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The tetrahedral intermediate in the reactions of imidates; the hydrolysis of 2-p-nitrophenyliminotetrahydrofuran and Y-hydroxy-p-nitrobutyranilide

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THE TETRAHEDRAL INTERMEDIATE IN THE REACTIONS OF IMIDATES

David Jonathan Sahn

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The Tetrahedral Intermediate in the Reactions of Imidates: The Hydrolysis of 2-p-nitrophenyliminotetrahydrofuran and γ - hydroxy-p-nitrobutyranilide.

A Thesis

Presented in Partial Fulfillment of the Requirements

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David Jonathan Sahn

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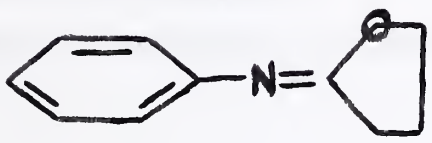
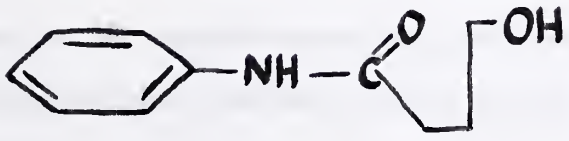
Introduction

The original intent of this project was to further elucidate the phenomena and hypotheses concerning the tetrahedral intermediate generated in the hydrolysis of the iminolactone 2-p-nitrophenyliminotetrahydrofuran:[1, 2]



The kinetics of the hydrolysis reaction were in accord with earlier work with thiazolines and oxazolines[3] but the ionization of a tetrahedral intermediate to an anionic species was suggested to explain the change of products with pH. Startling effects of bifunctional buffers on this product distribution were observed. It was proposed that this project entail the preparation of the p-methoxy and p-nitro substituted derivatives of the iminolactone and the study of the hydrolysis of both in terms of kinetics and product distribution.

The subsequent demonstration of predicted effects of buffers and pH on the hydrolysis of γ-hydroxybutyranilide:



(believed to hydrolyze through the same family of tetrahedral intermediates)[4], the extension of this work into the reactions of thioimidate esters[5], and difficulties encountered in the preparation and purification of the two labile iminolactone derivatives necessitated a change in the objectives of this project. Therefore, the γ-hydroxybutyranilide derivative corresponding to the

p-nitro substituted iminolactone was prepared. Upon its successful purification, we studied the hydrolyses of the p-nitro substituted iminolactone and its hydroxyanilide in order to delineate the nature of the family of tetrahedral intermediates common to both these hydrolyses.

This paper will be divided into four sections and an appendix.

Section I:

will be a brief review of the general state of the tetrahedral intermediate hypothesis and catalytic mechanisms involved in nucleophilic reactions at acyl centers; a review of the state of knowledge concerning iminolactones and specific compounds prepared during this project.

Section II:

will describe the properties of 2-p-nitrophenylimino-tetrahydrofuran; its preparation; the kinetics of its hydrolysis; and effects of buffers and pH on the products of hydrolysis.

Section III:

will describe the preparation and properties of γ-hydroxy-p-nitrobutyranilide and studies of the kinetics of its hydrolysis.

Section IV:

will contain a discussion and proposition of a mechanism to account for the results set forth in the previous two sections.

Appendix:

will contain descriptions of the preparation and properties of other compounds synthesized during this project.

Section I

Chapter 1

Nucleophilic Reactions at Acyl Centers--The Tetrahedral Intermediate Hypothesis--Catalytic Phenomena.

The reactions of nucleophiles at acyl centers are at the heart of biochemistry. The majority of reactions in protein and amino acid chemistry are among these. This is also the case in the chemistry of lipids. Furthermore, studies of the catalytic phenomena demonstrated in these reactions, are a reasonable approach (using simplified models) to the mechanisms of enzyme action. These reactions and their catalytic phenomena have recently been the subject of substantial reviews by Bender[6] and Johnson[7].

