.5	90.715			
	98.5	89.4	98.6	90.715			
10	98.5	85.3	98.4	86.642	1.1536	0.06206	9.4073
	98.3	85.1	98.0	86.704			
	98.1	85.1	98.2	86.704			
15	98.7	81.8	98.5	82.961	1.2058	0.08127	9.7656
	98.0	81.2	97.9	82.899			
	97.7	81.1	97.9	82.924			
20	97.5	77.4	97.4	79.425	1.2582	0.09785	10.1210
	97.6	77.6	97.7	79.467			
	97.7	77.5	97.3	79.527			
25	97.4	76.3	97.3	78.376	1.2754	0.105646	10.5150
	97.2	76.3	97.4	78.417			
	97.3	76.3	97.3	78.417			
30	97.8	72.2	97.9	73.684	1.3552	0.132004	10.928
	97.2	71.7	97.1	73.803			
	97.1	71.8	97.3	73.868			
35	96.9	69.3	96.7	71.590	1.3976	0.145386	11.286
	97.0	69.4	97.1	71.509			
	96.8	69.3	96.9	71.553			
40	96.8	65.7	96.6	67.942	1.4703	0.167407	11.904
	96.8	65.7	96.6	67.942			
	96.7	65.8	96.4	68.151			

Specific Rate Constant = 1.29 x 10^{-3} moles^{-1} min.$^{-1}$ Liters

Rate Of Disproportionation Of p-Methoxybenzoic-acetic Mixed Anhydride At 100°C. Reaction Followed By Measurement Of p-Methoxybenzoic-acetic Mixed Anhydride

Time (min.)	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$	Concentration^{-1} (liters.mole^{-1})
0	98.1	21.7	98.2	22.109	4.5285	0.65965	6.3973
	98.0	21.6	97.9	22.052			
	98.2	21.7	98.3	22.086			
5	98.0	23.7	98.1	24.171	4.1438	0.617398	6.8351
	98.1	23.7	98.0	24.171			
	98.0	23.7	98.0	24.183			
10	97.8	25.5	97.9	26.060	3.8328	0.583518	7.2319
	97.9	25.6	98.0	26.135			
	97.8	25.5	97.8	26.073			
15	97.8	27.2	97.7	27.826	3.5963	0.555856	7.5918
	97.6	27.1	97.5	27.780			
	98.1	27.3	98.2	27.814			
20	97.4	28.9	97.3	29.686	3.3668	0.527214	8.0043
	97.4	29.0	97.4	29.774			
	97.2	28.8	97.1	29.644			
25	97.5	30.8	97.5	31.589	3.1672	0.500678	8.4286
	97.5	30.7	97.4	31.503			
	97.7	30.8	97.8	31.508			
35	92.3	31.8	92.4	34.434	2.8953	0.461695	9.1402
	92.0	31.8	91.8	34.602			
	92.2	31.9	92.3	34.579			

Specific Rate Constant = 1.38×10^{-3} moles^{-1} min.$^{-1}$ Liters

Rate Of Disproportionation Of p-Methoxybenzoic-acetic Mixed Anhydride At 100°C Reaction Followed By Measurement Of p-Methoxybenzoic Anhydride

Time (min.)	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$	Concentration^{-1} (liters.moles^{-1})
0	98.1	91.7	98.2	93.428	1.0701	0.029411	8.7108
	98.0	91.6	98.0	93.469			
	98.1	91.7	98.2	93.428			
5	98.1	90.1	98.0	91.891	1.0884	0.036790	8.9206
	98.2	90.2	98.3	91.806			
	98.0	90.0	97.8	91.930			
10	97.9	88.0	97.9	89.887	1.1122	0.050068	9.1827
	97.9	88.0	97.9	89.887			
	97.9	88.1	98.0	89.943			
15	97.8	85.5	97.9	87.378	1.1441	0.058468	9.3370
	97.8	85.5	97.7	87.468			
	97.7	85.4	97.8	87.365			
20	98.0	83.9	98.0	85.612	1.1681	0.067477	9.5057
	97.9	83.8	97.9	85.597			
	97.8	83.8	98.0	85.597			
25	97.9	80.3	97.9	82.022	1.2205	0.086540	9.8814
	97.9	80.2	97.9	81.920			
	98.0	80.3	98.2	81.855			
35	97.7	74.9	97.7	76.663	1.3044	0.115412	10.515
	97.8	75.0	97.9	76.647			
	97.6	74.8	97.5	76.678			

Specific Rate Constant = 1.60 x 10^{-3} moles^{-1} min.$^{-1}$ Liters

Rate Of Disproportionation Of p-Methoxybenzoic-acetic Mixed Anhydride At 100°C Reaction Followed By Measurement Of p-Methoxybenzoic Anhydride

Time (min.)	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$	Concentration^{-1} (liters.moles^{-1})
0	97.7 97.7 97.5	91.3 91.2 91.3	97.6 97.7 97.7	93.497 93.346 93.545	1.0699	0.02934	8.8183
5	97.9 97.7 97.8	87.1 87.0 86.9	97.9 97.8 97.9	88.968 89.000 88.809	1.1245	0.050960	9.1996
10	97.8 97.7 97.8	84.0 83.9 84.1	97.9 97.9 97.9	85.845 85.787 85.947	1.1646	0.066178	9.4696
15	97.8 97.8 97.6	79.5 79.4 79.4	97.8 97.8 97.5	81.288 81.186 81.394	1.2301	0.089947	9.9502
20	97.8 97.9 97.5	76.5 76.6 76.4	97.8 98.0 97.6	78.220 78.203 78.318	1.2780	0.106530	10.309
25	97.8 97.6 97.9	73.5 73.4 73.6	97.9 97.5 98.0	75.114 75.243 75.140	1.3303	0.123949	10.729
30	97.8 97.7 97.6	70.5 70.5 70.4	97.7 97.9 97.6	72.122 72.085 72.131	1.3867	0.141984	11.185
35	97.8 97.8 97.6	68.1 68.2 68.0	97.7 97.9 97.5	69.667 69.698 69.703	1.4349	0.156820	11.600
40.5	97.8 97.8 97.7	66.0 66.0 66.0	97.8 97.7 97.7	67.484 67.519 67.553	1.4810	0.170560	12.019
45	97.8 97.7 97.9	64.1 64.0 64.2	97.8 97.5 98.0	65.541 65.573 65.543	1.5255	0.183310	12.422

Specific Rate Constant = 1.34 x 10^{-3} moles^{-1} min.$^{-1}$ Liters

Rate Of Disproportionation Of Benzoic-acetic Mixed Anhydride At 100°C. Reaction Followed By Measurement Of Benzoic-acetic Mixed Anhydride

Time (min.)	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$	Concentration^{-1} (liters.mole^{-1})
0	90.0	43.0	90.0	47.778	2.0987	0.321980	7.1184
	90.2	42.7	89.9	47.418			
	89.8	42.9	89.9	47.746			
5	86.9	43.9	86.8	50.546	1.9753	0.295636	7.7527
	86.8	44.0	86.8	50.691			
	86.7	43.9	86.7	50.634			
10	87.2	45.0	87.5	51.516	1.9411	0.288052	7.9568
	87.6	45.0	87.7	51.340			
	87.3	45.1	87.2	51.690			
15	86.3	46.8	86.0	54.323	1.8295	0.262325	8.7372
	85.8	46.9	85.7	54.693			
	85.3	46.8	85.0	54.961			
20	87.6	49.1	87.9	55.954	1.7812	0.25071	9.1420
	87.0	48.9	87.0	56.206			
	87.1	49.0	87.1	56.257			
25	89.0	52.2	89.0	58.651	1.7113	0.23325	10.1767
	89.3	52.1	89.3	58.342			
	89.4	52.1	89.3	58.310			
30	88.4	52.8	88.5	59.694	1.6733	0.223578	10.2514
	88.3	53.0	88.6	59.920			
	88.6	52.9	88.7	59.672			
35	88.2	54.5	88.2	61.791	1.6198	0.209466	10.9421
	88.2	54.5	87.8	61.931			
	88.3	54.2	88.0	61.486			

Specific Rate Constant = 2.28 x 10^{-3} moles^{-1} min.$^{-1}$ Liters

Rate Of Disproportionation Of Benzoic-acetic Mixed Anhydride At 100°C. Reaction Followed By Measurement Of Benzoic-acetic Mixed Anhydride

Time (min.)	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$	Concentration^{-1} (liters.mole^{-1})
0	90.0	42.2	90.0	46.888	2.1299	0.328260	6.9822
	90.0	42.3	90.0	47.000			
	90.1	42.5	90.0	46.961			
5	90.5	45.4	90.7	50.110	1.9875	0.29831	7.6832
	90.5	45.7	90.2	50.581			
	90.2	45.4	90.5	50.249			
10	89.5	47.2	89.5	52.737	1.8948	0.277564	8.2575
	89.5	47.3	89.6	52.819			
	89.4	47.2	89.5	52.766			
15	89.9	50.7	90.2	56.022	1.7871	0.252145	9.0900
	90.1	50.3	90.2	55.795			
	90.2	50.5	90.0	56.048			
20	90.9	53.2	91.2	58.784	1.7121	0.233526	9.8148
	91.3	53.1	91.1	58.223			
	91.0	53.0	91.0	58.209			
25	90.0	54.4	89.7	60.545	1.6562	0.219112	10.4604
	89.6	54.5	89.5	60.859			
	90.0	54.0	89.9	60.033			
30	91.2	57.0	91.3	62.465	1.6006	0.204282	11.2197
	91.3	57.0	91.1	62.500			
	91.2	56.9	91.0	62.458			

Specific Rate Constant = 2.82 x 10^{-3} moles^{-1} min.$^{-1}$ Liters

Rate Of Disproportionation Of Benzoic-acetic Mixed Anhydride At 100°C. Reaction Followed By Measurement Of Benzoic-acetic Mixed Anhydride

Time	Blank	Sample	Blank	%T	Ave $\frac{I_o}{I}$	Log ave. $\frac{I_o}{I}$	Concentration^{-1}
0	92.0	43.5	91.7	47.359	2.1159	0.325499	7.0414
	91.5	43.2	91.6	47.187			
	91.4	43.2	91.5	47.238			
5	91.8	45.8	91.6	49.836	2.0032	0.301724	7.5963
	92.0	45.9	91.8	49.945			
	91.7	45.8	91.6	49.972			
10	92.0	47.4	92.0	51.521	1.9392	0.287624	7.9687
	91.0	47.0	90.6	51.790			
	92.0	47.2	91.7	51.388			
15	90.4	48.7	90.2	53.931	1.8487	0.266868	8.2485
	90.2	49.0	90.5	54.233			
	90.5	48.9	90.3	54.092			
20	91.3	50.4	91.3	55.202	1.8043	0.256312	8.9422
	91.2	50.8	91.5	55.610			
	91.3	50.6	91.2	55.452			
25	91.8	53.4	92.0	58.106	1.7205	0.235655	9.7260
	92.0	53.5	92.0	58.152			
	92.1	53.4	91.7	58.106			
30	91.2	54.8	91.2	60.087	1.6729	0.223448	10.2574
	91.1	54.3	91.4	59.505			
	91.3	54.5	91.2	59.726			
35	92.0	57.1	92.3	61.964	1.6160	0.208440	10.9959
	92.4	57.0	92.0	61.822			
	92.1	57.0	92.2	61.855			
60	91.5	60.5	91.8	66.012	1.5176	0.181058	12.6589
	91.8	60.4	91.8	65.795			
	91.9	60.5	91.8	65.868			

Specific Rate Constant = 2.16 x 10^{-3} moles^{-1} min.$^{-1}$ Liters

Rate Of Disproportionation Of Benzoic-acetic Mixed Anhydride At 100°C. Reaction Followed By Measurement Of Benzoic-acetic Mixed Anhydride

Time	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log ave. $\frac{Io}{I}$	Concentration^{-1}
0	85.0	39.7	85.0	46.705	2.1422	0.33086	6.9274
	85.2	39.8	85.3	46.686			
	85.1	39.7	85.1	46.650			
5	83.7	41.7	83.7	49.820	2.0123	0.303693	7.5470
	84.0	41.7	84.0	49.642			
	84.3	41.8	84.2	49.614			
10	84.3	44.6	84.6	52.812	1.8942	0.277416	8.2619
	85.0	44.8	84.9	52.736			
	85.1	44.9	84.9	52.823			
15	85.6	46.2	85.5	54.000	1.8362	0.263918	8.6945
	83.5	45.6	83.5	54.610			
	83.5	45.7	83.4	54.763			
20	85.7	48.5	85.7	56.592	1.7696	0.247874	9.2466
	85.6	48.4	85.8	56.476			
	85.6	48.3	85.5	56.458			
25	85.6	50.2	85.6	58.644	1.7044	0.231570	9.8976
	85.8	50.4	85.9	58.707			
	85.8	50.3	85.7	58.658			
30	86.0	52.1	86.2	60.511	1.6460	0.216430	10.5900
	86.0	52.3	86.1	60.778			
	85.9	52.4	86.0	60.965			
45	83.0	53.2	83.2	65.222	1.5331	0.185569	12.3512
	83.3	53.3	83.4	65.146			
	83.4	53.4	83.2	65.306			
120	90.8	60.0	90.5	67.217	1.4867	0.172203	13.3098
	92.0	60.1	92.4	67.353			
	92.3	60.0	92.2	67.208			

Specific Rate Constant = 2.44×10^{-3} moles^{-1} min.$^{-1}$ Liters

Effect Of Added Substances On The Rate Of Disproportionation Of Benzoic-acetic Mixed Anhydride. Reaction Followed By Measurement Of Benzoic-acetic Mixed Anhydride At 100°C

Substance Added	Concentration Moles Liters^{-1}	Time min.	Blank	Sample	Blank	Ave. $\frac{Io}{I}$	Concentration^{-1}
Benzoic Acid	0.20	0	90.1 90.7 91.0	42.8 43.1 43.3	90.2 90.8 91.1	2.1049	7.0697
"	0.20	5	89.8 89.6 90.0	45.7 45.6 45.8	89.7 89.9 89.8	1.9650	7.8129
"	0.20	10	89.3 89.6 89.3	48.6 48.7 48.6	89.4 89.6 89.5	1.8392	8.6614
"	0.20	15	90.0 90.0 89.8	51.3 51.2 51.1	89.9 90.0 89.6	1.7555	9.3784
"	0.20	20	89.7 90.0 89.7	53.0 53.2 53.1	89.6 89.9 89.6	1.6896	10.063

Specific Rate Constant = 3.250 x 10^{-3} min.$^{-1}$ moles^{-1} Liters

Substance Added	Concentration Moles Liters^{-1}	Time min.	Blank	Sample	Blank	Ave. $\frac{Io}{I}$	Concentration^{-1}
Sodium Acetate	0.1 gms Suspension	0	90.2 90.7 90.9	42.9 43.2 43.1	90.1 90.7 90.8	2.1029	7.1001
"	"	5	90.4 90.6 90.8	47.0 47.1 47.2	90.5 90.5 91.0	1.9367	7.9844
"	"	10	90.5 90.4 90.4	50.2 50.1 50.1	90.4 90.4 90.3	1.8031	8.9522
"	"	15	90.6 90.8 91.0	52.7 52.8 52.9	90.7 90.9 91.1	1.7206	9.7246
"	"	20	91.2 91.4 91.7	56.0 56.1 56.3	91.3 91.5 91.8	1.6297	10.8067

Specific Rate Constant = 3.64 moles^{-1} min.$^{-1}$ Liters

Substance Added	Concentration Moles Liters^{-1}	Time min.	Blank	Sample	Blank	Ave. $\frac{Io}{I}$	Concentration^{-1}
Powdered Glass	Suspension 0.10 gm	0	92.1 91.4 91.4	43.6 43.3 43.1	91.8 91.7 91.6	2.1154	7.0437
"	"	5	83.7 84.1 84.3	41.8 41.6 41.9	83.9 84.0 84.2	2.0119	7.5494
"	"	10	87.1 87.7 87.3	45.1 44.9 45.1	87.4 87.6 87.3	1.9403	7.9619
"	"	15	85.4 83.6 83.6	46.3 45.7 45.7	85.3 83.6 83.7	1.8422	8.6379
"	"	20	87.8 87.2 87.5	49.2 48.8 48.7	88.0 87.4 87.3	1.7899	9.0653

Specific Rate Constant = 2.010 x 10^{-3} moles^{-1} min.$^{-1}$ Liters

___	___	0	85.1 85.3 85.2	39.7 39.9 39.6	85.1 85.3 85.0	2.1434	6.9223
___	___	5	85.2 85.3 85.6	42.6 42.8 42.9	85.0 85.5 85.7	1.9965	7.5693
___	___	10	84.4 85.3 85.2	44.7 44.5 44.5	84.8 85.1 85.0	1.9065	8.1788
___	___	15	86.3 86.8 87.2	46.4 46.6 46.9	86.5 87.0 87.4	1.8627	8.4844
___	___	20	85.8 85.9 85.7	48.7 48.6 48.7	85.0 85.5 85.6	1.7627	9.3103

Specific Rate Constant = 2.44 x 10^{-3} moles^{-1} min.$^{-1}$ Liters

Substance Added	Concentration Moles Liters^{-1}	Time min.	Blank	Sample	Blank	Ave. $\frac{Io}{I}$	$\text{Concentration}^{-1}$
Acetic Acid	0.2	0	92.1 91.6 91.4	43.6 43.1 43.2	91.7 91.5 92.5	2.1117	7.0601
"	0.2	5	93.2 93.3 93.4	47.6 47.7 47.8	93.1 93.4 93.7	1.9570	7.8722
"	0.2	10	93.9 94.1 94.0	51.4 51.6 51.5	93.7 94.3 94.4	1.8265	8.7607
"	0.2	15	93.6 93.8 93.5	53.3 53.3 53.2	93.9 93.5 93.7	1.7590	9.3448
"	0.2	20	93.6 93.5 93.3	55.8 55.8 55.7	93.4 93.4 93.5	1.6757	10.223

Specific Rate Constant = 3.0666 x 10^{-3} moles^{-1} min.^{-1} Liters

Substance Added	Concentration Moles Liters^{-1}	Time min.	Blank	Sample	Blank	Ave. $\frac{Io}{I}$	$\text{Concentration}^{-1}$
Acetic Acid	0.3	0	90.0 90.1 90.2	42.7 42.7 42.8	90.3 90.2 90.2	2.1096	7.0931
"	0.3	5	89.7 89.6 89.8	45.9 46.0 46.1	89.5 89.8 89.9	1.9503	7.8994
"	0.3	10	89.9 89.8 90.1	49.3 49.4 49.6	90.2 90.0 90.3	1.8216	8.8002
"	0.3	15	90.8 90.6 90.2	52.1 52.0 51.7	90.7 90.8 89.9	1.7426	9.5107
"	0.3	20	91.2 91.0 91.3	54.9 54.7 55.0	91.1 90.8 91.5	1.6591	10.424

Specific Rate Constant = 3.33 x 10^{-3} moles^{-1} min.^{-1} Liters

Summary Of Data For N-Acyl Para-Toluidine Systems

A. Principal Wave lengths

1. N-(acetyl)-p-toluidine --- 7.269 microns
2. N-(benzoyl)-p-toluidine --- 6.676 microns
3. N-(p-methoxybenzoyl)-p-toluidine --- 8.534 microns
4. N-(p-nitrobenzoyl)-p-toluidine --- 7.393 microns

B. Beer's Law Curves

1. N-(acetyl)-p-toluidine
    - a. At N-acetyl wave length

      $\text{Log}\ \frac{I_o}{I} = 1.271\ (\text{N-acetyl}) + 0.000$
    - b. At N-benzoyl wave length

      $\text{Log}\ \frac{I_o}{I} = 1.211\ (\text{N-acetyl}) + 0.00025$
    - c. At N-(p-methoxybenzoyl) wave length

      $\text{Log}\ \frac{I_o}{I} = 0.0858\ (\text{N-acetyl}) + 0.000$
    - d. At N-(p-nitrobenzoyl) wave length

      $\text{Log}\ \frac{I_o}{I} = 0.3150\ (\text{N-acetyl}) + 0.000$
2. N-(benzoyl)-p-toluidine
    - a. At N-acetyl wave length