A minority of these reactions, such as those with hindered acyl centers (eg: certain benzoic acid derivatives), or those with readily ionizable leaving groups have been felt to undergo nucleophilic displacement by an Sn_1 mechanism[6]. However, for most of these reactions, a reaction mechanism involving Sn_2 kinetics and a tetrahedral intermediate, and with a general scheme

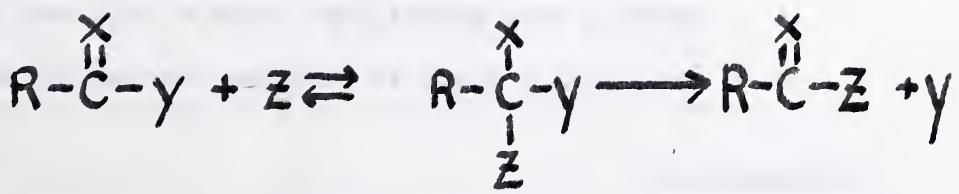


figure 1

has been proposed. The evidence for this proposition has come from various types of studies:

A-Interpretation of kinetic data have been used in support of this hypothesis.

1-Breaks in the pH rate profile (changes in slope)

The familiar bell shaped curve for pH rate profile of imidates.

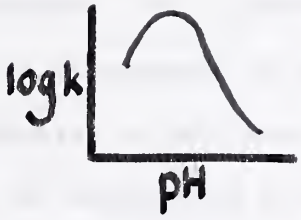
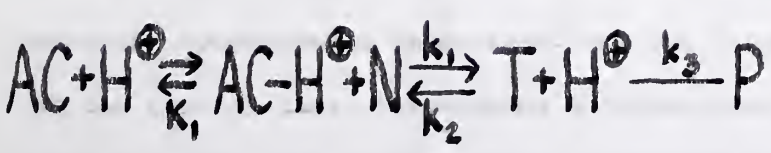


figure 2

The reversal of slope at low pH in this curve has been explained as a shift to protonation of water to hydronium ion which decreases its activity as a nucleophile. As a result the rate becomes slower with decreasing pH. This explanation has been proposed when the break occurs at very low pH. However, most often when the break occurs in weaker acid solutions, the explanation has been one of change from a reaction with rate limiting addition of nucleophile dependant on a pre-equilibrium for protonated substrate (activated for nucleophilic addition), to rate limiting breakdown of intermediate in acid solution where the high degree of protonation makes the addition step fast. Also the availability of protons transforms the previously rate limiting step into a rapid equilibrium step by acid catalysis of the back reaction of the addition step.



N-Nucleophile
AC-Acyl Center
T-Tetrahedral Intermediate
H -hydrogen ion
P-products

figure 3.

Thus at low pH the rate limiting step is the breakdown of the intermediate. The kinetically demonstrated steps involve the inherent assumption that a tetrahedral intermediate is formed. It is formed in the addition step and decomposes to products in the breakdown step.

2- Inconsistencies in rate-reactivity data: certain substitutions on the nucleophile or substrate lead to changes in kinetics which can be best explained by proposing a separation of the attack and breakdown steps by the formation of a tetrahedral intermediate.

3- There are other effects which are attributed to ionization or catalysis involving the tetrahedral intermediate and which favor one or another path of breakdown and lead to changes of the products of the reactions with or without changes in rate[1]. Further, there exist complex relationships of rate and catalysis which can best be explained by interaction of catalysts with several forms of tetrahedral intermediate and with different orders of dependance[8].

B- Certain data involving O^{18} exchange have supported acyl oxygen fission in ester hydrolysis and the formation of a tetrahedral intermediate.

C- Finally, whereas under some conditions, the addition of amines to carbonyl centers has been shown to take place in a fast pre-equilibrium step[9] and the detection of addition compounds in these reactions has been expected[6], at the present time, tetrahedral addition intermediates have been detected only under special circumstances and under

non hydrolytic conditions.[10] The accumulation of tetrahedral intermediate in reactions at acyl centers and its spectral detection (isolation being an endeavor for the future) is to be most expected when the addition step is fast, when there is little ground state stabilization of the substrate, and when breakdown is rate limiting.[11] One recent paper reports such detection during the reaction of amines with acyl chlorides in alkane solvents.

Aside from their importance in the elucidation of the mechanism of enzyme action, studies of catalysis in nucleophilic reactions at acyl centers have also given support to the tetrahedral intermediate hypothesis. These mechanisms of catalysis will be considered next.

We may define the process of catalysis as changes in rate of a reaction without changes in equilibrium. The catalytic substance appears in the rate expression for a total reaction or for one step in that reaction to a higher power than it appears in the stoichiometry. Often, as it is regenerated, it does not appear in the stoichiometry at all. The elucidation of mechanism of catalysis for a given catalyst often lies in experiments of buffer concentration and its effect on rate at different pH's. The following general categories of catalysis have been defined: 1- Electrophilic catalysis, or General acid catalysis, 2- General base-Nucleophilic catalysis, most often considered together, 3- Bifunctional general acid-general base catalysis, and 4- the combination of catalytic factiors the summation of which is

Enzymic catalysis.

General acid or electrophilic catalysis has, in general, been observed with two general mechanisms. The first involves the interaction of the electrophile with the carbonyl group at an acyl center so that the electron distribution about the center is rearranged in such a fashion as to activate the carbonyl group to nucleophilic attack. This may take the form of protonation of the carbonyl. The protonation occurs in a rapid pre-equilibrium step and determines the concentration of activated substrate available for the rate determining addition step. This type of interaction has been observed in NMR studies. Interaction with the electrophile may involve the tetrahedral intermediate itself and rearrange the electron distribution to favor departure of the leaving group (this may be the rate determining step) or to favor one leaving group over another and thus effect the product ratio. The electrophile may be a metal ion (which may be analogous to the requirement by certain enzymes of metallic coenzyme groups) or groups on a resin. Despite the polymeric character of resins, catalysis by resins does not approach the efficacy of that by enzymes. The electrophilic group involved in the catalyst may also be intramolecular. An example is found in the comparison of the rate of hydrolysis of phthalamic acid compared to that of benzamide where enhancement is seen with the acidic form of the substrate[12]. The concepts of neighboring group participation in organic chemistry, having been subjected to

kinetic analysis, have been interpreted as intramolecular forms of catalysis. In analogous situations, as would be expected, the efficiency of intramolecular catalysis is greater than that of intermolecular situations.

General base and Nucleophilic catalysis have been frequently grouped together as they are often hard to distinguish. Nevertheless, they entail different concepts of interaction, and Bender [6], discusses efforts to separate them.

Nucleophilic catalysis by definition involves the addition of the catalyst to the acyl group and the formation of a tetrahedral transient. The formation of this intermediate may speed the reaction by helping to expel the leaving group before the catalyst is itself expelled. Evidence for this direct interaction is seen in the differences in the catalytic abilities of buffers with the same basicities, but different nucleophilicities. Using buffers such as imidazole and phosphate, the susceptibility of a given reaction to nucleophilic catalysis may be assessed. Other experiments with more sterically hindered buffer compounds whose nucleophilicity has been greatly reduced distinguish the two types of catalysis.

General base catalysis is distinguished by catalytic enhancement of reaction rates in situations where nucleophilic addition by buffer could not possibly have an enhancing effect, such as the catalysis of the hydrolysis of acetic annhydride by acetate ion. In another example,

the reaction of acetyl imidazolium ion with acetate reacting via base catalysis is essentially non reversible and is not inhibited by imidazole. That occuring by nucleophilic catalysis is reversible as a result of the reverse reaction of the annhydride formed with imidazole. The inhibition of the reaction by imidazole accounts for only 22% of the total possible inhibition and thus only this small fraction occurs by nucleophilic catalysis[13].