      $\text{Log}\ \frac{I_o}{I} = 0.196\ (\text{N-benzoyl}) + 0.000$
    - b. At N-benzoyl wave length

      $\text{Log}\ \frac{I_o}{I} = 1.814\ (\text{N-benzoyl}) + 0.008$
3. N-(p-methoxybenzoyl)-p-toluidine
    - a. At N-acetyl wave length

      $\text{Log}\ \frac{I_o}{I} = 1.184(\text{N-p-Methoxybenzoyl}) + 0.000$
    - b. At N-(p-methoxybenzoyl)-p-toluidine wave length

      $\text{Log}\ \frac{I_o}{I} = 10.810\ (\text{N-p-methoxybenzoyl}) + 0.000$

4. N-(*p*-nitrobenzoyl) *p*-toluidine

   a. At N-acetyl wave length

      Log $\frac{Io}{I}$ = 0.7442 (N-*p*-nitrobenzoyl) + 0.000

   b. At N-*p*-nitrobenzoyl *p*-toluidine

      Log $\frac{Io}{I}$ = 6.560 (N-*p*-nitrobenzoyl) + 0.000

Simultaneous Equations Derived From Above Data

A. For the benzoic-acetic mixed anhydride

at 7.269 microns; 0.196 (benzoyl) + 1.271 (acetyl) = Log $\frac{Io}{I}$

at 6.676 microns; 1.814 (benzoyl) + 1.211 (acetyl) = Log $\frac{Io}{I}$ - 0.0083

B. For *p*-methoxybenzoic-acetic mixed anhydride

at 7.269 microns; 1.184 (methoxybenzoyl) + 1.271 (acetyl) = Log $\frac{Io}{I}$

at 8.534 microns; 10.81 (methoxybenzoyl) + 0.0858 (acetyl) = Log $\frac{Io}{I}$

C. For *p*-nitrobenzoic-acetic mixed anhydride

at 7.269 microns; 0.7442 (nitrobenzoyl) + 1.271 (acetyl) = Log $\frac{Io}{I}$

at 7.393 microns; 6.565 (nitrobenzoyl) + 0.3150 (acetyl) = Log $\frac{Io}{I}$

Beer's Law Curve For N-Benzoyl p-toluidine At N-Acetyl-p-toluidine Wave Length 7.269 Microns In Chloroform

Concentration	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
0.1908	98.2	90.1	98.1	91.798	1.0895	0.03720
	98.0	90.0	98.2	91.743		
	98.3	90.2	98.2	91.806		
0.1526	98.5	91.8	98.4	93.245	1.0712	0.029870
	98.3	91.7	98.2	93.333		
	98.2	91.7	98.1	93.428		
0.1145	98.5	93.5	98.4	94.972	1.0524	0.022184
	98.2	93.5	98.4	95.116		
	98.3	93.4	98.4	94.966		
0.07632	98.5	95.2	98.5	96.649	1.0337	0.014394
	98.5	95.3	98.4	96.800		
	98.4	95.2	98.4	96.747		

$$\text{Log } \frac{Io}{I} = 0.1960 \text{ (N-Benzoyl tolu)} + 0.000$$

Beer's Law Curve For N-Benzoyl-*p*-toluidine At Its Peak 6.676 Microns In Chloroform

Concentration	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
0.1908	98.2 98.1 98.2	43.7 43.6 43.7	98.2 98.3 98.3	44.501 44.399 44.478	2.2492	0.352028
0.1526	98.2 98.1 98.1	51.0 51.1 51.0	98.0 98.2 98.0	51.987 52.063 52.014	1.9219	0.283728
0.1145	98.6 98.5 98.7	60.4 60.5 60.6	98.5 98.4 98.8	61.288 61.452 61.367	1.6294	0.212028
0.7632	97.0 97.0 96.9	67.8 67.6 67.7	97.1 96.9 96.9	69.860 69.726 69.865	1.4323	0.156033
0.5724	98.3 98.3 98.4	76.0 76.0 75.9	98.4 98.5 98.5	77.275 77.235 77.094	1.2953	0.112372
0.3816	98.6 98.6 98.5	82.5 82.4 82.5	98.7 98.5 98.6	83.628 83.612 83.713	1.1954	0.077514
0.1908	98.6 98.5 98.5	89.6 89.6 89.7	98.5 98.5 98.4	90.826 90.965 91.112	1.0992	0.041078

$$\text{Log } \frac{Io}{I} = 1.814 \text{ (Benzoyl tolu)} + 0.008$$

Beer's Law Curve For N-Acetyl-p-toluidine At N-(p-Nitrobenzoyl)-p-toluidine Wave Length 7.393 Microns

Concentration	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
0.2121	97.8	84.0	97.8	85.889	1.1623	0.065328
	97.8	84.1	97.6	86.079		
	97.7	84.2	97.8	86.138		
0.1697	97.7	86.4	97.8	88.388	1.1320	0.053850
	97.8	86.3	97.7	88.286		
	97.8	86.4	97.8	88.343		
0.1273	97.7	90.1	97.6	92.268	1.0834	0.034790
	97.6	90.0	97.5	92.260		
	97.5	90.0	97.4	92.355		
0.08495	97.7	92.2	97.6	94.418	1.0588	0.024818
	97.5	92.1	97.6	94.413		
	97.7	92.2	97.7	94.370		

$$\text{Log } \frac{Io}{I} = 0.3150 \text{ (Acetyl tolu)} + 0.000$$

Beer's Law Curve For N-(acetyl)-p-toluidine At N-(Benzoyl)-p-toluidine Peak 6.677 Microns In Chloroform

Concentration	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
0.2176	98.2	64.2	98.1	65.410	1.5330	0.18554
	98.1	63.8	98.1	65.035		
	98.2	64.0	98.0	65.239		
0.17408	98.2	70.5	98.2	71.792	1.3944	0.144384
	98.3	70.3	98.0	71.625		
	98.2	70.4	98.1	71.726		
0.13056	98.2	76.0	98.0	77.471	1.2872	0.109648
	98.0	76.4	98.1	77.919		
	98.1	76.2	98.1	77.675		
0.08704	98.0	83.0	98.1	84.650	1.1826	0.072836
	98.2	82.9	98.2	84.419		
	98.0	83.0	98.2	84.607		
0.04352	98.3	89.9	98.4	91.408	1.0939	0.038980
	98.3	89.8	98.2	91.399		
	98.5	90.0	98.4	91.416		
0.02176	98.3	98.9	98.2	95.572	1.0469	0.019908
	98.0	93.7	98.1	95.563		
	98.2	93.8	98.4	95.422		

$$\text{Log } \frac{Io}{I} = 1.211 \text{ (Acetyl tolu)} + 0.00025$$

Beer's Law Curve For N-Acetyl-p-toluidine At Its Wave Length 7.269 Microns In Chloroform

Concentration moles Liters^{-1}	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
0.2677	97.6	44.3	97.5	45.412	2.1931	0.341060
	97.8	44.7	98.0	45.658		
	97.6	44.6	97.5	45.720		
0.2176	98.1	53.0	98.0	54.054	1.8491	0.266964
	98.1	53.0	98.1	54.026		
	98.1	53.1	98.0	54.156		
0.1741	97.7	59.0	98.0	60.296	1.6589	0.219823
	98.0	59.2	98.1	60.377		
	98.1	59.1	98.1	60.244		
0.1306	98.1	67.6	98.2	68.874	1.4640	0.16554
	98.2	67.6	98.3	68.804		
	98.4	67.6	98.3	68.734		
0.0870	97.9	76.3	98.0	77.896	1.2841	0.108603
	98.0	76.2	97.9	77.794		
	97.9	76.3	97.9	77.936		
0.0435	98.0	86.4	98.0	88.163	1.1345	0.050860
	98.0	86.5	98.1	88.220		
	98.2	86.4	98.1	88.028		
0.02176	97.9	92.2	97.9	94.177	1.0622	0.026202
	98.0	92.0	97.7	94.069		
	97.7	92.1	97.9	94.171		

$$\text{Log}\ \frac{Io}{I} = 1.271\ (\text{Acetyl tolu}) + 0.000$$

Beer's Law Curve For N-Acetyl-*p*-toluidine At (*p*-Methoxybenzoyl)-*p*-toluidine Wave Length 8.534 Microns In Chloroform

Concentration moles liters^{-1}	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
0.262	95.4	90.5	95.4	94.863	1.0545	0.023045
	95.8	90.8	95.6	94.879		
	95.2	90.1	95.0	94.742		
0.2096	96.4	92.3	96.3	95.746	1.0431	0.018322
	96.2	92.2	96.1	95.891		
	96.1	92.3	96.3	95.945		
0.1834	95.8	92.7	96.0	96.663	1.0343	0.014646
	95.9	92.6	95.8	96.609		
	95.7	92.7	95.9	96.764		
0.1332	96.2	93.9	96.3	97.558	1.0254	0.010892
	96.1	93.6	96.0	97.449		
	96.4	94.0	96.3	97.560		

$$\text{Log}\ \frac{Io}{I} = 0.0858\ (\text{Acetyl tolu}) + 0.000$$

Beer's Law Curve For N-(p-methoxybenzoyl)-p-toluidine At N-acetyl-p-toluidine 7.269 Microns In Chloroform

Concentration	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
0.0692	97.1	80.2	97.0	82.637	1.2101	0.082825
	96.8	79.9	96.6	82.626		
	96.7	80.0	96.9	82.644		
0.0554	96.8	83.5	97.0	86.171	1.1610	0.06483
	97.2	83.8	97.4	86.125		
	97.4	83.9	97.5	86.095		
0.0415	96.9	86.5	97.1	89.129	1.1216	0.049828
	97.0	86.6	97.2	89.186		
	96.9	86.3	96.7	89.152		
0.0277	95.6	88.6	95.7	92.629	1.0793	0.03314
	95.8	88.7	95.7	92.637		
	95.6	88.5	95.4	92.670		
0.0138	95.8	92.2	95.7	96.292	1.0386	0.016452
	95.8	92.3	95.9	96.296		
	96.0	92.5	96.2	96.253		

Log $\frac{Io}{I}$ = 1.184 (p-MeO tolu) + 0.000

Beer's Law Curve For N-(p-Methoxybenzoyl)-p-toluidine At Its Wave Length 8.534 Microns

Concentration	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
0.02995	97.5	46.8	97.6	47.975	2.0881	0.319752
	97.7	46.7	97.6	47.823		
	97.6	46.6	97.5	47.770		
0.02395	98.4	55.2	98.5	56.069	1.7837	0.251318
	98.3	55.1	98.4	56.024		
	98.4	55.2	98.4	56.097		
0.01796	98.4	63.2	98.4	64.227	1.5543	0.191628
	98.3	63.2	98.2	64.325		
	98.4	63.1	98.4	64.126		
0.01198	98.4	73.1	98.4	74.288	1.3452	0.128786
	98.4	73.2	98.4	74.390		
	98.3	73.1	98.4	74.326		
0.00598	98.2	85.4	98.3	86.921	1.1524	0.061502
	98.3	85.3	98.4	86.731		
	98.3	85.2	98.3	86.673		

Log $\frac{Io}{I}$ 10.81 (pMeOtolu) + 0.000

Beer's Law Curve For N-(p-Nitrobenzoyl)-p-toluidine At N-Acetyl-p-toluidine Wave Length 7.269 Microns In Chloroform

Concentration moles Liters^{-1}	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
0.0940	96.8	82.3	96.9	84.977	1.1757	0.07030
	96.2	82.0	96.4	85.150		
	96.3	81.9	96.1	85.135		
0.0752	95.8	84.1	95.9	87.741	1.1386	0.056368
	96.1	84.4	96.0	87.870		
	96.4	84.6	96.2	87.850		
0.0564	95.9	86.9	95.8	90.662	1.1032	0.042658
	95.7	86.9	95.9	90.709		
	95.8	86.8	95.7	90.652		
0.0376	96.2	90.2	96.1	93.811	1.0657	0.027596
	96.3	90.3	96.2	93.818		
	96.2	90.4	96.4	93.873		
0.01880	95.9	92.9	95.8	96.922	1.0329	0.014058
	95.8	92.7	95.7	96.814		
	95.6	92.5	95.7	96.706		

$$\text{Log } \frac{Io}{I} = 0.7442 \text{ (p-Nitro tolu)} + 0.000$$

Beer's Law Curve For N(p-Nitrobenzoyl)-p-toluidine At Its Wave Length 7.393 Microns In Chloroform

Concentration	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
0.03484	97.8	58.8	97.9	60.091	1.6656	0.221666
	97.9	58.7	97.9	59.959		
	97.8	58.8	98.0	60.061		
0.02788	97.8	64.4	97.8	65.848	1.5170	0.18099
	97.7	64.4	97.7	65.916		
	97.8	64.5	97.7	65.984		
0.02093	97.6	70.9	97.7	72.606	1.3776	0.139116
	97.6	70.9	97.6	72.643		
	97.5	70.9	97.5	72.717		
0.01394	97.6	79.0	97.6	80.942	1.2347	0.091565
	97.6	79.0	97.6	80.942		
	97.5	79.1	97.6	81.086		
0.006974	97.5	87.7	97.6	89.810	1.1134	0.046656
	97.6	87.7	97.6	89.856		
	97.6	87.6	97.6	89.754		

$$\text{Log } \frac{Io}{I} = 6.560 \text{ (p-Nitro tolu)} + 0.000$$

Cleavage Of Benzoic-acetic Mixed Anhydride With p-Toluidine

Reaction Medium	Wave Length	Blank	Sample	Blank	%T	Ave. $\frac{I_o}{I}$	Log $\frac{I_o}{I}$
Benzene	7.269	93.4	26.2	93.5	28.036	3.5558	0.550936
		93.5	26.3	93.6	28.113		
		93.6	26.4	93.4	28.220		
	6.676	93.3	26.4	93.2	28.289	3.5324	0.548068
		93.3	26.5	93.3	28.403		
		93.2	26.3	93.1	28.234		

Melting point of mixture = 149-150°C

Concentration N-acetyl = 0.431956

Concentration N-benzoyl = 0.0098277

Acetylation ratio = 44.0

50 w/w Acetone-Benzene	7.269	93.5	50.0	93.5	53.475	1.8722	0.272356
		93.2	49.8	93.3	53.404		
		93.1	49.7	93.2	53.354		
	6.676	93.7	50.2	94.0	53.489	1.8718	0.272262
		94.0	50.3	94.1	53.482		
		93.8	50.1	94.0	53.354		

Melting point of mixture = 148.6-150°C

Concentration N-acetyl = 0.213414

Concentration N-benzoyl = 0.005665

Acetylation ratio = 37.7

Reaction Medium	Wave Length	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
Acetone	7.269	94.5	36.4	94.4	38.538	2.5964	0.414368
		94.0	36.2	94.2	38.469		
		94.1	36.3	94.3	38.535		
	6.676	95.3	36.1	95.2	37.900	2.6358	0.420916
		95.1	36.1	95.0	37.980		
		95.0	36.0	94.8	37.934		

Melting point of mixture = 148.4-150°C

Concentration N-acetyl = 0.32425

Concentration N-benzoyl = 0.0109437

Acetylation ratio = 29.6

50 w/w Acetone-Water	7.269	94.5	29.9	94.7	31.606	3.1606	0.499768
		94.6	29.8	94.3	31.551		
		94.5	30.0	94.7	31.712		
	6.676	94.3	29.5	94.2	31.299	3.1949	0.504456
		94.1	29.4	94.2	31.226		
		94.3	29.6	94.4	31.372		

Melting point of mixture = 149-150°C

Concentration N-acetyl = 0.49747

Concentration N-benzoyl = 0.0117124

Acetylation ratio = 42.5

Cleavage Of p-Methoxybenzoic-acetic Mixed Anhydride With p-Toluidine

Reaction Medium	Wave Length	Blank	Sample	Blank	%T	Ave $\frac{Io}{I}$	Log $\frac{Io}{I}$
Bezene	7.269	97.8	64.9	97.9	66.326	1.5095	0.178835
		97.9	64.8	98.1	66.088		
		98.0	65.0	98.0	66.326		
	8.534	97.8	92.0	97.8	94.069	1.0634	0.026694
		97.8	91.9	97.7	94.015		
		97.8	92.0	97.9	94.021		

Melting point of mixture = 143°C-144°C

Concentration N-acetyl = 0.139427

Concentration N-p-methoxybenzoyl = 0.00136202

Acetylation ratio = 102

Reaction Medium	Wave Length	Blank	Sample	Blank	%T	Ave $\frac{Io}{I}$	Log $\frac{Io}{I}$
50 w/w Acetone-	7.269	97.0	45.6	97.2	46.961	2.1297	0.328320
		97.1	45.7	97.3	47.016		
		97.2	45.5	96.9	46.883		
	8.534	96.8	88.0	97.0	90.815	1.1009	0.041750
		97.1	88.3	97.3	90.843		
		97.2	88.2	97.0	90.834		

Melting point of mixture = 143.5°C=144.6°C

Concentration N-acetyl = 0.256616

Concentration N-p-methoxybenzoyl = 0.00182417

Acetylation ratio = 140

Reaction Medium	Wave Length	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
Acetone	7.269	98.9	54.6	98.7	55.263	1.8089	0.257396
		98.6	54.5	98.6	55.273		
		98.9	54.7	98.9	55.308		
	8.534	98.9	91.1	98.8	92.159	1.0846	0.035270
		98.9	91.2	98.9	92.214		
		98.7	91.1	98.9	92.206		

Melting point of mixture = 143°C-144°C

Concentration N-acetyl = 0.200965

Concentration N-p-methoxybenzoyl = 0.0016676

Acetylation ratio = 120

50 w/w Acetone-Water	7.269	97.6	35.7	97.9	36.521	2.7379	0.437414
		97.9	35.8	98.0	36.549		
		98.0	35.8	98.1	36.511		
	8.534	98.2	83.8	98.1	85.379	1.1709	0.068523
		98.3	83.8	98.1	85.492		
		98.5	84.0	98.4	85.322		

Melting point of mixture = 144°C=145°C

Concentration N-acetyl = 0.340763

Concentration N-p-methoxybenzoyl = 0.00363418

Acetylation ratio = 93.7

Cleavage Of p-Nitrobenzoic-acetic Mixed Anhydride With p-toluidine

Reaction Medium	Wave Length	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
Benzene	7.269	95.3	61.6	95.6	64.536	1.5487	0.189966
		95.6	61.7	95.4	64.607		
		95.3	61.5	95.2	64.566		
	7.393	95.8	44.1	95.9	46.009	2.1724	0.336940
		95.9	44.2	96.0	46.065		
		96.1	44.2	96.0	46.017		

Melting point of mixture = 125-131°C

Concentration N-acetyl = 0.12286

Concentration N-p-nitrobenzoyl = 0.045428

Acetylation ratio = 2.70

Reaction Medium	Wave Length	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
50 w/w Acetone-	7.269	94.8	47.0	95.0	49.525	2.0212	0.305612
		94.9	47.0	94.8	49.551		
		94.7	46.8	94.6	49.445		
	7.394	94.5	40.5	94.4	42.879	2.3470	0.37051
		94.6	40.5	94.6	42.811		
		94.6	40.4	94.5	42.728		

Melting point of mixture = 126-132°C

Concentration N-acetyl = 0.213477

Concentration N-p-nitrobenzoyl = 0.046194

Acetylation ratio = 4.66

Reaction Medium	Wave Length	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
Acetone	7.269	93.4	39.4	93.3	42.206	2.3718	0.375020
		93.1	39.2	93.0	42.127		
		92.9	39.2	93.1	42.150		
	7.394	93.2	26.2	93.1	28.126	3.5644	0.551988
		93.0	26.2	93.4	28.111		
		93.5	26.1	93.4	27.929		

Melting point of mixture = 126.5-131°C

Concentration N-acetyl = 0.25308

Concentration N-p-nitrobenzoyl = 0.071937

Acetylation ratio = 3.52

Reaction Medium	Wave Length	Blank	Sample	Blank	%T	Ave. $\frac{Io}{I}$	Log $\frac{Io}{I}$
50 w/w Acetone-	7.269	92.8	48.0	93.0	51.668	1.9341	0.286482
		93.0	48.1	93.1	51.692		
		93.1	48.2	93.2	51.744		
	7.394	92.9	33.5	93.0	36.040	2.7768	0.443548
		93.1	33.5	93.2	35.963		
		93.2	33.6	93.3	36.032		

Melting point of mixture = 124.8-133.1°C

Concentration N-acetyl = 0.19121

Concentration N-p-nitrobenzoyl = 0.058387

Acetylation ratio = 3.28

Apparent First Order Rate Constants For The Hydrolysis Of Para Substituted Benzoic-acetic Mixed Anhydrides

A. p-Methoxybenzoic-acetic Mixed Anhydride

x_∞ = 9.630 milliliters

t(min)	$x_\infty - x_t$	t(min)	$x_\infty - x_t$
19.0	5.09	234.5	3.40
39.2	4.59	255.0	3.25
59.0	4.06	295.0	3.08
79.0	4.03	324.0	2.95
99.0	4.10	360.0	2.89
119.0	3.97	395.0	2.72
159.0	3.83	455.0	3.09
179.0	3.83	485.0	2.40
202.5	3.56	515.0	2.21

Corrected first order rate constant = 1.41 x 10^{-5} Sec.$^{-1}$

B. p-Nitrobenzoic-acetic Mixed Anhydride

x_∞ = 22.35 Milliliters

t(min)	$x_\infty - x_t$	t(min)	$x_\infty - x_t$
1	10.77	15	9.37
2	10.35	18	9.15
3	10.33	21	9.00
4	10.25	24	8.82
6	9.93	28	8.77
8	9.83	32	8.26
10	9.73	37	8.04
12	9.53	43	7.74
		51	7.33

Corrected first order rate constant = 23.7 x 10^{-5} Sec.$^{-1}$

C. Benzoic-acetic Mixed Anhydride

x_∞ = 14.885 milliliters

t(min)	$x_\infty - x_t$	t(min)	$x_\infty - x_t$
1.2	7.311	32.0	6.525
4.0	7.204	36.0	6.406
8.5	7.085	39.0	6.335
12.5	6.985	43.0	6.262
16.0	6.898	47.5	6.205
20.0	6.845	54.0	5.035
24.0	6.715	60.0	5.935
28.0	6.655	70.0	5.765
		80.0	5.527

Corrected first order rate constant = 3.77×10^{-5} Sec.$^{-1}$

PART II. STUDIES OF THE ALPHA-CHYMOTRYPSIN CATALYZED HYDROLYSIS OF SPECIFIC SUBSTRATES

THE KINETICS OF THE α CHYMOTRYPSIN CATALYZED HYDROLYSIS OF THREE N-ACYL-L-TYROSINHYDRAZIDES

It has been known for a long time that the enzyme α chymotrypsin can catalyze the hydrolysis of certain acylated and unacylated amino acid esters, amides and hydrazides.