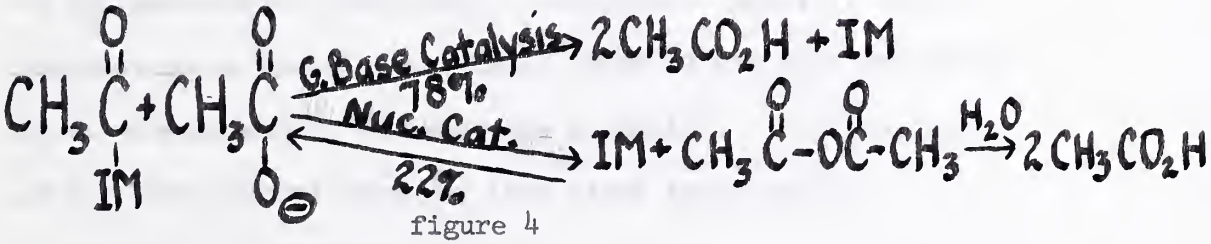
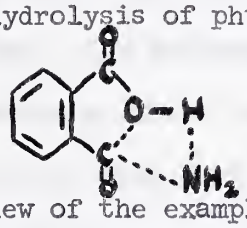


figure 4

The interactions involved in general base catalysis do not involve direct addition of the catalyst to the carbonyl carbon. The base may activate the nucleophile by rearranging its electron distribution or by extracting a proton as a specific example. It may also extract a proton from the tetrahedral intermediate and favor expulsion of the leaving group.

Bifunctional catalysis, or electrophilic-nucleophilic catalysis, has been demonstrated in the extra and intramolecular situations, usually by the enhanced catalytic capacity of multifunctional groups such as phosphate. The interactions may be of a multistep variety, but are often viewed as a concerted interaction between the nucleophile and both "arms" of the catalyst rearranging the electron distribution of the substrate in an activating fashion. In intramolecular situations

the groups interacting with the acyl center may be on different parts of the molecule as long as the stereochemistry allows interaction. An example is the interaction proposed for the hydrolysis of phthalamic acid [12].



This is another view of the example of what was earlier discussed as intramolecular electrophilic catalysis. However, this explanation is one of facilitated zwitterionic type activation by an intramolecular bifunctional mechanism. Interactions of the bifunctional type may take place involving the tetrahedral intermediate as well.

The characteristics of enzymic catalysis which sets it apart from the simpler means of catalysis are those of specificity and greater efficiency. Both may be viewed as resulting from the specific configuration of the enzyme. This allows for the existence of a specific area of the protein molecule which is stereospecifically suited to a specific type of substrate in order to allow its interaction with what probably amounts to several functional groups on the enzyme. Thus, multiple interactions with functional groups may be involved. These may be of electrophilic, general base-nucleophilic, or bifunctional nature. In addition, the binding of the substrate to the enzyme by functional groups at a specific site and prior to its activation by other subsequent interactions, allows the enhancements of rate to be of the greater magnitude

characteristic of intramolecular catalysis. In addition we may consider the binding of the cosubstrates as reducing the thermodynamic requirements for proper collision of these with the substrate. The co-substrates may also be activated in these interactions. The determinations of the active sites of enzymes and some of the dependancies of activity on pH have supported the view that the functional groups are similar in nature to those involved in the simpler molecules participating in the various types of catalysis previously mentioned. These groups are probably serine hydroxide groups, cysteine sulfhydril, imidazole etc.

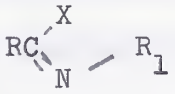
The multiple possibilities for interaction of tetrahedral intermediates in reactions at acyl centers which have been elaborated, and the studies of catalytic mechanisms, have been the subject of rapidly growing interest and rapidly expanding knowledge. In addition to their importance in the understanding of basic organic chemical mechanisms, these fields of interest are essential for the elucidation of the presently poorly understood complex interactions of biochemistry.

Section I

Chapter 2

The Mechanism of Hydrolysis of Imidates: Nature of the Tetrahedral Intermediate

The class of compounds posessing the imino function:



includes such groups as oxazolines, thiazolines, and imino-lactones (cyclic counterparts of imidates and thiolimidates). The tetrahedral intermediate formed in the hydrolysis of these substances is similar to that presumably formed during the hydrolysis of amides (such as peptides), the aminolysis of esters, or thiolesters, and the reactions of Schiff bases. They have been studied in some detail because of their importance in biochemistry.