The work which is reported here is concerned with the kinetics of the hydrolysis of three N-acyl-L-tyrosinhydrazides; the N-trimethylacetyl, the N-dichloroacetyl and the N-formyl-L-tyrosinhydrazides. The results are shown in Table 1.

Table 1

The Kinetic Constants for Three N-Acyl-L-tyrosinhydrazides

	k_3(min.$^{-1}$)x10^{-3}	K_sx10^{-3}
N-Formyl-L-tyrosinhydrazide	0.068	11.8
N-Trimethylacetyl-L-tyrosinhydrazide	0.30	29.2
N-Dichloroacetyl-L-tyrosinhydrazide	0.53	3.84

At the time that this work was undertaken it was believed that the course of the hydrolysis of the N-acyl-L-tyrosinhydrazides could be predicted upon the basis of the classical Michaelis-Menten formulation. However, it has subsequently been shown by other investigators in these laboratories that this belief may not be valid. The values of the various kinetic constants which are reported here are calculated on the basis of the classical mechanism and must be regarded as provisional. Therefore no attempt has been made to correlate any of the results either among themselves or with respect to data determined by other investigators.

The classical approach, which was found to be valid for the hydrolysis of the amides, assumes that the enzyme and substrate react reversibly with one another to form an enzyme-substrate complex. This complex may then undergo a transformation resulting in the formation of the free enzyme and the products of hydrolysis.

$$E + S \underset{}{\overset{K_S}{\rightleftharpoons}} [ES] \xrightarrow{k_3} E + P_1 + P_2$$

$$K_S = \frac{[E][S]}{[ES]} \; ; \quad \frac{d[P]}{dt} = k_3[ES]$$

On the basis of this reaction mechanism it is possible to derive the following relationship:

$$-\frac{dS}{dt} = V_t = k_3[ES] = \frac{k_3[E][S]}{K_S + [S]} \quad \text{— A}$$

Where [S] = Substrate concentration at time t

[E] = Total enzyme concentration

[ES]= Enzyme-substrate complex concentration

V_t = Velocity at time t

By taking the reciprocal of the above equation, the following equation is obtained:

$$1/V = \frac{K_S + [S]}{k_3[E][S]} = \frac{K_S}{k_3[S]}\left(1/[S]\right) + \frac{1}{k_3[E]} \quad \text{— B}$$

When $t=0$, then $S=S_0$ and $V=V_0$
equation B becomes

$$1/V_0 = \frac{K_S}{k_3[E]}\left(1/[S_0]\right) + \frac{1}{k_3[E]} \quad \text{— C}$$

Equation C establishes a relationship which enables one to estimate the kinetic constants K_S and k_3 from a knowledge of the initial velocities at various substrate concentrations.

A complicating factor arises when the products of hydrolysis combine with the enzyme and hence prevent its further interaction with the substrate. The effect of product inhibition may be included in the basic formulation by defining the following equilibrium:

$$E + P \overset{K_P}{\rightleftharpoons} [EP]$$

$$K_P = \frac{[E][P]}{[EP]}$$

This results in the modification of equation B to include K_p:

$$1/V = \frac{K_S}{k_3[E]}\left(1/S\right) + \frac{1}{k_3[E]} + \frac{K_S}{[E][K_P]}\left(\frac{S_0 - S}{S}\right) \text{—} B$$

It is possible to neglect the effect of product inhibition if the experiment is designed so that only a small percentage of hydrolysis is followed and if it is assumed that $K_s > K_p$.

This is the basis upon which the experiments which led to the results shown in Tables 3, 5 and 7 were carried out. It is predicated upon the validity of equation B.

By integration of equation B' it is possible to derive equation D.

$$k_3 E = K_S\left(1 + S_0/K_P\right)\frac{\ln S_0/S}{t} + \frac{S_0 - S}{t}\left(1 - K_S/K_P\right) \text{—} D$$

From this relationship it is seen that a plot of $\frac{\ln S_o/S}{t}$ versus $\frac{S_o - S}{t}$ should be a straight line whose slope is a function of S_o, K_p, and K_s. For the hydrolysis of several N-acyl-L-tyrosinamides this linear result has been obtained.

W. Lands of these laboratories has recently observed the hydrolysis of N-acetyl-L-tyrosinhydrazide over a relatively large extent of reaction. When the data for this reaction were examined according to equation D a decided deviation from linearity was obtained. The data indicate that the interaction of the enzyme and product may be greater than any that has been observed so far, with the apparent K_p much greater than K_s.

The data for the hydrolysis of N-formyl-L-tyrosinhydrazide and N-trimethylacetyl-L-tyrosinhydrazides (Tables 3 and 5) do not show any influence of product interaction. This is presumably due to the small extent of reaction which was observed and the tendency of any random error in the data to be magnified when plotted according to equation D.

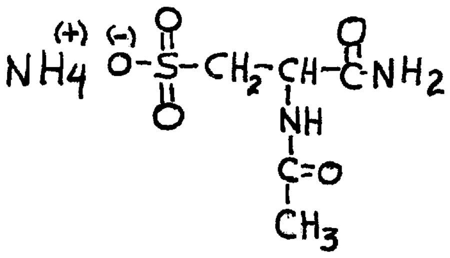
N-Dichloroacetyl-L-tyrosinhydrazide hydrolyzes at a somewhat more rapid rate. When the data for its hydrolysis were examined by equation D a classical K_p of approximately 3.8×10^{-3} was indicated (Figure 8). The effect of this apparent classical product inhibition was corrected for by application of the Lands modification of the Jennings correction (recorded as f_o in Table 7).

N-Trimethylacetyl-L-tyrosinhydrazide was found to be an extremely soluble substrate. It was possible to work with solutions which were as concentrated as 0.24F. This substrate concentration was much higher than had ever been obtained before.

The Ammonium Salt Of N-Acetyl-L-Cysteic Acid Carboxylamide As A Substrate

During his work on the structure of insulin, Sanger observed that α chymotrypsin catalyzed the hydrolysis of a peptide bond which involved the carboxyl group of cysteic acid.(1) This was the first time it was suggested that α chymotrypsin would act upon cysteic acid peptides.

It was hoped that this would prove to be a general observation and that simple cysteic acid derivatives would serve as suitable substrates. N-Acetyl-L-cysteic acid carboxylamide (I) was synthesized to serve as a model substrate.



$$\overset{(+)}{NH_4}\ \overset{(-)}{O}-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{S}}-CH_2-\underset{\substack{|\\ NH\\ |\\ C=O\\ |\\ CH_3}}{CH}-\overset{\overset{O}{\|}}{C}-NH_2 \quad \text{— (I)}$$

It was found, however, that it was not possible to demonstrate (Table 8) that any appreciable hydrolysis had occurred when α chymotrypsin was allowed to act upon (I). In like manner it was not possible to demonstrate that (I) has any appreciable ability to act as a competitive inhibitor (Table 9). Under these conditions the ammonium salt of N-acetyl-L-cysteic carboxylamide has no ability to influence the behavior of α chymotrypsin.

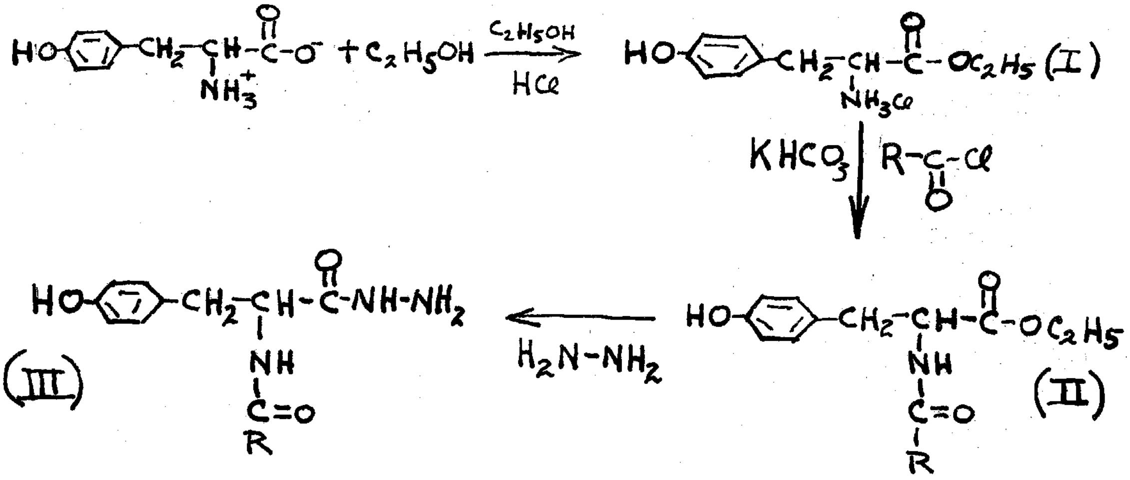
There is an accumulation of evidence to indicate that a negative charge is associated with the active site of α chymotrypsin. In view of this evidence it is not surprising that the negatively charged cysteyl ion fails to interact with the enzyme.

The cysteic acid peptide link in insulin which was hydrolyzed is a bond in which the amino group of serine is involved. It is suggested(2) that at some time, perhaps during the preliminary treatment of the insulin molecule with performic acid, an "N" to "O" shift had occurred. In this process the carboxyl group of the cysteic acid residue had migrated to the adjacent hydroxyl group converting the peptide link into an ester bond. It is well known that α chymotrypsin will catalyze the hydrolysis of a wide variety of esters.

As a corollary to this work an experiment was run to determine the effect of sulfate ion on the activity of α chymotrypsin. The results are shown in Table 10. It is evident that under these conditions sulfate ion does not affect the enzyme.

The Synthesis Of The N-Acyl-L-tyrosinhydrazides

The synthetic route to N-trimethylacetyl-L and N-dichloroacetyl-L-tyrosinhydrazides is outlined below.



L-Tyrosine Ethyl Ester (I)

One mole of L-tyrosine (Merck & Co.) was suspended in 2½ liters of absolute ethanol(3) contained in a five liter three neck round bottom flask. The center neck contained a mechanically sealed electrically driven stirrer. One of the side necks contained a drying tube filled with calcium chloride. Into the remaining neck was inserted a delivery tube for the admission of anhydrous hydrogen chloride. The whole assembly was packed in ice and maintained at 0°C.

The cold ethanolic suspension was saturated with anhydrous hydrogen chloride, the process taking 2½ hours. A clear solution was obtained and allowed to stand over night at room temperature.

The amber colored ethanolic solution was then evaporated to dryness under reduced pressure (25 mm.) on a steam bath.

The crude L-tyrosine ethyl ester hydrochloride was ground to a fine powder and suspended, with the aid of a mechanical stirrer, in one liter of dry chloroform cooled to 0°C. Two hundred ml. of a cold saturated solution of ammonia in chloroform was added over a period of 30 minutes. After an additional $\frac{1}{2}$ hour the precipitated ammonium chloride was filtered off and the chloroform evaporated to dryness under reduced pressure.

The crude L-tyrosine ethyl ester was recrystallized twice from dry ethyl acetate.

Final M.P = 108.5-109°C

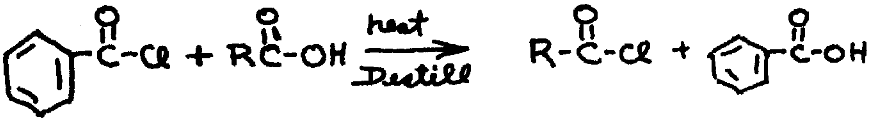
$[\alpha]_D^{25} = +20.5$ (methanol)

Yield = 69%

The material was kept in the dark and stored at 5°C until used.

Alkyl Acid Chlorides

Trimethyl, dichloro and trichloroacetyl chlorides were synthesized by the method of H. C. Brown.(4) This method is based upon the reaction of the desired acid with benzoyl chloride. The method is applicable only to acid chlorides which have a boiling point lower than benzoyl chloride.



	B.P.	Yield
Trimethylacetyl Chloride	104-105°C	85%
Dichloroacetyl Chloride	106-107.5°C	74%
Trichloroacetyl Chloride	116-118°C	53%

Acylation of L-Tyrosine Ethyl Ester

Five one-hundredths mole of L-tyrosine ethyl ester was dissolved in 300 ml. of dry ethyl acetate contained in one liter Erlenmeyer flask. The solution was cooled to -5 C in an ice salt bath. The cold solution was agitated with an emulsifying, vibrating, electrically driven stirrer. Twenty-five thousandths mole of the acid chloride was then added. After two minutes 50 ml. of 0.05 N potassium carbonate solution was added, two minutes later another 0.025 mole portion of the acid chloride was added followed two minutes later by another 50 ml. portion of potassium carbonate. The process was repeated until four samples of acid chloride and the potassium carbonate solution had been added. During the process of addition the entire system was completely emulsified.

The cold solution was allowed to separate into two phases. The ethyl acetate phase was washed with 6 N HCl and then twice with cold distilled water. The ethyl acetate solution was dried by being shaken with calcium chloride and then with anhydrous potassium carbonate.

The ethyl acetate was removed by evaporation under reduced pressure. The product that was obtained was not treated further but converted directly to the hydrazide.

	Crude Yield	Physical State
N-Trimethylacetyl-L-Tyrosine Ethyl Ester	55%	oil
N-Dichloroacetyl-L-Tyrosine Ethyl Ester	48%	gummy solid

The Conversion of the N-Acyl-L-tyrosine Ethyl Esters to the Corresponding Hydrazides.

The crude esters were dissolved in 2½ times their weight of anhydrous methanol. The solutions were heated to boiling and a 50% excess of hydrazine hydrate was quickly added. The cool mixtures were allowed to stand over night.

The volumes of the methanolic solutions were reduced and the hydrazides allowed to crystallize. The yield of the crude materials averaged 80%.

The compounds were recrystallized from methanol-water mixtures.

N-Trimethylacetyl-L-tyrosinhydrazide	N-Dichloroacetyl-L-tyrosinhydrazide
M.P. = 179-180° C	M.P. = 193-193.5° C
$[\alpha]_D^{25}$ = 29.0° (water)	$[\alpha]_D^{26}$ = +12.5° (40% ethanol)

Elementary Analysis

C	Calc	= 60.19
	Found	= 60.17
H	Calc	= 7.53
	Found	= 7.52
N	Calc	= 15.05
	Found	= 15.12

Overall yield from
tyrosine ethyl ester = 18%

Elementary Analysis

C	Calc	= 43.20
	Found	= 43.17
H	Calc	= 4.25
	Found	= 4.24
N	Calc	= 13.70
	Found	= 13.75
Cl	Calc	= 23.23
	Found	= 23.31

Overall yield from
tyrosine ethyl ester = 13%

N-Formyl-L-tyrosinhydrazide

The synthesis of N-formyl-L-tyrosinhydrazide was similar to the above sequence except that the acylation was carried out in a different manner.

Five one-hundredths mole of L-tyrosine ethyl ester was dissolved in 250 ml. of 80% formic acid and placed in a 500 ml. three neck, round bottom flask equipped with a mechanically sealed electrically driven stirrer. The flask was protected from the atmosphere by a calcium chloride drying tube. 43 ml. (0.5 mole) of acetic anhydride was slowly added to the agitated mixture in the flask. The rate of addition was such that the temperature did not rise above 70°C. One half hour was required for the addition.

The mixture was allowed to stand over night at room temperature and then concentrated to a syrup under vacuum.

The syrup was dissolved in 200 mls. of ethyl acetate and thoroughly washed with 3 N sodium hydroxide solution, then with 6 N HCl and finally with distilled water. The ethyl acetate was removed by evaporation under reduced pressure.

The syrup obtained (60% yield) was then treated with hydrazine in the manner indicated above.

N-Formyl-L-tyrosinhydrazide

M.P. = 218°C (Decomp.)

$[\alpha]_D^{25} = +21.4$ (30% ethanol)

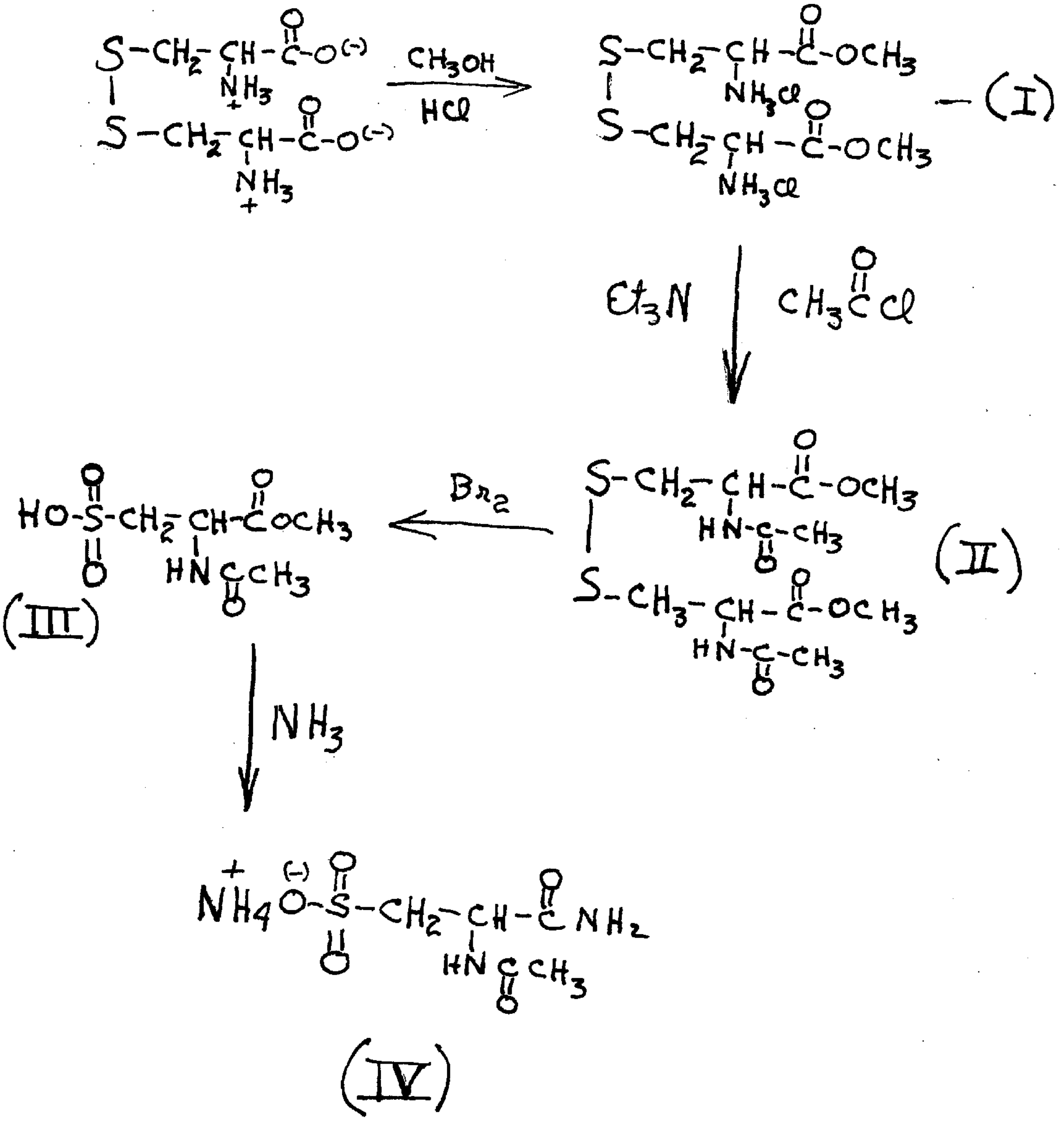
Elementary Analysis

C	Calc = 53.75	Found = 53.70
H	Calc = 5.95	Found = 6.04
N	Calc = 18.85	Found = 18.80

Over all yield from
L-Tyrosine Ethyl Ester = 15%

The Synthesis of the Ammonium Salt of N-Acetyl-L-cysteic Acid Carboxylamide

The synthetic route used to obtain this compound is outlined below.



L-Cystine Dimethyl Ester Dihydrochloride (I)

This compound was synthesized from commercially available L-cystine in a manner exactly analogous to the synthesis of L-tyrosine ethyl ester (p. 164). The product was recrystallized from methanol. Yield 89%

N,N' Diacetyl-L-cystine Dimethyl Ester (II)

The free base, cystine dimethyl ester, is an unstable syrupy substance, which slowly decomposes to free sulfur, alamine and other substances.(5) Therefore it is necessary to carry out the acetylation of this compound without first preparing the free base.

One tenth mole of the finely powdered diester dihydrochloride was suspended in two liters of dry tetrahydrofuran contained in a five liter three neck round bottom flask protected from the atmosphere by a drying tube filled with calcium chloride. The suspension was cooled to -5°C. One mole of dry redistilled triethylamine was added. The suspension was maintained for one half hour. Twenty-two hundredths mole of freshly redistilled acetyl chloride was slowly added. The mixture was allowed to stir for four hours.

The triethylamine hydrochloride was removed by filtration and the solvent removed by distillation under reduced pressure. The yield of the crude crystalline material was 71%.

N-Acetyl-L-cysteic Acid Carboxylmethyl Ester (III)

Five-hundredths mole of the diacetyl dimethyl cystine derivative was placed in a 500 ml. three neck flask, and 250 ml. of distilled water containing 60 ml. of concentrated hydrochloric acid was added. The mixture was slowly stirred with a mechanical agitator. After solution was complete, liquid bromine was slowly added. The addition was continued until the bromine color was no longer discharged. (about 0.25 mole required.)

The resulting solution was evaporated under reduced pressure. The product crystallized from the residue in a 75% yield. M.P. = 186 (With Decomposition.)

The Ammonium Salt of N-Acetyl-L-cysteic Acid Carboxylamide (IV)

Four hundredths mole of the crude N-acetyl methyl ester derivative was dissolved in one liter of dry methanol contained in a two liter single neck round bottom blask and cooled to 0°C. The solution was saturated with anhydrous ammonia and while still at 0°C the glass stopper was wired in place. The flask was placed in a heavy walled crock and allowed to stand at room temperature for five days.

The solvent was stripped off at reduced pressure and the product recrystallized from anhydrous ethanol.

M.P. = 201-202°C

$[\alpha]_D^{25} = -9.27^\circ$ (water)

Elementary Composition

	Calc	Found
C	26.39	26.35
H	5.74	5.77
N	18.50	18.50
S	14.10	13.96

Overall yield from
Amino Acid = 18%

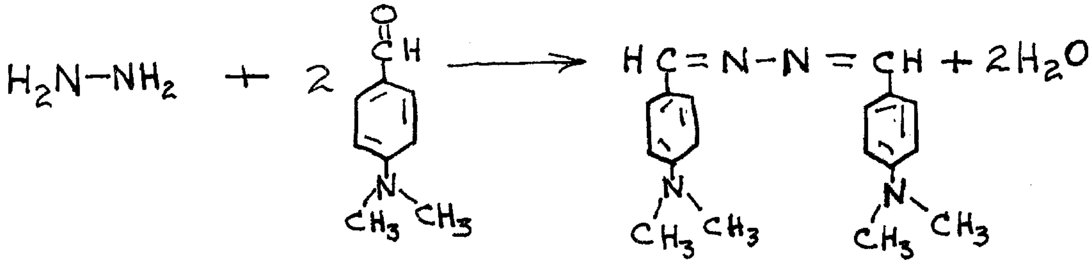
Analytical Procedure

In order to get the information necessary to calculate k_3 and K_s the following general procedure was adopted.

A buffer solution was made up by dissolving enough tris hydroxymethylaminomethane (Tham) to make a 0.2F solution. Concentrated hydrochloric acid was added drop wise to the solution, which was maintained at 25°C until the desired pH was approached. The pH of the stock buffer solution was read on a Beckman pH meter. The stock buffer solution was then diluted ten fold and its pH observed. Final adjustments were made to the stock solution until the diluted solution had the desired pH value.

All the runs were carried out at a buffer concentration of 0.02 F (except where noted) and at a pH of 7.8. The pH optimum curves (Figures 1, 3 and 5) of the three substrates seem to indicate a pH optimum which is slightly lower than this. However, since the previous work was done at 7.8 it was decided to carry out these investigations at this value also.

The reaction between hydrazine and p-dimethylamino benzaldehyde is the basis of the colorimetric procedure used to follow the course of the hydrolysis.(6)



To start the procedure of a typical run, eight ml. of the substrate solution, equilibrated to 25°C were pipetted into a 10 ml. volumetric flask. One ml. of the stock 25°C buffer solution was then added. At zero time, one ml. of 25°C enzyme solution was pipetted into the volumetric flask, the pipette allowed to drain for ten seconds, the flask stoppered and inverted 12 times and placed in a 25° ± 0.02°C constant temperature bath.

At various times aliquot samples were pipetted into a 10 ml. volumetric flask which contained one ml. of 1.60 F HCl and one ml. of a 10% solution of p-dimethylamino benzaldehyde in 100% ethanol. The solution was then diluted to the calibration mark and the optical density determined at 455 millimicrons with a Beckman model D spectrophotometer. The color samples were allowed to stand about 15 minutes at room temperature before the optical density was determined. Subsequently it was found that it was advantageous that the azine solution should be incubated at 25°C before the optical density was determined.

At the conclusion of each run the pH was checked to be certain that it had remained constant.

The enzyme concentration was one milligram of protein per ml. of solution. The enzyme sample analyzed 14.44% protein nitrogen.

Often it was noted that the enzyme solutions were quite turbid, which was presumably due to the precipitation of the enzyme from the acidic solution. This causes the plot of optical density versus time to intersect the optical density axis rather than pass through the origin (Figure 6). This necessitates that a blank correction be applied to each optical density reading. This blank correction must be obtained for each run by extrapolation of the optical density versus time curve until it intersects the optical density axis. Subsequently it was found that the turbidity could be greatly reduced by adding seven ml. of water to the 10 ml. volumetric flask which contained the standard acidified p-dimethylaminobenzaldehyde solution. In this way the enzyme solution was pipetted into a solution whose initial acid concentration was lower and precipitation did not occur.

The observed optical densities were corrected by subtraction of the blank values and recorded in Tables 3 to 10 as D_t(corr.). From these values the first order term was calculated. V_o was estimated both from a first order plot and a zero order plot. The average of the two values was used in the calculation of K_s and k_3. The final results were obtained by a "least squares" calculation on the data as expressed by equation B.

REFERENCES

(1) F. Sanger, E. O. P. Thompson - Biochem. J. 53 353 (1953)

(2) D. F. Elliott - Biochem. J. 50 542 (1952)

(3) L. F. Fieser - Experiments in Organic Chemistry - page 358 (1941) D. C. Heath and Company

(4) H. C. Brown - J.A.C.S. 60 1325 (1938)

(5) R. D. Coghill - J. Biol. Chem. 114 419-424 (1936)

(6) R. Lutwack - Ph.D. Dissertation, California Institute of Technology (1954)

Table 2

The Relative Rates Of The α Chymotrypsin Catalyzed Hydrolysis Of N-Trimethylacetyl-L-tyrosinhydrazide At Different pH Values

Time of Hydrolysis = 122 min.

So = 2.56×10^{-3} molar

Initial pH	Observed Optical Density	Final pH
6.25	0.706	6.23
6.65	1.142	6.63
7.13	1.399	7.12
7.30	1.475	7.25
7.40	1.495	7.37
7.75	1.519	7.72
8.14	1.352	8.12
8.30	1.350	8.27
8.42	1.226	8.37
7.36	1.472	7.33
7.50	1.505	7.48
7.63	1.534	7.60
7.65	1.521	7.62
7.71	1.491	7.69
7.84	1.480	7.83
8.00	1.478	7.96

Figure 1

pH Optimum Curve for

Trimethylacetyl-L-tyrosinhydrazide

Optical Density vs. pH

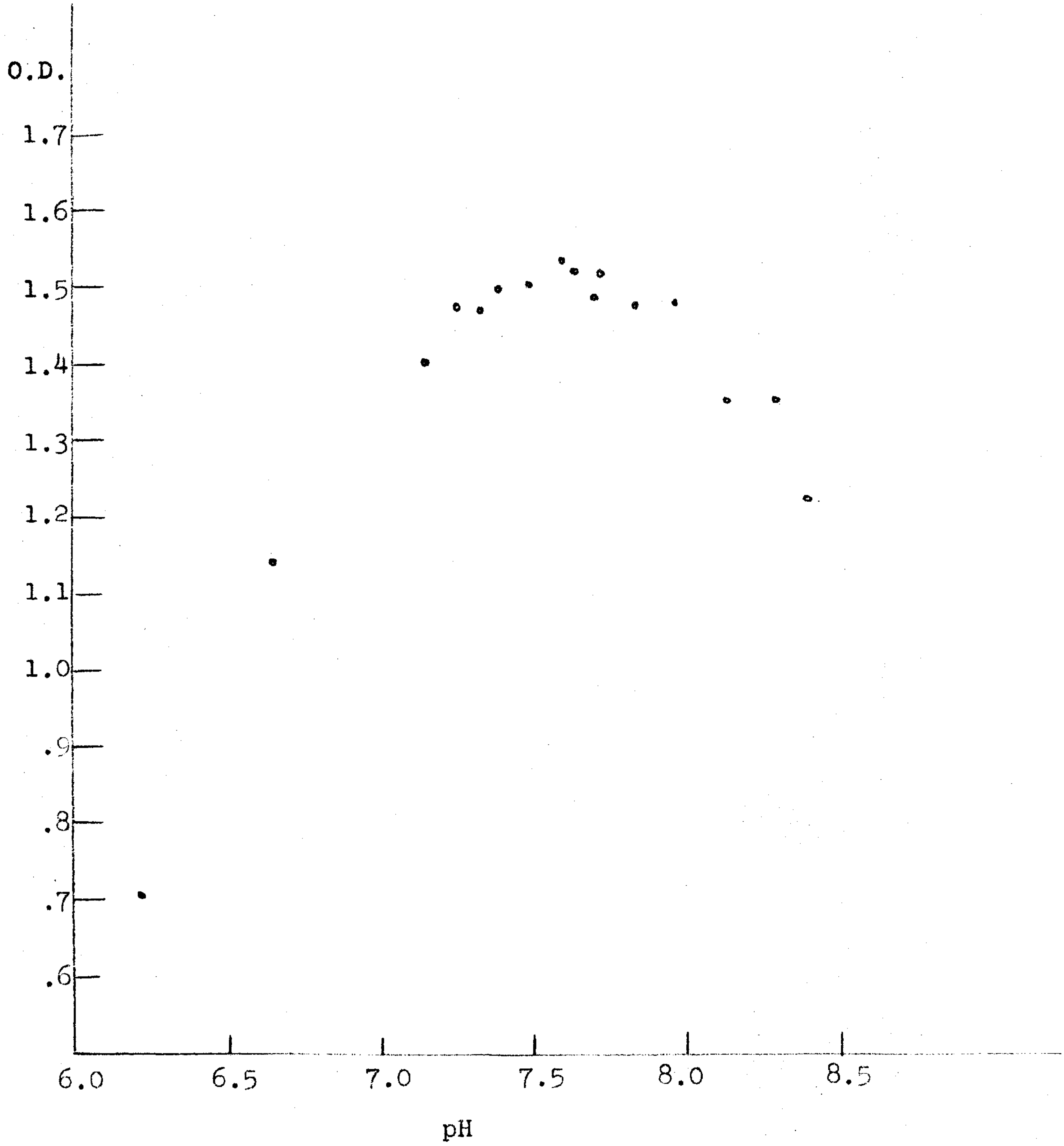


Table 3

The α Chymotrypsin Catalyzed Hydrolysis Of N-Trimethylacetyl-L-tyrosinhydrazide

Initial Substrate Concentration = 0.240 Molar

Extent of Hydrolysis = 0.48%
Blank = 1.65

Time (Min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln\left(\frac{D_\infty}{D_\infty - D_t}\right)$
2	0.33	6.81872	0.00203
4	0.89	6.81811	0.00264
6	1.19	6.81778	0.00297
8	1.65	6.81729	0.00346
10	2.17	6.81672	0.00403
12	2.55	6.81620	0.00455
14	2.99	6.81577	0.00498
16	3.31	6.81535	0.00540

$V_o = 5.68 \times 10^{-5}$ moles.min.$^{-1}$

Initial Substrate Concentration = 0.20 Molar

Extent of Hydrolysis = 0.22%
Blank = 0.830

Time (min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln\left(\frac{D_\infty}{D_\infty - D_t}\right)$
2	0.194	6.63724	0.00133
4	0.444	6.63691	0.00166
6	0.860	6.63662	0.00195
8	1.036	6.63612	0.00245
10	1.016	6.63615	0.00242
12	1.322	6.63575	0.00285
14	1.532	6.63547	0.00310
16	1.706	6.63524	0.00330

$V_o = 4.34 \times 10^{-5}$ moles·min.$^{-1}$

---

*This column was obtained by subtraction of the blank value from the original data

Table 3 Continued

Initial Substrate Concentration = 1.21×10^{-1} Molar

Extent of Hydrolysis = 1.19%
Blank = 1.35

Time (Min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.73	6.12962	0.00234
8	1.39	6.12818	0.00378
12	2.11	6.12661	0.00535
16	2.69	6.12534	0.00062
20	3.41	6.12376	0.00820
24	4.09	6.12259	0.00937
28	4.85	6.12068	0.01128
32	5.53	6.11911	0.01285

$V_o = 4.28 \times 10^{-5}$ moles·min^{-1}

Initial Substrate Concentration = 3.02×10^{-1} Molar

Extent of Hydrolysis = 1.27%
Blank = 0.360

Time (min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
2	0.105	4.74405	0.00403
4	0.270	4.74261	0.00537
6	0.365	4.74091	0.00717
8	0.565	4.73916	0.00982
10	0.768	4.73739	0.01169
12	0.933	4.73673	0.01225
14	1.113	4.73489	0.01319
16	1.253	4.73401	0.01407
18	1.470	4.73209	0.01599

$V_o = 2.18 \times 10^{-5}$ moles·min.$^{-1}$

---

*This column was obtained by subtraction of the blank value from the original data

Table 3 Continued

Initial Substrate Concentration = 1.048×10^{-2}

Extent of Hydrolysis = 2.10%
Blank = 0.033

Time (Min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
$2\frac{1}{4}$	0.027	3.68541	0.00421
4	0.217	3.68345	0.00617
6	0.310	3.68083	0.00879
8	0.414	3.67819	0.01143
10	0.514	3.67567	0.01495
12	0.610	3.67304	0.01658
14	0.742	3.66996	0.01966
16	0.840	3.66751	0.02211

$V_o = 1.38 \times 10^{-5}$ moles·min.$^{-1}$

Initial Substrate Concentration = 7.56×10^{-3}

Extent of Hydrolysis = 2.70%
Blank = 0.195

Time (min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.103	5.65533	0.01057
8	0.248	5.65124	0.01446
12	0.412	5.6446	0.02124
16	0.490	5.64134	0.02436
20	0.654	5.63586	0.02984
24	0.761	5.63203	0.03367
28	0.885	5.62758	0.03812
32	1.006	5.62322	0.04248
36	1.107	5.61957	0.04613

$V_o = 8.33 \times 10^{-6}$ moles·min.$^{-1}$

---

*This column was obtained by subtraction of the blank value from the original data

Table 3 Continued

Initial Substrate Concentration = 7.49×10^{-3}

Extent of Hydrolysis = 3.80%
Blank = 0.114

Time (min.)	$D_t(\text{corr})^*$	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
3	0.061	3.34686	0.00614
6	0.163	3.34361	0.00939
9	0.283	3.33905	0.01395
12	0.488	3.33535	0.01763
17	0.581	3.32848	0.02452
20	0.683	3.32472	0.02828
24	0.836	3.31921	0.03379
28	0.996	3.31339	0.03961

$V_o = 9.76 \times 10^{-6}$ moles·min.$^{-1}$

Initial Substrate Concentration - 5.99×10^{-3}

Extent of Hydrolysis = 3.67%
Blank = 0.172

Time (min.)	$D_t(\text{corr})^*$	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
2	0.030	3.12047	0.00848
4	0.098	3.11745	0.01200
6	0.150	3.11514	0.01431
8	0.211	3.11242	0.01703
10	0.276	3.10954	0.01991
12	0.309	3.10807	0.02138
14	0.404	3.08823	0.02557
16	0.426	3.07921	0.02668

$V_o = 7.41 \times 10^{-6}$ moles·min.$^{-1}$

---

*This column was obtained by subtraction of the blank value from the original data

Table 3 Continued

Initial Substrate Concentration = 4.49×10^{-3}

Extent of Hydrolysis = 1.87%
Blank = 0.150

Time (min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.088	2.82821	0.01389
8	0.185	2.82233	0.02077
14	0.340	2.81294	0.02916
16	0.393	2.80973	0.03237
20	0.476	2.80569	0.03641
24	0.560	2.79954	0.04256
28	0.653	2.79396	0.04814
32	0.749	2.78815	0.05395

$V_o = 6.23 \times 10^{-6}$ moles·min.$^{-1}$

Initial Substrate Concentration = 2.99×10^{-3}

Extent of Hydrolysis = 4.35%
Blank = 0.152

Time (min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
10	0.018	1.70992	0.03379
15	0.154	1.70339	0.04032
20	0.203	1.69442	0.04929
25	0.256	1.68649	0.05722
30	0.272	1.68074	0.06297
35	0.365	1.66421	0.07950
40	0.398	1.65794	0.08577

$V_o = 2.38 \times 10^{-6}$ moles·min.$^{-1}$

---

*This column was obtained by subtraction of the blank value from the original data