The hydrolysis of imidates shows a characteristic pH-rate profile (bell shaped curves A).

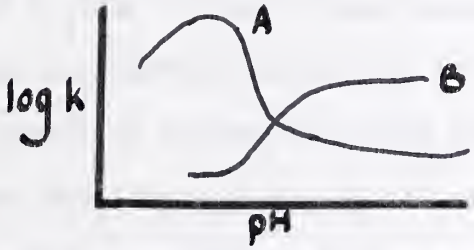
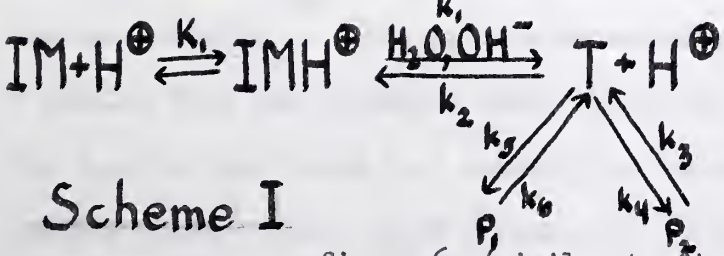


figure 5

The following reaction mechanism has been proposed for these compounds to explain the kinetics.



IM-imidate
t-tetrahedral intermediate
p-products

Scheme I

figure 6 (similar to figure 3)

This mechanism contains the following features:

1) at high pH, rate limiting attack of the nucleophile on the protonated imidate.

2) at low pH there may occur a rapid pre-equilibrium between imidate and intermediate such that the rate limiting step becomes the breakdown of the tetrahedral intermediate.

3) the pH independant region which has been attributed to the rate limiting attack of hydroxide on the protonated imidate (or the kinetically indistinguishable attack of water on a neutral intermediate). As the concentration of hydroxide anion increases, there is a corresponding decrease in the concentration of the protonated imidate ion.

This general mechanism has been found relevant for the hydrolyses of oxazolines [14, 15], thiazolines [3, 16], Schiff bases [17], thiolimidates [5], N substituted imidate esters [18], and iminolactones [1]. In further elucidation of this mechanism, Schmir [3] studied a group of substituted thiazoline derivatives and showed good correlation between the pK_1 calculated from the kinetic expression derived from this mechanism, and those pK's that could be measured by titration. A variation of the bell shaped curve (figure 5 B) is seen with certain imidate derivatives which have higher pK's such as one of the more basic N substituted acetimidate derivatives which has been studied [18]. The pH rate dependance seen in curve B results from the situation where the pK is so high for the imidate that there is a substantial amount of protonated imidate still present at pH's where the hydroxide ion

concentration is high enough to make the pH independant reaction rate faster than that seen in acid. These studies tell us much about the formation paths available for this tetrahedral intermediate but little about the paths available for the breakdown of the intermediate. Studies of the products derived from these hydrolyses give us the latter kind of information.

The above mechanism (figure 6) predicts a product distribution independant of pH. However, the pH-product dependance found first for the iminolactone 2-phenylimino-tetrahydrofuran is far from pH independance.

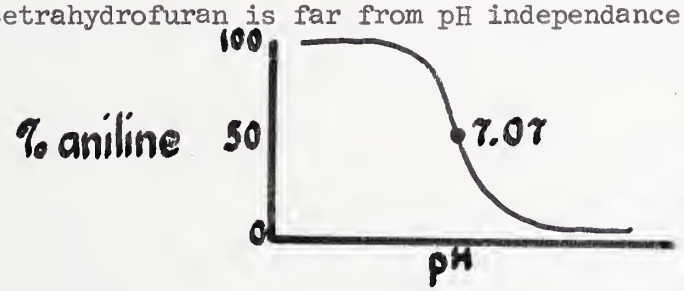


figure 7

The products of the hydrolysis of this compound are either aniline and butyralactone from CN bond cleavage, or γ-hydroxybutyranilide from CO bond cleavage[1]. The similarity of the curve in figure 7 to a titration curve of a weak acid with pK 7.07 led to the hypothesis that the hydrolysis involved two tetrahedral intermediates differing in state of ionization. Product-pH dependancies such as this were also obtained for thiolimidates[5], and other N-substituted imidate esters[18]. Therefore the following modifications of the above scheme were proposed. (These are summarized from a theoretical paper by Schmir)[19].