Table 3 Continued

Initial Substrate Concentration = 2.99×10^{-3}

Extent of Hydrolysis = 4.35%
Blank 0.152

Time (min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.043	2.41859	0.01691
8	0.114	2.41235	0.02315
10	0.144	2.40971	0.02579
15	0.247	2.40078	0.03472
20	0.316	2.35330	0.05220
24	0.372	2.38006	0.05544
28	0.439	2.37434	0.06126
32	0.512	2.36812	0.6723

$V_o = 4.12 \times 10^{-6}$ moles·min.$^{-1}$

Initial Substrate Concentration = 1.08×10^{-3}

Extent of Hydrolysis = 7.20%
Blank = 0.067

Time (min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.038	1.39158	0.02568
8	0.063	1.38604	0.03117
12	0.084	1.38003	0.03718
16	0.113	1.37270	0.04451
20	0.152	1.36533	0.05188
24	0.159	1.36098	0.05623
28	0.174	1.35196	0.06525
32	0.204	1.34937	0.06784

$V_o = 1.59 \times 10^{-6}$ moles·min.$^{-1}$

---

*This column was obtained by subtraction of the blank value from the original data

Table 3 Continued

Initial Substrate Concentration = 7.49×10^{-4}

Extent of Hydrolysis = 6.15%
Blank = 0.107

Time (min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
5	0.023	1.00404	0.04654
10	0.040	0.99780	0.5278
15	0.066	0.98816	0.06242
20	0.081	0.98257	0.06801
25	0.103	0.97340	0.07628
30	0.125	0.96595	0.08463
35	0.145	0.95831	0.09227
40	0.168	0.94945	0.10113

$V_o = 1.12 \times 10^{-6}$ moles·min.$^{-1}$

Initial Substrate Concentration = 6.59×10^{-4}

Extent of Hydrolysis = 6.15%
Blank = 0.09

Time (min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
5	0.021	0.87705	0.04562
10	0.041	0.86919	0.05348
15	0.060	0.86120	0.06147
20	0.074	0.85527	0.06740
25	0.096	0.84587	0.07680
30	0.115	0.83768	0.08499
35	0.136	0.82855	0.09412
40	0.154	0.82066	0.10201

$V_o = 1.02 \times 10^{-6}$ moles·min.$^{-1}$

---

*This column was obtained by subtraction of the blank value from the original data

Table 3 Continued

Initial Substrate Concentration = 5.76×10^{-4}

Extent of Hydrolysis = 7.15%
Blank = 0.082

Time (min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
5	0.003	0.74953	0.03963
10	0.020	0.74146	0.04770
15	0.038	0.73237	0.05679
20	0.057	0.72368	0.06548
25	0.077	0.71393	0.07523
30	0.094	0.70557	0.08359
35	0.111	0.69350	0.09566
40	0.125	0.69014	0.09902

$V_o = 8.50 \times 10^{-7}$ moles·min.$^{-1}$

Initial Substrate Concentration = 4.91×10^{-4}

Extent of Hydrolysis = 8.55%
Blank = 0.083

Time (min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.012	0.57672	0.05285
12	0.035	0.56588	0.06374
20	0.052	0.55446	0.07521
28	0.078	0.54582	0.08385
36	0.097	0.52886	0.10081
44	0.119	0.51581	0.11386
52	0.142	0.50197	0.12768
60	0.162	0.48980	0.13987

$V_o = 7.27 \times 10^{-7}$ moles·min.$^{-1}$

---

*This column was obtained by subtraction of the blank value from the original data

Table 3 Continued

Initial Substrate Concentration = 4.94×10^{-4}

Extent of Hydrolysis = 8.10%
Blank = 0.050

Time (Min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
6	0.011	0.60213	0.03391
12	0.030	0.59167	0.04437
18	0.070	0.57987	0.05517
24	0.067	0.57098	0.06406
30	0.081	0.56531	0.06973
36	0.100	0.55158	0.08446
42	0.114	0.53606	0.09098
48	0.124	0.52176	0.09797
54	0.152	0.5406	0.11328

$V_o = 7.12 \times 10^{-7}$ moles·min^{-1}

---

*This column was obtained by subtraction of the blank value from the original data

FIGURE 2

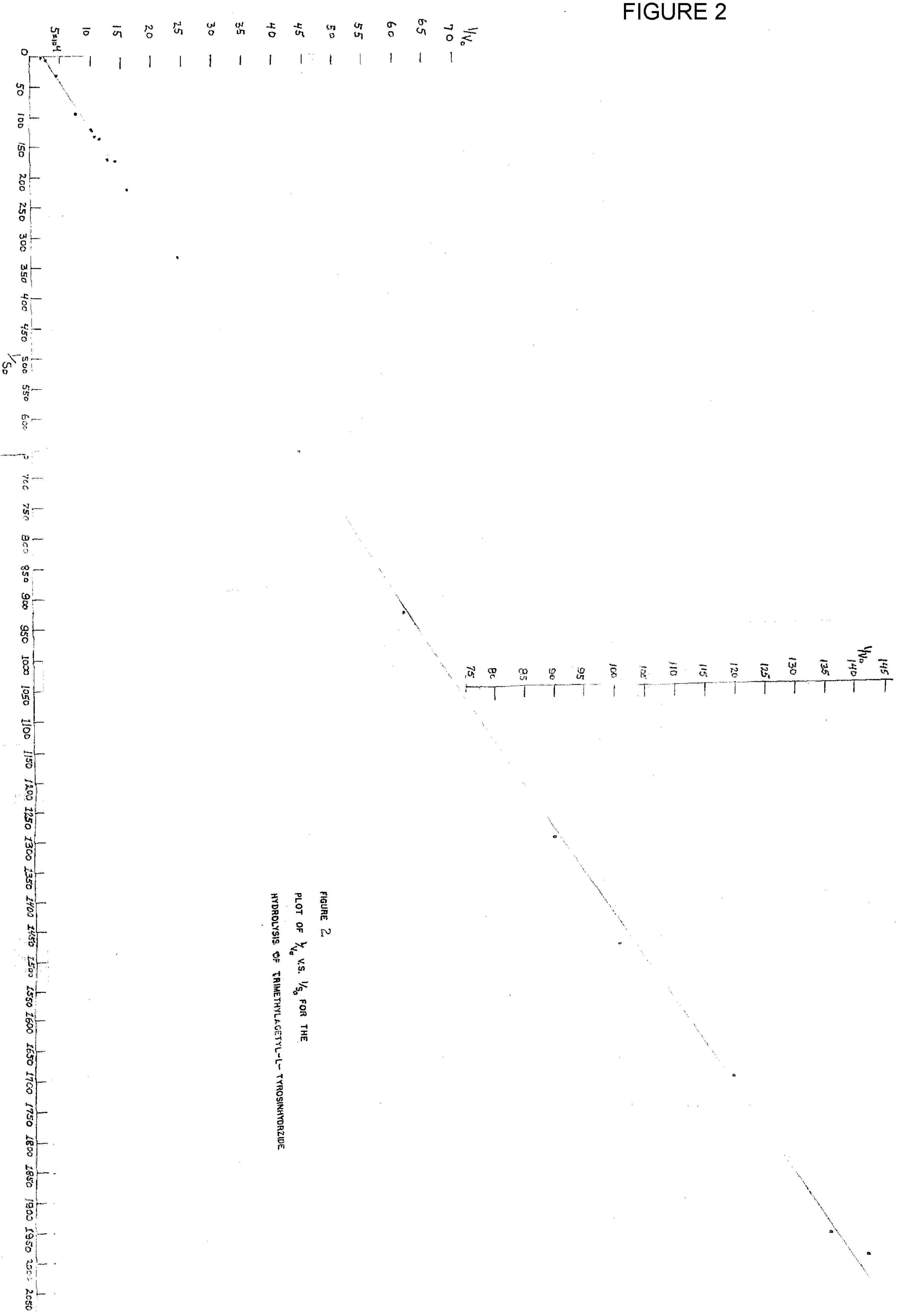


FIGURE 2

PLOT OF $1/V_0$ V.S. $1/S_0$ FOR THE HYDROLYSIS OF TRIMETHYLACETYL-L-TYROSINHYDRAZIDE

Table 4

The Relative Rates Of The α Chymotrypsin Catalyzed Hydrolysis Of N-Formyl-L-tyrosinhydrazide At Different pH Values

Time of Hydrolysis = 135 min.
[S_0] = 1.2×10^{-4} molar

Initial pH	Observed Optical Density	Final pH
6.20	0.252	6.20
6.70	0.318	6.65
7.00	0.382	6.92
7.25	0.411	7.21
7.50	0.420	7.47
7.80	0.422	7.78
8.25	0.360	8.27
8.42	0.324	8.45
8.35	0.365	8.30
8.63	0.322	8.54
7.80	0.390	7.79
7.88	0.420	7.85
7.78	0.434	7.77
7.75	0.434	7.72
7.70	0.438	7.66
7.65	0.395	7.64
7.60	0.439	7.62
7.54	0.429	7.51
7.58	0.436	7.55

Figure 3

pH Optimum Curve for

N-Formyl-L-tyrosinhydrazide

Optical Density vs. pH

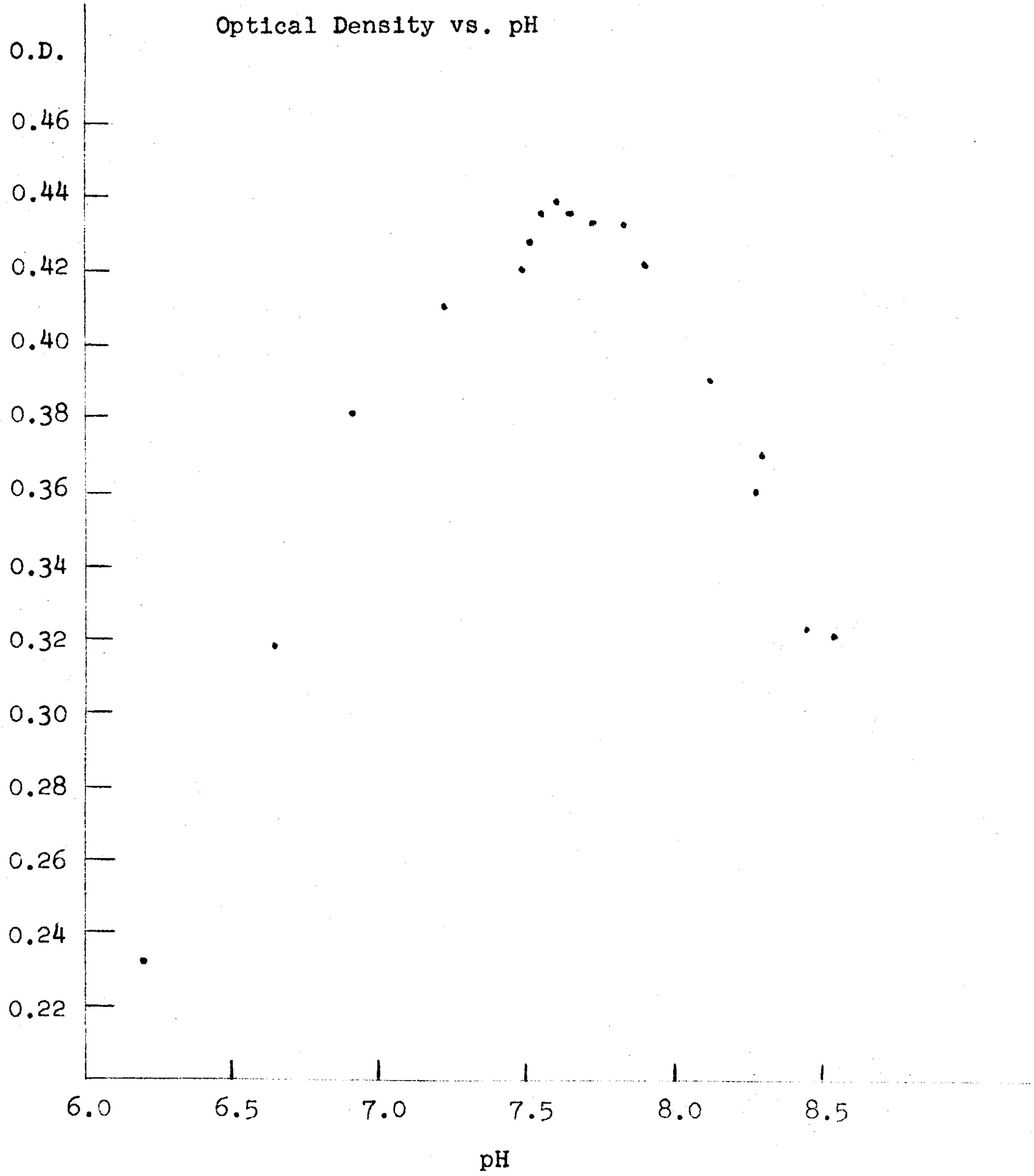


Table 5

The α Chymotrypsin Catalyzed Hydrolysis Of N-Formyl-L-tyrosinhydrazide

Initial Substrate Concentration = 13.47×10^{-3} Molar

Extent of Hydrolysis = 1.23%
Blank = 0.220

Time (Min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.040	6.23830	0.00501
8	0.112	6.23690	0.00641
12	0.194	6.23529	0.00802
16	0.270	6.23380	0.00957
20	0.334	6.23255	0.01076
24	0.406	6.23113	0.01218
28	0.497	6.22933	0.01398
32	0.561	6.22808	0.01523
36	0.622	6.22687	0.01644

$V_o = 49.40 \times 10^{-7}$ moles·min.$^{-1}$

Initial Substrate Concentration = 11.23×10^{-3} Molar

Extent of Hydrolysis = 1.37%
Blank = 0.210

Time (Min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.029	6.05547	0.00559
8	0.106	6.05367	0.00741
12	0.174	6.05216	0.00890
16	0.241	6.05049	0.01057
20	0.308	6.04890	0.01216
24	0.375	6.04732	0.01374
28	0.439	6.04581	0.01525
32	0.530	6.04365	0.01741
36	0.586	6.04232	0.01874

$V_o = 45.50 \times 10^{-7}$ moles·min.$^{-1}$

---

*This column was obtained by subtraction of the blank value from the original data

Table 5 Continued

Initial Substrate Concentration = 8.98×10^{-3} molar

Extent of Hydrolysis = 1.46%
Blank = 0.200

Time (min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.030	3.52862	0.00668
8	0.085	3.52701	0.00829
12	0.141	3.52536	0.00994
16	0.216	3.52316	0.01215
20	0.278	3.52132	0.01398
24	0.324	3.51996	0.01534
28	0.375	3.51845	0.01685
32	0.440	3.51652	0.01878
36	0.502	3.51468	0.02062

$V_o = 38.90 \times 10^{-7}$ moles·min.$^{-1}$

Initial Substrate Concentration = 8.00×10^{-3} Molar

Extent of Hydrolysis = 1.52%
Blank = 0.155

Time (min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.026	3.41375	0.00581
8	0.075	3.41214	0.00742
12	0.143	3.40939	0.00967
16	0.195	3.40517	0.01139
20	0.247	3.40695	0.01311
24	0.312	3.40151	0.01526
28	0.371	3.40131	0.01725
32	0.427	3.40054	0.01902

$V_o = 37.82 \times 10^{-7}$ moles·min.$^{-1}$

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*This column was obtained by subtraction of the blank value from the original data

Table 5 Continued

Initial Substrate Concentration = 6.74×10^{-3} Molar

Extent of Hydrolysis = 1.66%
Blank = 0.173

Time (Min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.027	3.23985	0.00780
8	0.077	3.23789	0.00976
12	0.126	3.23557	0.01208
16	0.178	3.23392	0.01373
20	0.227	3.23175	0.01590
24	0.275	3.22969	0.01796
28	0.337	3.22771	0.01994
32	0.386	3.22577	0.02188
36½	0.428	3.22410	0.02355

$V_o = 33.74 \times 10^{-7}$ moles·min.$^{-1}$

Initial Substrate Concentration = 5.56×10^{-3} Molar

Extent of Hydrolysis = 1.72%
Blank = 0.120

Time (min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.068	3.05055	0.00886
8	0.093	3.04937	0.01003
12	0.142	3.04704	0.01236
16	0.189	3.04481	0.01459
20	0.231	3.04280	0.01660
24	0.279	3.04051	0.01889
28	0.317	3.03869	0.02071
32	0.370	3.03572	0.02368

$V_o = 30.05 \times 10^{-7}$ moles·min.$^{-1}$

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*This column was obtained by subtraction of the blank value from the original data

Table 5 Continued

Initial Substrate Concentration = 4.63×10^{-3} Molar

Extent of Hydrolysis = 1.9%
Blank = 0.111

Time (Min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.048	2.86340	0.00982
8	0.074	2.86271	0.01051
12	0.136	2.85916	0.01406
16	0.170	2.85721	0.01601
20	0.203	2.85532	0.01795
24	0.249	2.85233	0.02089
28	0.269	2.85152	0.02175
32	0.326	2.84822	0.02505

$V_o = 26.78 \times 10^{-7}$ moles·min.$^{-1}$

Initial Substrate Concentration = 3.90×10^{-3} Molar

Extent of Hydrolysis = 2.66%
Blank = 0.150

Time (Min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.040	2.68847	0.01276
8	0.077	2.68590	0.01533
12	0.110	2.68233	0.01840
16	0.159	2.68115	0.02005
20	0.192	2.67856	0.02267
24	0.236	2.67509	0.02614
28	0.265	2.67297	0.02826
32	0.308	2.67000	0.03123
36	0.372	2.66556	0.03567

$V_o = 23.3 \times 10^{-7}$ moles·min.$^{-1}$

---

*This column was obtained by subtraction of the blank value from the original data

Table 5 Continued

Initial Substrate Concentration = 3.71×10^{-3} Molar

Extent of Hydrolysis = 2.37%
Blank = 0.120

Time (Min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.048	2.63813	0.001193
8	0.066	2.63684	0.01322
12	0.112	2.63354	0.01652
16	0.148	2.63095	0.01901
20	0.185	2.62828	0.02178
24	0.218	2.62590	0.02416
28	0.242	2.62416	0.02590
30	0.279	2.62147	0.02859
38	0.348	2.61645	0.03351

$V_o = 23.55 \times 10^{-7}$ moles·min.$^{-1}$

Initial Substrate Concentration = 2.08×10^{-3} Molar

Extent of Hydrolysis = 2.43%
Blank = 0.130

Time (Min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
4	0.029	2.05386	0.02019
8	0.038	2.05271	0.02134
12	0.062	2.04953	0.02414
16	0.086	2.04653	0.02752
20	0.104	2.04440	0.02965
24	0.127	2.04122	0.03283
28	0.153	2.03780	0.03625
32	0.171	2.03680	0.03725
36	0.192	2.03275	0.04130

$V_o = 23.40 \times 10^{-7}$ moles·min.$^{-1}$

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*This column was obtained by subtraction of the blank value from the original data

Table 5 Continued

Initial Substrate Concentration = 0.926×10^{-3} Molar

Extent of Hydrolysis = 3.27%
Blank = 0.110

Time (Min.)	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
5	0.017	1.22730	0.03655
10	0.015	1.22788	0.03597
15	0.027	1.22434	0.03948
20	0.024	1.22515	0.03870
25	0.060	1.21461	0.04925
30	0.046	1.21877	0.04508
40	0.090	1.20567	0.05718
45	0.100	1.20267	0.06118
50	0.113	1.19876	0.06509

$V_o = 6.55 \times 10^{-7}$ moles·min.$^{-1}$

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*This column was obtained by subtraction of the blank value from the original data

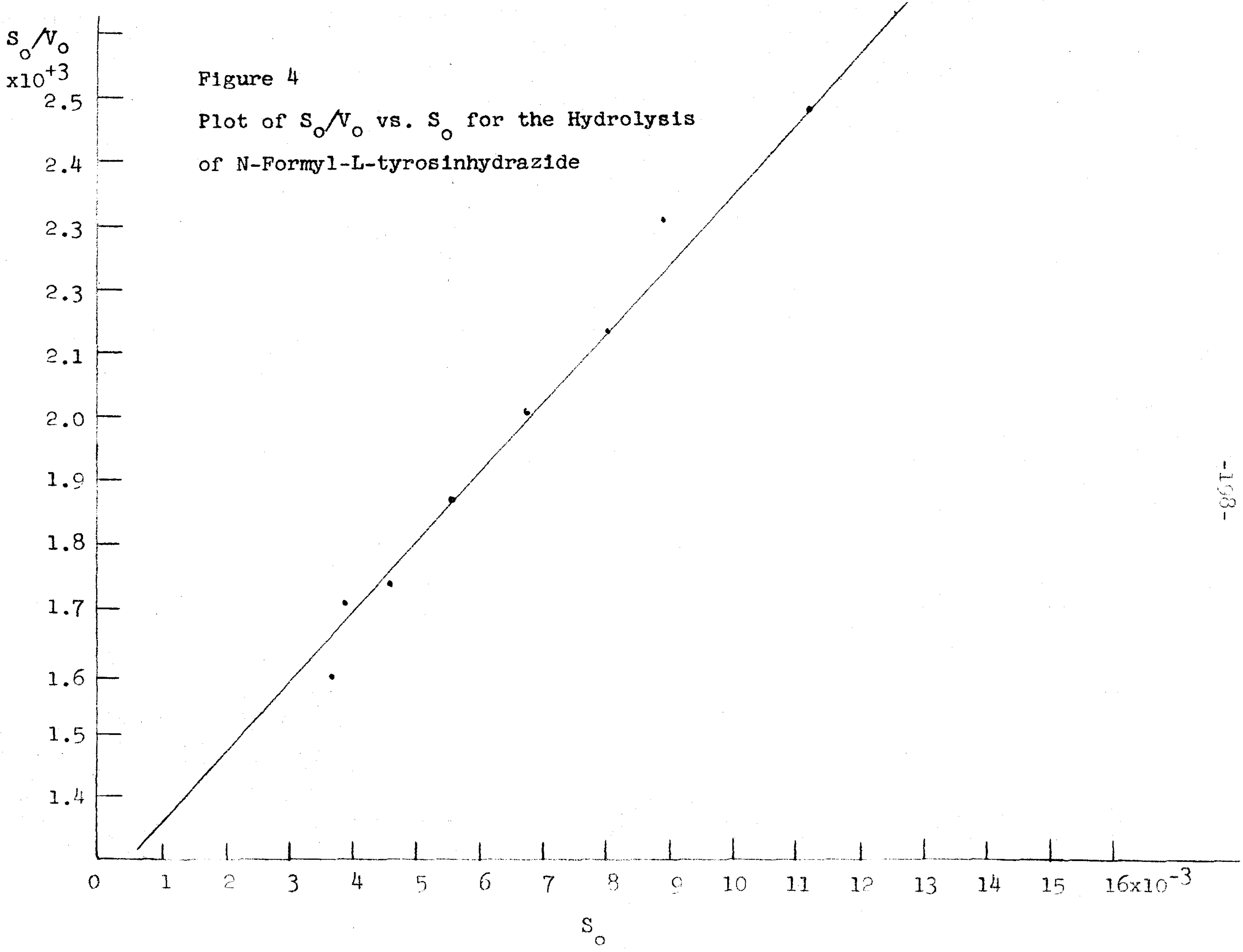

Figure 4

Plot of S_o/V_o vs. S_o for the Hydrolysis of N-Formyl-L-tyrosinhydrazide

Table 6

The Relative Rates Of The α Chymotrypsin Catalyzed Hydrolysis Of N-Dichloroacetyl-L-tyrosinhydrazide At Different pH Values

Time of Hydrolysis = 40 min.
$[S_0] = 6 \times 10^{-4}$ molar

Initial pH	Observed Optical Density	Final pH
6.20	0.370	6.20
6.70	0.571	6.68
7.09	0.702	6.93
7.25	0.852	7.10
7.80	0.869	7.78
8.32	0.685	8.35
8.25	0.750	8.25
8.43	0.660	8.40
8.00	0.833	7.94
7.88	0.883	7.85
7.78	0.887	7.75
7.75	0.895	7.72
7.61	0.894	7.66
7.65	0.905	7.64
7.60	0.909	7.62
7.54	0.907	7.51
7.40	0.892	7.40
7.30	0.875	7.26

Figure 5

ph Optimum Curve for

Dichloroacetyl-L-tyrosinhydrazide

Optical Density vs. pH

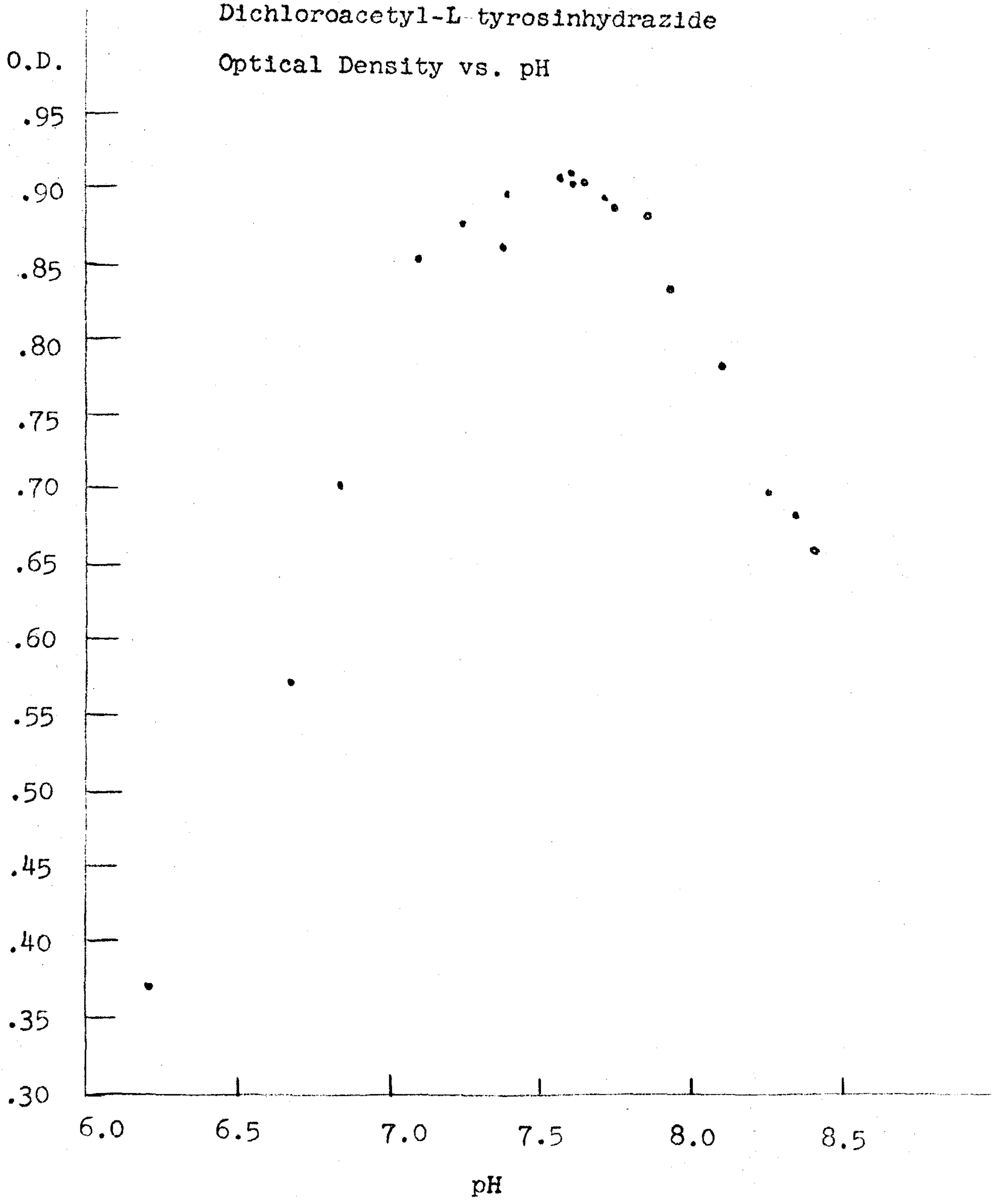

Table 7

The Kinetics of the α Chymotrypsin Catalyzed Hydrolysis of N-Dichloroacetyl-L-tyrosinhydrazide

Initial Substrate Concentration = 3.82×10^{-3} Molar

Extent of Hydrolysis = 9.96%
Blank = 0.150

t	D_t(corr)*	$\ln\frac{D_\infty}{D_\infty - D_t}$	f_o
2	0.298	0.01952	0.9893
3	0.458	0.03113	0.9854
4	0.580	0.4227	0.9790
5	0.758	0.05285	0.9739
6	0.905	0.06344	0.9689
7	1.032	0.07401	0.9637
9	1.338	0.09482	0.9542
10	1.454	0.10499	0.9492

$V_o = 4.20 \times 10^{-5}$ moles·min.$^{-1}$

Initial Substrate Concentration = 3.26×10^{-3} Molar

Extent of Hydrolysis = 11.52%
Blank = 0.120

t	D_t(corr)*	$\ln\frac{D_\infty}{D_\infty - D_t}$	f_o
2	0.298	0.02562	0.9879
3	0.440	0.03748	0.9865
5	0.730	0.06216	0.9753
6	0.872	0.07448	0.9644
7	1.039	0.08915	0.9576
8	1.170	0.10083	0.9522
9	1.300	0.11254	0.9468
10	1.435	0.12584	0.9411

$V_o = 4.04 \times 10^{-5}$ moles·min.$^{-1}$

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*The data in this column were obtained by subtraction of the blank value from the original data

Table 7 Continued

Initial Substrate Concentration = 3.234×10^{-3} Molar

Extent of Hydrolysis = 10.80%
Blank = 0.125

t	D_t(corr)*	$\ln\frac{D_\infty}{D_\infty - D_t}$	f_o
2	0.275	0.02400	0.9886
3	0.421	0.03410	0.9833
4	0.545	0.04566	0.9773
5	0.708	0.05728	0.9718
6	0.819	0.06960	0.9659
7	0.933	0.08013	0.9607
8	1.103	0.09163	0.9554
9	1.197	0.10303	0.9502
10	1.345	0.11422	0.9448

$V_o = 3.84 \times 10^{-5}$ moles·min.$^{-1}$

Initial Substrate Concentration = 2.855×10^{-3} Molar

Extent of Hydrolysis = 12.24%
Blank = 130

t	D_t(corr)*	$\ln\frac{D_\infty}{D_\infty - D_t}$	f_o
2	0.268	0.03874	0.9876
3	0.398	0.05112	0.9815
4	0.510	0.06812	0.9762
5	0.602	0.07038	0.9701
6	0.770	0.08621	0.9661
7	0.902	0.10949	0.9579
8 1/4	1.055	0.11544	0.9508
9 1/4	1.192	0.12976	0.9396
10 1/2	1.339	0.14522	0.9374

$V_o = 3.47 \times 10^{-5}$ moles·min.$^{-1}$

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*The data in this column were obtained by subtraction of the blank value from the original data

Table 7 Continued

Initial Substrate Concentration = 2.45 x 10^{-3} Molar

Extent of Hydrolysis = 11.13%
Blank = 0.100

t	D_t(corr)*	$\ln\frac{D_\infty}{D_\infty - D_t}$	f_o
3	0.362	0.04000	0.9801
4	0.471	0.05098	0.9747
5	0.574	0.07341	0.9640
6	0.730	0.08600	0.9588
7	0.830	0.09577	0.9536
8	0.940	0.10802	0.9478
9	1.062	0.11790	0.9433

V_o = 3.39 x 10^{-5} moles·min.$^{-1}$

Initial Substrate Concentration = 2.31 x 10^{-3} Molar

Extent of Hydrolysis = 11.31%
Blank = 0.130

t	D_t(corr)*	$\ln\frac{D_\infty}{D_\infty - D_t}$	f_o
2	0.229	0.02351	0.9882
3	0.286	0.03531	0.9827
4	0.396	0.04711	0.9765
5	0.515	0.05868	0.9711
6	0.608	0.07078	0.9602
7	0.702	0.08295	0.9595
8	0.805	0.09514	0.9538
9	0.925	0.10761	0.9480
10	0.971	0.12000	0.9423

V_o = 2.86 x 10^{-5} moles·min.$^{-1}$

*The data in this column were obtained by subtraction of the blank value from the original data

Table 7 Continued

Initial Substrate Concentration = 1.85×10^{-3} Molar

Extent of Hydrolysis = 12.54%
Blank = 0.062

t	D_t(corr)*	$\ln \frac{D_\infty}{D_\infty - D_t}$	f_o
2	0.183	0.025134	0.9810
3	0.288	0.03922	0.9809
4	0.349	0.05309	0.9737
5	0.469	0.06697	0.9672
6	0.603	0.08068	0.9605
7	0.624	0.09409	0.9543
8	0.719	0.10760	0.9480
9	0.787	0.12087	0.9419
10	0.888	0.13402	0.9359

$V_o = 2.54 \times 10^{-5}$ moles·min.$^{-1}$

Initial Substrate Concentration = 1.50×10^{-3} Molar

Extent of Hydrolysis = 12.16%
Blank = 0.130

t	D_t(corr)*,	$\ln \frac{D_\infty}{D_\infty - D_t}$	f_o
2	0.160	0.05187	0.9861
3	0.220	0.06285	0.9809
4	0.280	0.07040	0.9851
5	0.360	0.08931	0.9682
6	0.435	0.10176	0.9614
7	0.502	0.11675	0.9558
8	0.565	0.14717	0.9489
9	0.608	0.15674	0.9459
10	0.697	0.17372	0.9379

$V_o = 2.23 \times 10^{-5}$ moles·min.$^{-1}$

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*This column was obtained by subtraction of the blank value from the original data

Table 7 Continued

Initial Substrate Concentration = 9.24×10^{-4} Molar

Extent of Hydrolysis = 12.97%
Blank = 0.095

t	D_t(corr)*	$\ln\frac{D_\infty}{D_\infty - D_t}$	f_o
2	0.073	0.04876	0.9892
3	0.175	0.07041	0.9792
4	0.198	0.08666	0.9715
5	0.214	0.09472	0.9689
6	0.327	0.12732	0.9530
7	0.348	0.13410	0.9499
8	0.409	0.15406	0.9395
9	0.458	0.17039	0.9337

$V_o = 1.45 \times 10^{-5}$ moles·min.$^{-1}$

Initial Substrate Concentration = 9.09×10^{-4} Molar

Extent of Hydrolysis = 15.016%
Blank = 0.100

t	D_t(corr)*	$\ln\frac{D_\infty}{D_\infty - D_t}$	f_o
2	0.134	0.07611	0.9831
3	0.148	0.08044	0.9783
4	0.202	0.09733	0.9704
5	0.259	0.11548	0.9620
6	0.318	0.13461	0.9558
7	0.356	0.14713	0.9477
8	0.405	0.16351	0.9405
9	0.456	0.18085	0.9327
10	0.523	0.23982	0.9228

$V_o = 1.54 \times 10^{-5}$ moles·min.$^{-1}$

---

*The data in this column were obtained by subtraction of the blank value from the original data

Table 7 Continued

Initial Substrate Concentration = 3.02 x 10^{-4} Molar

Extent of Hydrolysis = 16.03%
Blank = 0.085

t	$D_t(\text{corr})^*$	$\ln \frac{D_\infty}{D_\infty - D_t}$	f_o
2	0.039	0.10369	0.9831
3	0.071	0.13989	0.9689
4	0.076	0.14991	0.9666
5	0.095	0.16891	0.9581
6	0.115	0.18991	0.9489
7	0.143	0.21991	0.9367
8	0.149	0.22491	0.9339
9	0.167	0.24591	0.9257
10	0.185	0.26691	0.9129

$V_o = 5.83 \times 10^{-6}$ moles•min.$^{-1}$

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*The data in this column were obtained by subtraction of the blank value from the original data