Scheme II

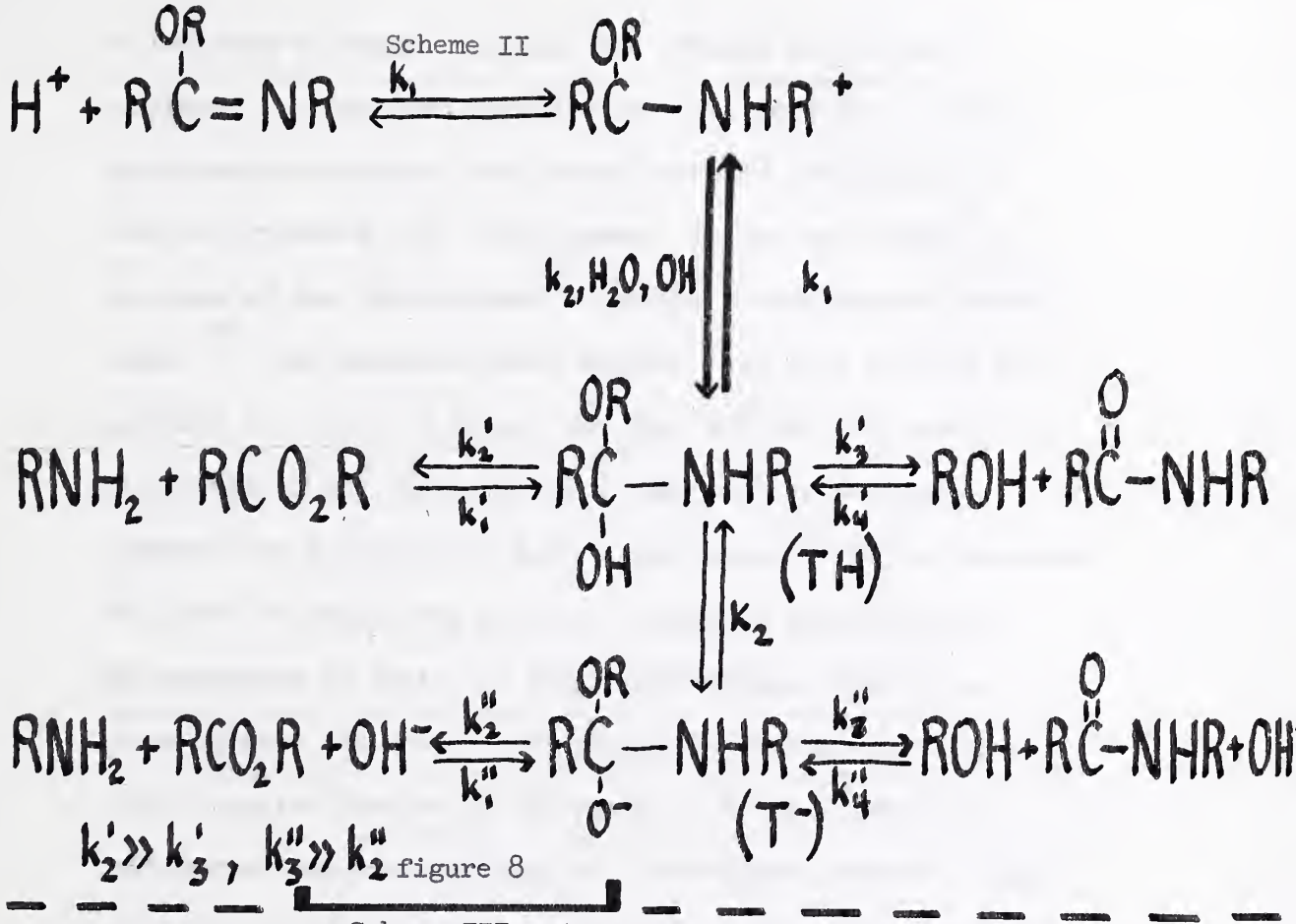


figure 8

Scheme III

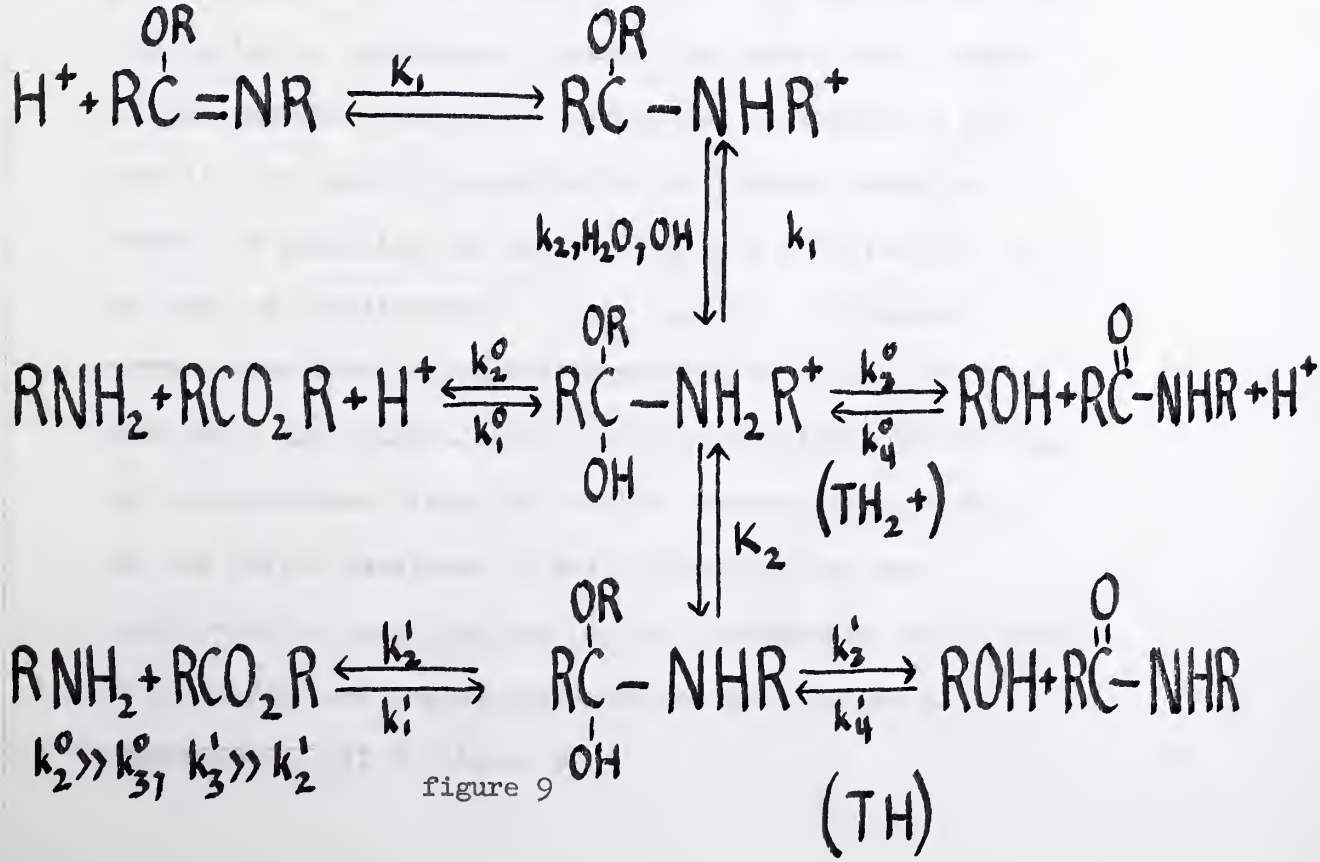


figure 9

In addition to these findings, the ability of buffers to influence the reaction products of these reactions without significantly changing the overall rate of hydrolysis has been incorporated into this scheme. It has been found in the case of the iminolactone[2] and the N substituted imidate esters[18] that monofunctional buffers have some ability to increase the yield of amine, but that buffers with both electrophilic and nucleophilic groups such as phosphate, acetate, and bicarbonate, have a startling ability to increase the yield of amine when present in minimal concentrations. The mechanism of action of the monofunctional buffers in these systems has not been thoroughly investigated. However, more intensive studies of the yield of amine formed as a function of the concentration of bifunctional buffer present at different pH have been carried out. The results have been interpreted as interaction between the bifunctional buffer and the neutral form of the tetrahedral intermediate such that it more closely resembles the zwitterion which is capable of expelling the amine group more efficiently. In the case of thiolimidates[17], the catalytic