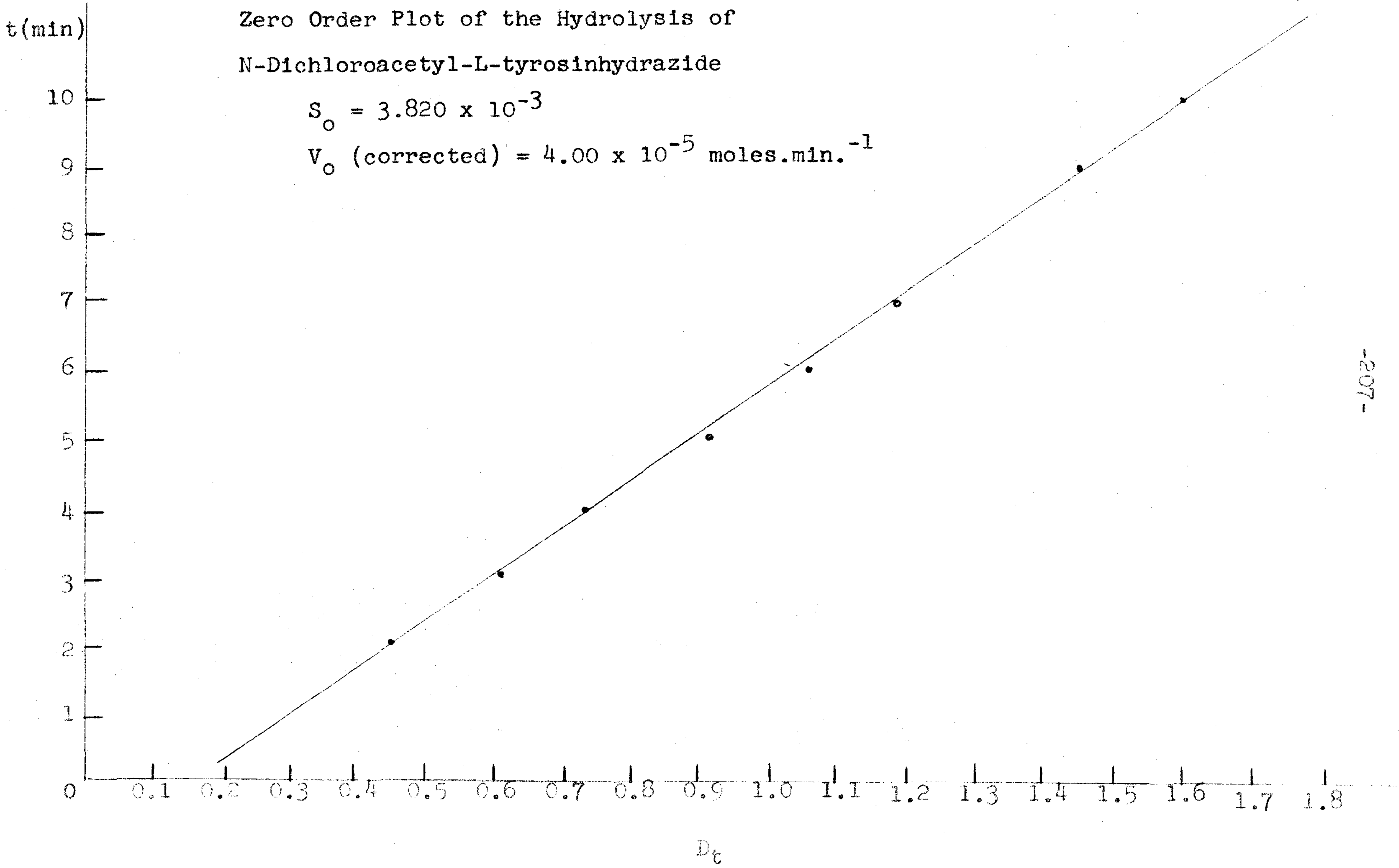

Figure 6

Zero Order Plot of the Hydrolysis of N-Dichloroacetyl-L-tyrosinhydrazide

$S_o = 3.820 \times 10^{-3}$

V_o (corrected) $= 4.00 \times 10^{-5}$ moles.min.$^{-1}$

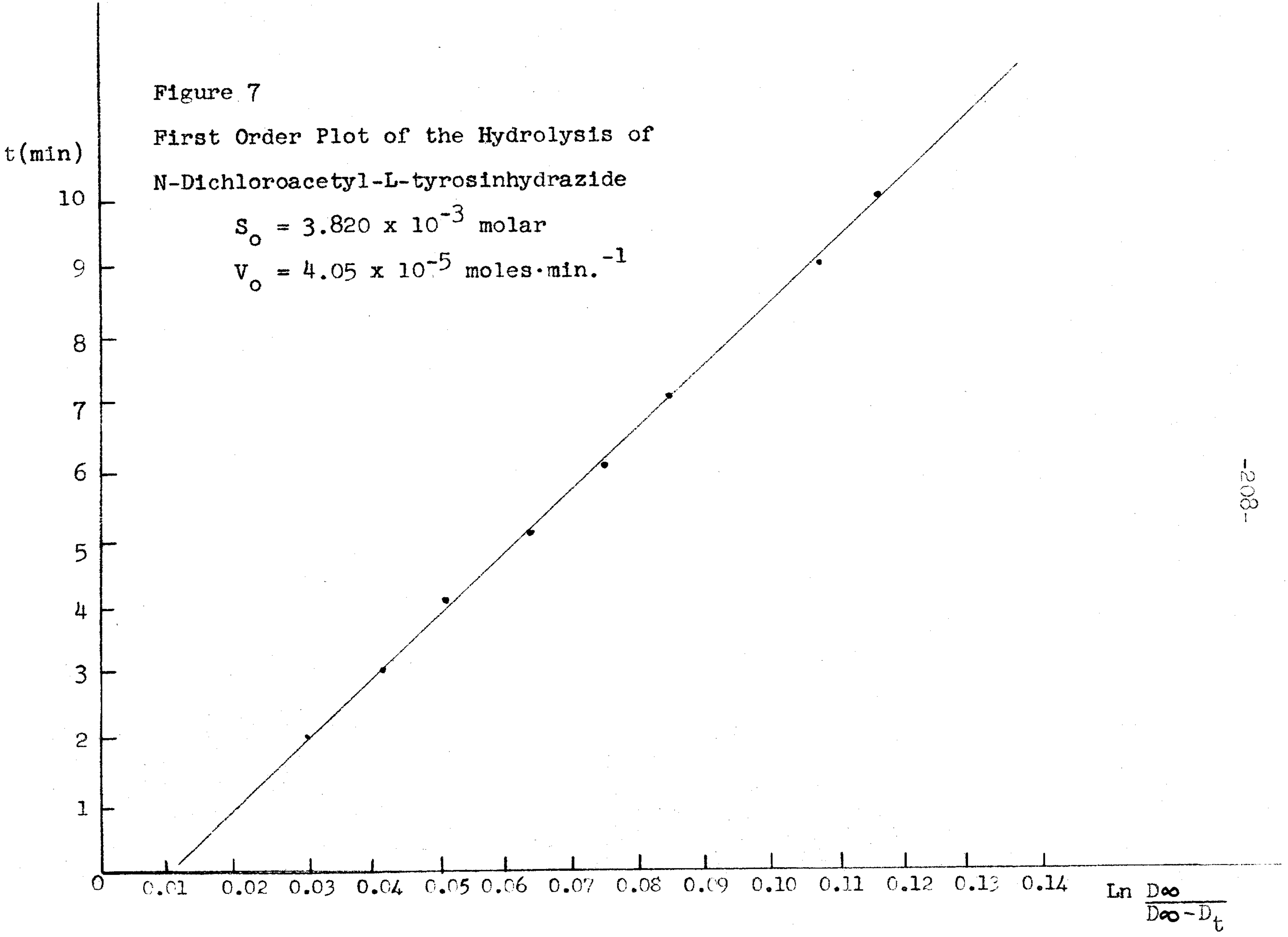
Figure 7

First Order Plot of the Hydrolysis of N-Dichloroacetyl-L-tyrosinhydrazide

$S_o = 3.820 \times 10^{-3}$ molar

$V_o = 4.05 \times 10^{-5}$ moles·min.$^{-1}$

Figure 8

Plot According to Integrated Equation - D

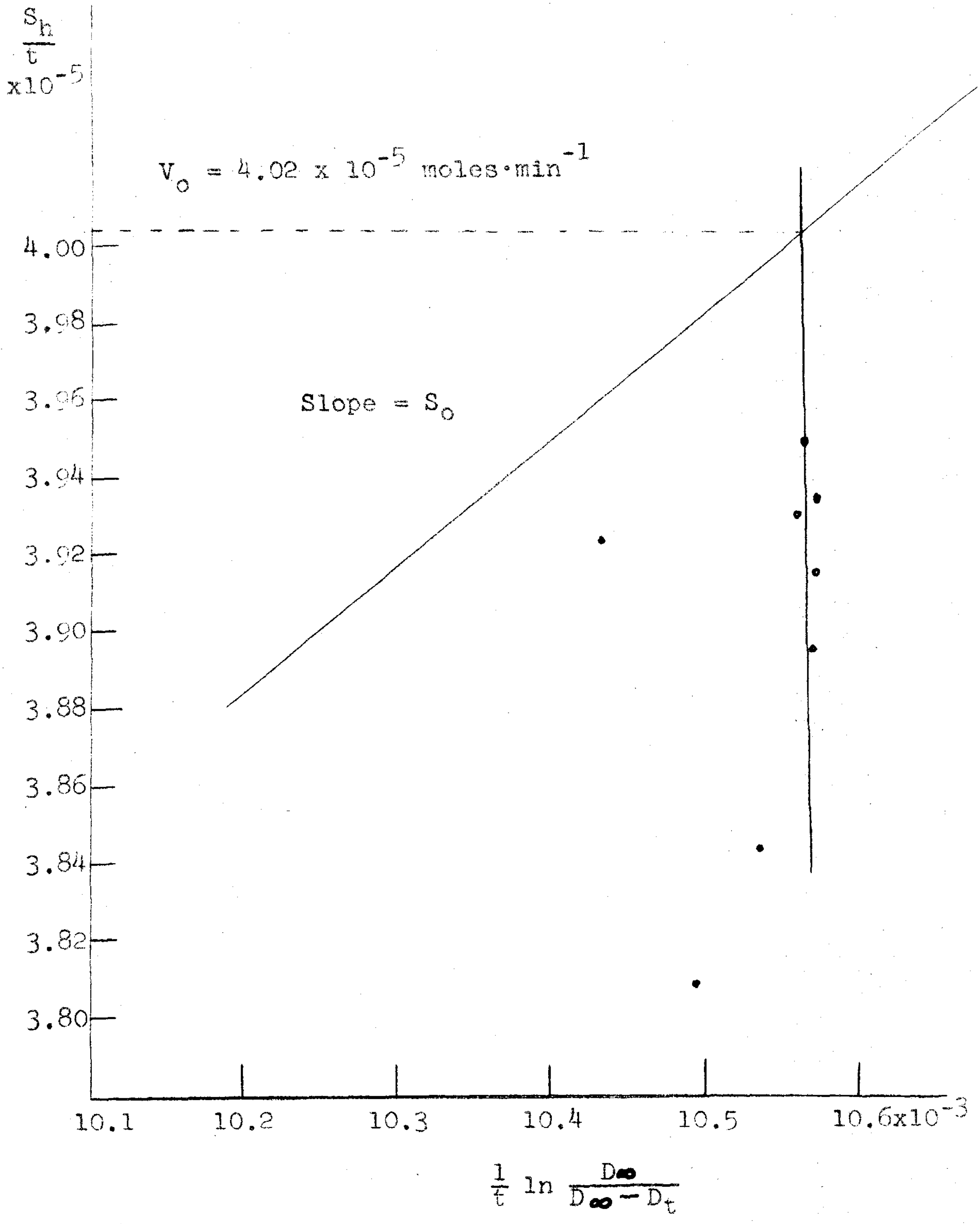

Figure 9

Plot of S_o/V_o vs. S_o for the Hydrolysis of N-Dichloroacetyl-L-Tyrosinhydrazide

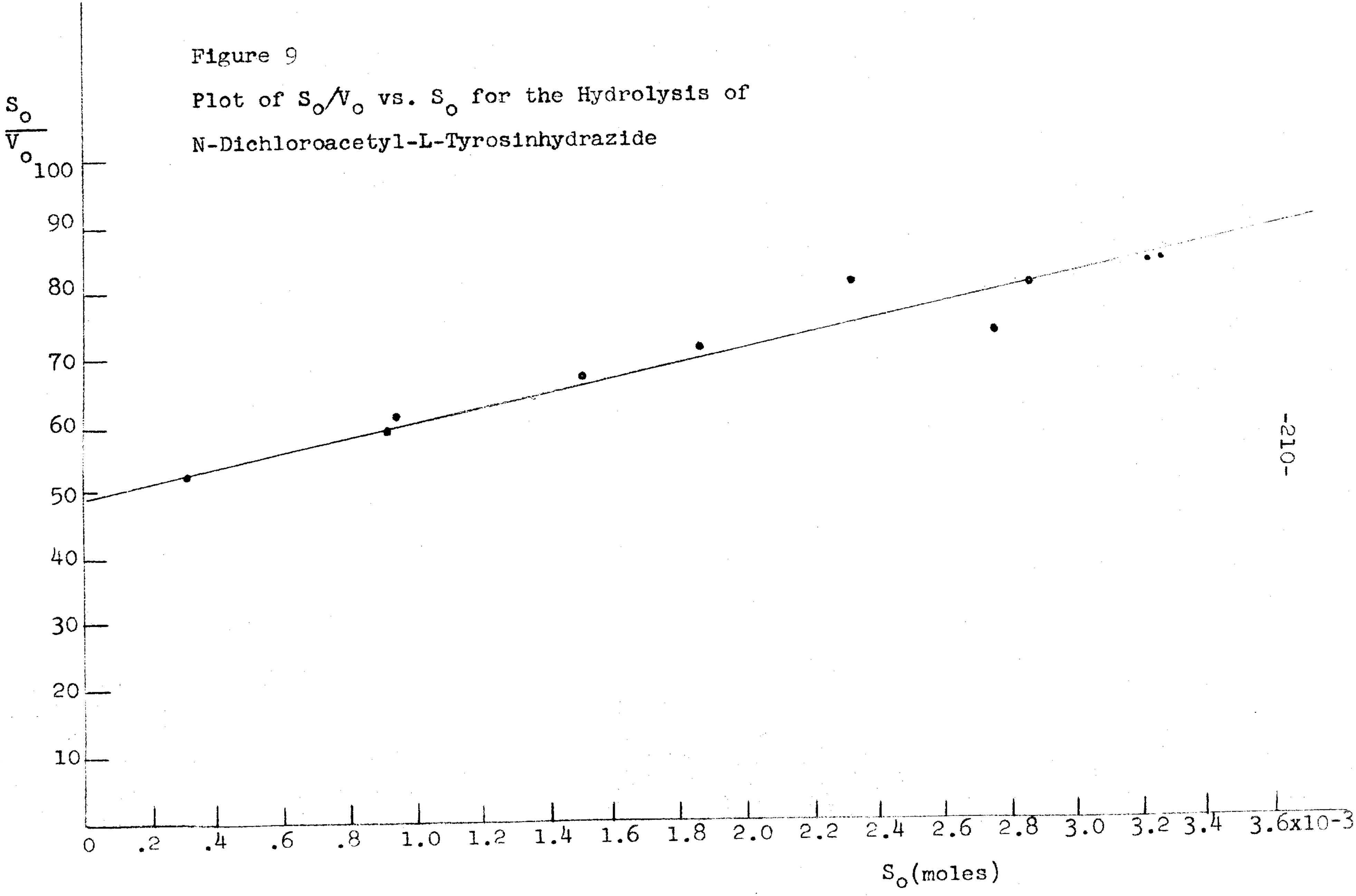

Table 8

The α Chymotrypsin Catalyzed Hydrolysis Of The Ammonium Salt Of N-Acetyl-L-cysteic Acid Carboxylamide

Substrate Concentration = 200 u moles·milliliter^{-1}

Normality of Base used in titration* = 0.10 N

Milliliters of Base equivalent to total substrate in titration = 2.0

Initial ph = 6.20
Final pH = 6.18

	T=0	T=2½hr.	T=8½hr.	T=22hr.	T=27½hr.
End Point(ml.)	0.339	0.340	0.350	0.350	0.350

Initial pH = 6.85
Final pH = 6.79

	T=0	T=2½hr.	T=8½hr.	T=22hr.	T=27½hr.
End Point(ml.)	0.398	0.414	0.420	0.425	0.420

Initial pH = 7.25
Final pH = 7.20

	T=0	T=2½hr.	T=8½hr.	-----	T=27½hr.
End Point(ml.)	0.255	0.200	0.385		0.390

Initial pH = 7.32
Final pH = 7.30

	T=0	T=2½hr.	T=8½hr.	T=22hr.	T=27½hr.
End Point(ml.)	0.390	0.400	0.400	0.420	0.420

Initial pH = 7.95
Final pH = 7.94

	T=0	T=2½hr.	T=8½hr.	T=22hr.	T=27½hr.
End Point(ml.)	0.320	0.335	0.345	0.370	0.380

Initial pH = 8.30
Final pH = 8.25

	T=0	T=2½hr.	T=8½hr.	T=22hr.	T=27½hr.
End Point(ml.)	0.290	0.310	0.324	0.350	0.355

---

*H. T. Huang and Carl Niemann - J.A.C.S. 73 1541 (1951)

Table 9

The Kinetics Of α Chymotrypsin Catalyzed Hydrolysis Of N-Acetyl-L-tyrosinhydrazide In The Presence of N-Acetyl-L-cysteic Acid-carboxylamide As An Inhibitor

(Tham Concentration = 0.5F for this set of runs)

Initial Substrate Concentration = 6.67×10^{-3} Molar

Blank = 0.100
Extent of Hydrolysis = 6.23%
Concentration of Inhibitor = 0.000 Molar

t	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
2	0.212	25.162	0.01247
4	0.380	24.994	0.01916
6	0.568	24.806	0.02672
8	0.728	24.646	0.03317
10	0.899	24.474	0.04015
12	1.095	24.279	0.04819
14	1.258	24.116	0.05493
16	1.428	23.946	0.06392
18	1.582	23.784	0.07232

$V_o = 2.41 \times 10^{-5}$ moles·min.$^{-1}$

Initial Substrate Concentration = 6.67×10^{-3} Molar

Blank = 0.100
Extent of Hydrolysis = 7.04%
Concentration of Inhibitor = 10.26×10^{-3} Molar

t	D_t(corr)*	$\ln(D \quad - D_t)$	$\ln \frac{D}{(D \quad - D_t)}$
2	0.209	3.22515	0.00122
4	0.390	3.21844	0.01941
6	0.618	3.20426	0.02859
8	0.809	3.20165	0.03820
10	0.906	3.19405	0.04350
12	1.172	3.17835	0.05951
14¼	1.382	3.17755	0.05993
16	1.560	3.17049	0.06736
18	1.750	3.16248	0.07535

$V_o = 2.63 \times 10^{-5}$ moles·min.$^{-1}$

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*This column obtained by subtraction of the blank value from the original data

Table 9 Continued

Initial Substrate Concentration = 4.23×10^{-3} Molar

Blank = 0.090
Extent of Hydrolysis = 7.40%
Concentration of Inhibitor = 0.000 Molar

t	$D_t(\text{corr})^*$	$\ln(D_\infty - D_t)$	$\ln \dfrac{D_\infty}{(D_\infty - D_t)}$
2	0.153	2.76642	0.01605
4	0.280	2.75931	0.02316
$6\frac{1}{4}$	0.440	2.74912	0.03315
8	0.550	2.74206	0.04041
10	0.669	2.73437	0.04810
12	0.810	2.72516	0.05731
14	0.916	2.71819	0.06428
16	1.086	2.70689	0.07558
18	1.197	2.69948	0.08299

$V_o = 1.79 \times 10^{-5}$ moles.min.$^{-1}$

Initial Substrate Concentration = 4.23×10^{-3} Molar

Blank = 0.090
Extent of Hydrolysis = 7.50%
Concentration of Inhibitor = 10.26×10^{-3} Molar

t	$D_t(\text{corr})^*$	$\ln(D_\infty - D_t)$	$\ln \dfrac{D_\infty}{(D_\infty - D_t)}$
2	0.148	2.76764	0.01553
4	0.395	2.75200	0.03047
6	0.415	2.75072	0.03175
8	0.535	2.74337	0.03910
12	0.830	2.72485	0.05762
14	0.930	2.71727	0.06520
16	1.064	2.70838	0.07409
18	1.220	2.69793	0.08454

$V_o = 1.76 \times 10^{-5}$ moles·min.$^{-1}$

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*This column obtained by subtraction of the blank value from the original data

Table 9 Continued

Initial Substrate Concentration = 2.53 x 10^{-3} Molar

Blank = 0.105
Extent of Hydrolysis = 6.75%
Concentration of Inhibitor = 0.000 Molar

t	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
2	0.075	2.24918	0.01931
4	0.128	2.24411	0.02438
6	0.237	2.23248	0.03601
8	0.396	2.22613	0.04236
10	0.375	2.21758	0.05091
12	0.468	2.20629	0.06220
14	0.530	2.20055	0.06794
16	0.582	2.19477	0.07372
18	0.650	2.18718	0.08131

V_o = 1.05 x 10^{-5} moles·min.$^{-1}$

Initial Substrate Concentration = 2.53 x 10^{-3} Molar

Blank = 0.075
Extent of Hydrolysis = 8.94%
Concentration of Inhibitor = 10.26 x 10^{-3} Molar

t	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
2	0.119	2.24887	0.01962
4	0.161	2.24379	0.02470
6	0.280	2.23109	0.03740
8	0.363	2.22215	0.04635
10	0.416	2.21638	0.05211
12	0.519	2.20508	0.06341
14	0.601	2.19600	0.07249
16	0.675	2.18774	0.08075
18½	0.777	2.17623	0.09226

V_o = 1.16 x 10^{-5} moles·min.$^{-1}$

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*This column obtained by subtraction of the blank value from the original data

Table 9 Continued

Initial Substrate Concentration = 1.69×10^{-3} Molar

Blank = 0.105
Extent of Hydrolysis = 7.13%
Concentration of Inhibitor = 0.000 Molar

t	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
2	0.048	1.84130	0.02429
4	0.097	1.83322	0.03210
6	0.155	1.82391	0.04140
8	0.204	1.81596	0.04936
10	0.245	1.81091	0.05441
12	0.304	1.79956	0.06576
14	0.317	1.79741	0.06791
16	0.376	1.78759	0.06873
18½	0.461	1.77326	0.08306

$V_o = 6.85 \times 10^{-6}$ moles·min.$^{-1}$

Initial Substrate Concentration = 1.69×10^{-3} Molar

Blank = 0.100
Extent of Hydrolysis = 6.4%
Concentration of Inhibitor = 10.26×10^{-3} Molar

t	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
2	0.044	1.84246	0.02286
4	0.094	1.83450	0.03082
6	0.138	1.82747	0.03785
8	0.175	1.82148	0.04384
10	0.243	1.81042	0.05490
12	0.275	1.80516	0.06016
14	0.313	1.79888	0.06644
16	0.386	1.78675	0.07857
18	0.412	1.78238	0.08294

$V_o = 6.08 \times 10^{-6}$ moles·min.$^{-1}$

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*This column obtained by subtraction of the blank value from the original data

Table 9 Continued

Initial Substrate Concentration = 1.27 x 10^{-3} Molar

Blank = 0.080
Extent of Hydrolysis = 7.41%
Concentration of Inhibitor = 0.000

t	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
2	0.058	1.55033	0.02886
4	0.078	1.54607	0.03312
6	0.120	1.53708	0.04211
8	0.173	1.52562	0.05357
10	0.193	1.52176	0.05793
12	0.258	1.50696	0.07223
14	0.298	1.49806	0.08113
16	0.305	1.49649	0.08270
18¼	0.358	1.48455	0.09464

$V_o = 5.25 \times 10^{-6}$ moles·min.$^{-1}$

Initial Substrate Concentration = 1.27 x 10^{-3} Molar

Blank = 0.110
Extent of Hydrolysis = 7.58%
Concentration of Inhibitor = 10.26 x 10^{-3} Molar

t	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
2	0.033	1.54773	0.03154
4	0.083	1.53704	0.04223
6	0.145	1.52401	0.05526
8	0.178	1.51798	0.06129
10	0.212	1.51050	0.06877
12	0.253	1.50170	0.7757
14	0.272	1.49717	0.08210
16	0.325	1.48523	0.09404
18	0.368	1.47545	0.10382

$V_o = 5.61 \times 10^{-6}$ moles·min.$^{-1}$

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*This column obtained by subtraction of the blank value from the original data

Table 10

The Kinetics Of Chymotrypsin Catalyzed Hydrolysis Of N-Acetyl-L-tyrosinhydrazide In The Presence Of Added Sodium Sulfate

(Tham Concentration = 0.82F for this set of runs)

Initial Substrate Concentration = 1.82×10^{-3} Molar

Blank = 0.250
Concentration of Sodium Sulfate = 0.000

t	D_t(corr)*	$\ln(D \quad -D_t)$	$\ln\frac{D}{(D \quad -D_t)}$
2 1/15	0.165	1.92161	0.01627
4	0.325	1.89295	0.04493
6	0.508	1.86191	0.07597
8	0.711	1.82986	0.10802
10	0.898	1.79940	0.13848
12	1.082	1.76849	0.16939
14	1.227	1.74344	0.19449
16	1.420	1.70729	0.22336
18	1.600	1.69457	0.23069

$V_o = 2.28 \times 10^{-5}$ moles·min.$^{-1}$

Initial Substrate Concentration = 1.82×10^{-3} Molar

Blank = 0.190
Concentration of Sodium Sulfate = 0.000

t	D_t(corr)*	$\ln(D \quad -D_t)$	$\ln\frac{D}{(D \quad -D_t)}$
2	0.170	1.91513	0.02275
4	0.383	1.88114	0.05674
6	0.559	1.85395	0.08393
8	0.774	1.81970	0.11918
10	0.955	1.78983	0.14805
12	1.130	1.76027	0.17761
14¼	1.324	1.72633	0.21155
16	1.463	1.70129	0.23659
18	1.632	1.66997	0.26791

$V_o = 2.23 \times 10^{-5}$ moles·min.$^{-1}$

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*This column obtained by subtraction of the blank value from the original data

Table 10 Continued

Initial Substrate Concentration = 1.82×10^{-3} Molar

Blank = 0.171
Concentration of Sodium Sulfate = 1×10^{-3} Molar

t	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
2	0.179	1.83780	0.10210
4	0.393	1.80105	0.13683
6	0.594	1.76730	0.17058
8	0.776	1.73572	0.20216
10	1.012	1.69323	0.24465
12	1.168	1.66412	0.27376
14	1.217	1.65479	0.28309
16	1.482	1.60282	0.33506
18	1.565	1.56716	0.37072

$V_o = 2.52 \times 10^{-5}$ moles·min.$^{-1}$

Initial Substrate Concentration = 1.82×10^{-3} Molar

Blank = 0.190
Concentration of Sodium Sulfate = 5×10^{-3} Molar

t	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
2	0.169	1.91324	0.02464
4	0.371	1.88297	0.05491
6	0.582	1.85034	0.08754
8	0.793	1.81661	0.12127
10	0.971	1.78725	0.15063
12¼	1.169	1.75354	0.18434
14	1.311	1.72864	0.20924
16	1.421	1.70892	0.22896
18	1.618	1.67162	0.26626

$V_o = 2.56 \times 10^{-5}$ moles·min.$^{-1}$

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*This column obtained by subtraction of the blank value from the original data

Table 10 Continued

Initial Substrate Concentration = 1.82×10^{-3} Molar

Blank = 0.200
Concentration of Sodium Sulfate = 10×10^{-3} Molar

t	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
2	0.182	1.91132	0.02656
4	0.374	1.88251	0.05537
6	0.582	1.85034	0.08754
8	0.778	1.80270	0.13518
10	0.943	1.79193	0.14595
12	1.112	1.76336	0.17452
14	1.312	1.72846	0.20942
16	1.494	1.69562	0.24226
18	1.621	1.67203	0.26585

$V_o = 2.43 \times 10^{-3}$ moles·min.$^{-1}$

Initial Substrate Concentration = 1.82×10^{-3} Molar

Blank = 0.170
Concentration of Sodium Sulfate = 40×10^{-3} Molar

t	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
2	0.188	1.91043	0.02831
4	0.373	1.88266	0.05608
6	0.586	1.84971	0.08853
8	0.750	1.80730	0.12094
10	0.952	1.79076	0.14798
12	1.118	1.76140	0.17784
14	1.287	1.73289	0.20535
16	1.448	1.70402	0.23422
18	1.598	1.67635	0.26189

$V_o = 2.51 \times 10^{-5}$ moles·min.$^{-1}$

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*This column obtained by subtraction of the blank value from the original data

Table 10 Continued

Initial Substrate Concentration = 1.82×10^{-3} Molar

Blank = 0.200
Concentration of Sodium Sulfate = 50×10^{-3} Molar

t	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
2	0.209	1.90733	0.03055
4	0.438	1.87272	0.06516
6	0.623	1.84388	0.09400
8	0.846	1.80796	0.12992
10	1.072	1.74432	0.19356
12	1.247	1.73994	0.19794
14	1.410	1.71091	0.22703
$16\frac{1}{4}$	1.009	1.67429	0.26365
18	1.769	1.64384	0.29410

$V_o = 2.60 \times 10^{-5}$ moles.min.$^{-1}$

Initial Substrate Concentration = 1.82×10^{-3} Molar

Blank = 0.185
Concentration of Sodium Sulfate = 100×10^{-3} Molar

t	D_t(corr)*	$\ln(D_\infty - D_t)$	$\ln \frac{D_\infty}{(D_\infty - D_t)}$
$2\frac{1}{4}$	0.195	1.90939	0.02846
4	0.353	1.88570	0.05215
6	0.515	1.86081	0.07707
8	0.688	1.83354	0.10434
10	0.866	1.80467	0.13321
12	1.046	1.77461	0.16327
14	1.224	1.74397	0.19391
16	1.356	1.72060	0.21728
18	1.468	1.70220	0.22568

$V_o = 2.25 \times 10^{-5}$ moles.min.$^{-1}$

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* This column obtained by subtraction of the blank value from the original data

APPENDIX

THE SYNTHESIS OF N-TRICHLOROACETYL-L-TYROSINAMIDE

This compound is a desirable substrate to study because of its usefulness in providing evidence pertaining to the theory which relates the magnitude of k_3 and the molar volume of the N-acyl function. It is predicted that the compound will have the largest k_3 of any amide yet studied in this laboratory.

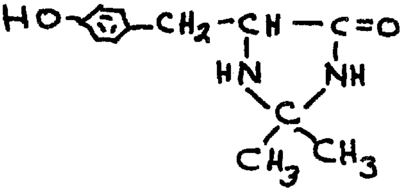
At first it was thought that this compound could be formed by the acylation of tyrosinamide with trichloroacetyl chloride. The reaction was attempted under the same conditions that tyrosine ethyl ester was acylated (p.166). However the products which were obtained by this procedure had a low chlorine content. This was presumably due to the fact that water very rapidly attacked trichloroacetyl chloride and prevented it from reacting with tyrosinamide. Under these conditions the trichloroacetyl amide bond was also probably hydrolyzed.

Next a nonaqueous acylation was tried. The conditions were similar to those employed for the acetylation of cystine dimethyl ester dihydrochloride (p.171). The product from the reaction of one mole of acid chloride and one mole of amino acid derivative was analyzed and found to have a high chlorine content. This indicates that some O-N diacylation had taken place. Attempts to selectively hydrolyze the phenyl ester without cleavage of the trichloroacetyl amide linkage resulted in failure.

It was next proposed to protect the phenyl hydroxyl group by forming the carbobenzoxyl ester and then to acetylate under anhydrous conditions. The free phenyl hydroxyl group could then be regenerated by catalytic hydrogenolysis of the carbobenzoxy function. This idea was abandoned when it was found that under the conditions of the hydrogenolysis the chlorine atoms were cleaved from the trichloroacetyl rest. Thus when trichloroacetyl anilide was treated with hydrogen in the presence of a catalysis at atmospheric pressure, mono and dichloroacetyl anilide were formed.

Finally it was determined to attempt the synthesis by the use of an amide-ester interchange. To this end ethylthio trichloroacetate was synthesized. The reaction was carried out in a Soxhlet Extractor. L-Tyrosinamide was placed in an extraction thimble, which was then placed in the upper portion of the extractor. A 20% solution containing 1.2 equivalents of ethylthio trichloroacetate and acetone was placed in the boiler. After one hour of operation all of the tyrosinamide was transferred to the boiler. The solution was a very dark brown color. Upon cooling, a crystalline mass settled out. This material was identified as L-tyrosinamide hydrochloride. No other material could be identified from reaction mixture.

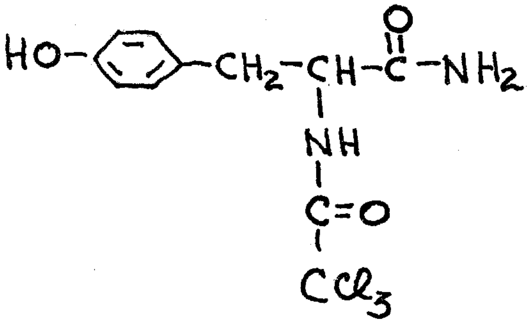
The experiment was then repeated using ethyl trichloroacetate in place of the thioester. Midway through the extraction a crystalline solid began separating out. At the conclusion of the run, the solution above the crystalline solid was clear with no evidence of decomposition. The crystalline material was recrystallized to a melting point of 184-185°C. It contained no chlorine. On the basis of the elementary composition it is suggested that this material is 2,2 dimethyl-5-(p-hydroxybenzyl)-tetrahydroimidazole-4-one. This material presumably arises by a condensation of acetone with the tyrosinamide.



Elementary composition	C	Calc = 65.50	H	Calc = 7.29
		Found = 65.14		Found = 7.44
	N	Calc = 12.72		
		Found = 12.27		

$[\alpha]_D^{25}$ = -18.2 (30% ethanol)

The experiment was repeated a third time using anhydrous ethyl acetate as the solvent. When the solvent was allowed to cool a new crystalline substance separated out of a colorless solution. This material contained chlorine and proved to be the desired product. The yield of the crude material was 65% M.P. 158-160°C. The material was recrystallized using chlorobenzene, methanol, water-methanol and anhydrous ethanol-benzene as solvents.



M.P. = 160-161° C

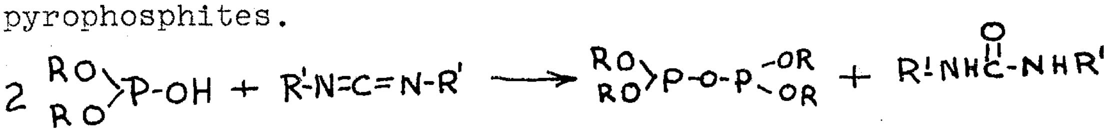
$[\alpha]_D^{25}$ = +30.5° (30% ethanol)

Elementary composition

C	Calc = 41.89	Found = 41.83	N	Calc = 8.87	Found = 8.91
H	Calc = 3.38	Found = 3.39	Cl	Calc =33.79	Found =33.68

PROPOSITIONS

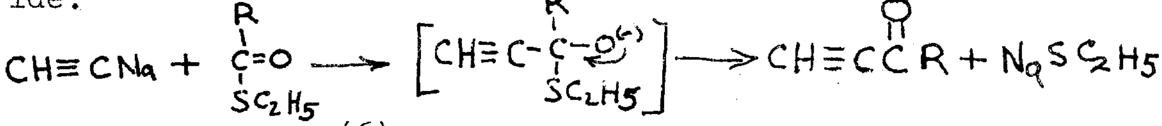
1. Dialkylcarbodiimines have been shown to be active agents in the synthesis of anhydrides.(1) It is therefore proposed that they be used in the synthesis of tetra-alkyl pyrophosphites.



$$2\ \begin{matrix}RO\\RO\end{matrix}\!\!>\!P\text{-}OH + R'\text{-}N{=}C{=}N\text{-}R' \longrightarrow \begin{matrix}RO\\RO\end{matrix}\!\!>\!P\text{-}O\text{-}P\!<\!\begin{matrix}OR\\OR\end{matrix} + R'\text{-}NH\overset{O}{\overset{\|}{C}}\text{-}NHR'$$

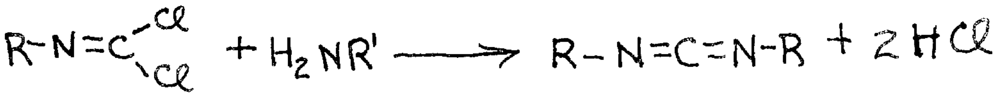
Tetra-alkyl pyrophosphites have been shown to be useful as intermediates in peptide synthesis,(2) but heretofore have been prepared only in low yields.(3)

2. Thiol esters have been shown to possess a greater reactivity than the corresponding oxygen esters.(4) In particular their carbonyl group appears to be more susceptible to nucleophilic attack. It is therefore proposed that a synthetic route to alkyl acetylenic ketones lies in the reaction of thiol esters and sodium acetylide.(5)



$$CH{\equiv}CNa + \overset{R}{\underset{SC_2H_5}{C}}{=}O \longrightarrow \left[CH{\equiv}C\text{-}\overset{R}{\underset{SC_2H_5}{C}}\text{-}O^{(-)}\right] \longrightarrow CH{\equiv}C\overset{O}{\overset{\|}{C}}R + NaSC_2H_5$$

3. Dihaloisonitriles(6) are compounds which can be considered to be structurally analogous to phosgene. It is therefore proposed that these compounds offer a synthetic approach to dialkylcarbodiimines.

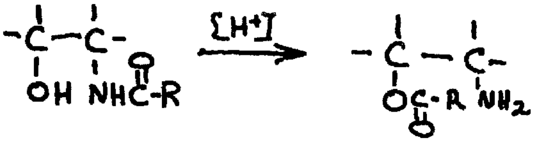


$$R\text{-}N{=}C\!<\!\begin{matrix}Cl\\Cl\end{matrix} + H_2NR' \longrightarrow R\text{-}N{=}C{=}N\text{-}R + 2HCl$$

4. Carbazole is an amine which displays acidic properties. The carbonyl group of N-acyl carbazoles(7) should then be subject to nucleophilic attack. It is therefore proposed that N-acyl carbazoles offer possibilities as mild, selective acylating agents.

5. Heretofore the reaction of anhydrides with amines of alcohols has been followed by titrametric procedures.(8) It is desirable that a procedure be developed which is more convenient and of greater accuracy than the titrametric procedure. It is proposed that hydroxylamine(9) offers the basis of such a colorimetric procedure. Concentrated solutions of hydroxylamine may effectively quench the reaction and convert the anhydride to a hydroxamic acid.

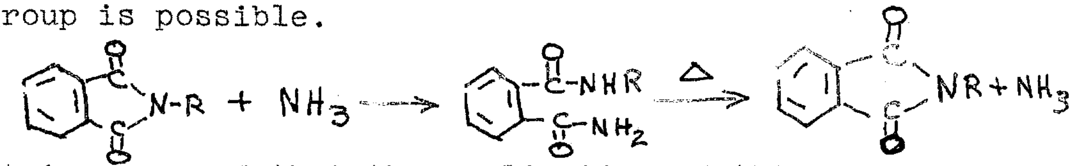
6. It has been shown(10) that, under mild acidic conditions, the acyl group of N-acyl-α hydroxyl alkyl compounds easily migrates to the adjacent hydroxyl group.



$$-\underset{OH}{\overset{|}{C}}-\underset{NH\overset{O}{\overset{\|}{C}}-R}{\overset{|}{C}}- \xrightarrow{[H^+]} -\underset{O\underset{O}{\underset{\|}{C}}-R}{\overset{|}{C}}-\underset{NH_2}{\overset{|}{C}}-$$

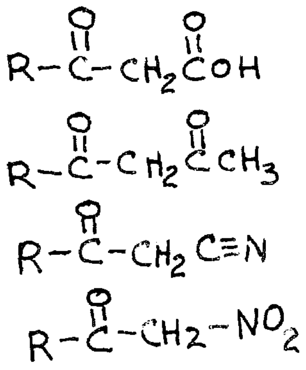
It is proposed that these "N to O" shifts are important phenomena in the acid denaturation of proteins which contain serine or threonine.

7. N-phthalamido-L-amino acid hydrazides could be of important use in the field of amino acid chemistry. The reaction between the phthalamido group and hydrazine make these compounds difficult to prepare.(11) It has been indicated(12) that by treating the phthalamido moiety with ammonia a reversible cleavage of the imide group is possible.



It is proposed that the application of this reaction could greatly simplify the synthesis of N-phthalamido-L-amino acid hydrazides.

8. It has been shown that chymotrypsin can cause the hydrolysis of β keto acids. Cyclopentadiene reacts with aldehydes and ketones in the presence of base to form highly colored fulvenes.(13) It is therefore proposed that cyclopentadiene can form the basis of a colorimetric procedure which can be used to follow the chymotrypsin catalyzed hydrolysis of the compounds listed below.

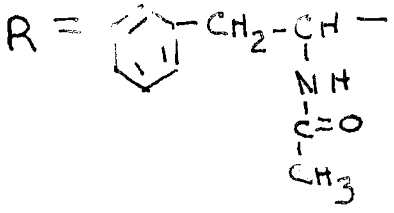


$$R-\overset{O}{\overset{\|}{C}}-CH_2\overset{O}{\overset{\|}{C}}OH$$

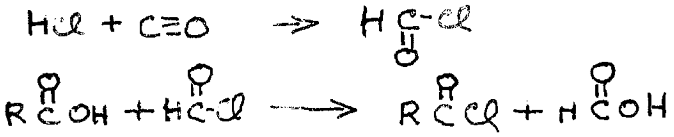
$$R-\overset{O}{\overset{\|}{C}}-CH_2\overset{O}{\overset{\|}{C}}CH_3$$

$$R-\overset{O}{\overset{\|}{C}}-CH_2C\equiv N$$

$$R-\overset{O}{\overset{\|}{C}}-CH_2-NO_2$$



9. Acid chlorides are capable of interacting with other acids to form a mixture of acid chlorides and acids.(14) It is therefore proposed that under conditions of high temperature and pressure a new industrial synthesis of acid chlorides could be developed using formyl chloride as an active intermediate.(15)



$$HCl + C{\equiv}O \rightarrow H\underset{\underset{O}{\|}}{C}\text{-}Cl$$

$$R\overset{\overset{O}{\|}}{C}OH + H\overset{\overset{O}{\|}}{C}\text{-}Cl \longrightarrow R\overset{\overset{O}{\|}}{C}Cl + H\overset{\overset{O}{\|}}{C}OH$$

10. It is proposed that the makers of "Sta Cold" are guilty of misleading advertising.

REFERENCES

(1) N. S. Corby, G. W. Kenner, and A. R. Todd - J. Chem. Soc. 1234 (1952)

(2) G. W. Anderson, J. Blodinger, A. D. Welcher - J.A.C.S. <u>74</u> 5309 (1952)

(3) A. E. Arbusov and B. A. Arbusov - Ber. <u>65</u> 195 (1932)

(4) F. Lipmann - Amer. Scientist <u>43</u> 37 (1955)

(5) J. A. Nievuland and R. R. Vogt - "The Chemistry of Acetylene" Reinhold Publishing Co., p. 93 (1945)

(6) E. Sell - Ber. <u>7</u> 1228 (1874)

(7) G. M. Ford - Iowa State Coll. J. Sci. <u>12</u> 121 (1937) Chem. Abstracts <u>32</u> 4943 (1938)

(8) V. Gold and E. G. Jefferson - J. Chem. Soc. 1409 (1953)

(9) D. M. Yost and H. Russell - "Systematic Inorganic Chemistry" Prentice-Hall, p. 97 (1946)

(10) G. Foder, U. Bruckner, J. Kiss and G. Chegyi - J. Org. Chem. <u>14</u> 337 (1949)

(11) K. Hoffmann - J.A.C.S. <u>74</u> 470 (1952)

(12) Unpublished experiments of Paul E. Peterson <u>et al</u>.

(13) J. H. Day - Chem. Reviews <u>53</u> 167 (1953)

(14) H. C. Brown - J.A.C.S. <u>60</u> 1326 (1938)

(15) H. Hopff - Angew Chem. <u>60A</u> 245 (1948)