effects of buffers have been interpreted according to Scheme III. In this case, the catalytic ability of bifunctional buffers has not been different from that of the monofunctional buffers and the buffer catalysis of amine formation has been interpreted as involving interaction between the acidic form of the buffer and the neutral form of the tetrahedral intermediate. (T in figure 9).

The validity of this set of mechanistic schemes for reactions involving these compounds has been demonstrated by the study of the kinetics of the acyl transfer reactions which lead to the interconversion of the products of the hydrolysis of imidates (an interconversion which, according to Scheme II or III takes place through the same family of tetrahedral intermediates). Certain relationships between imidate hydrolysis and the corresponding acyl transfer reactions are summarized in a recent theoretical paper by Schmir[19] as follows:

1) The rate limiting step for these acyl transfer reactions may be either the addition of the nucleophile or the breakdown of the tetrahedral intermediate formed by that addition. The determination of the rate limiting step depends on the direction of the interconversion between the products with reference to the favored path of breakdown for the form of the tetrahedral intermediate which is predominantly present at the pH studied. For example, when the aminolysis of esters occurs in the pH range where the tetrahedral intermediate present breaks down primarily to amide, addition of the nucleophile will be the rate determining step.

2) The general appearance of the plot of rate vs pH for these reactions will be determined whether they fall into Scheme III or Scheme II (whether they involve positively charged and neutral intermediates or whether they involve neutral and anionic ones). At present, evidence exists to show that the hydrolysis of hydroxyanilides or the aminolysis of esters occurs via Scheme II while the aminolysis of thiol

esters probably occurs via Scheme III. The shape of the curve does not depend on which steps are rate limiting, but only on the state of dissociation of the tetrahedral intermediates involved and the transitions between them. A more thorough description of these concepts as well as the derivation of the kinetic expressions describing them will be found in the reference cited. However, one example which is particularly relevant is the pH-rate profile of the hydrolysis of a γ-hydroxyanilide to aniline and butyrolactone[4]. As such it will now be discussed.

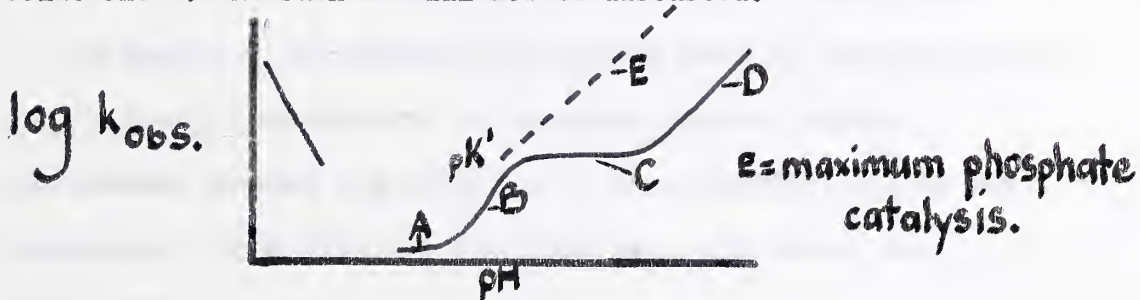
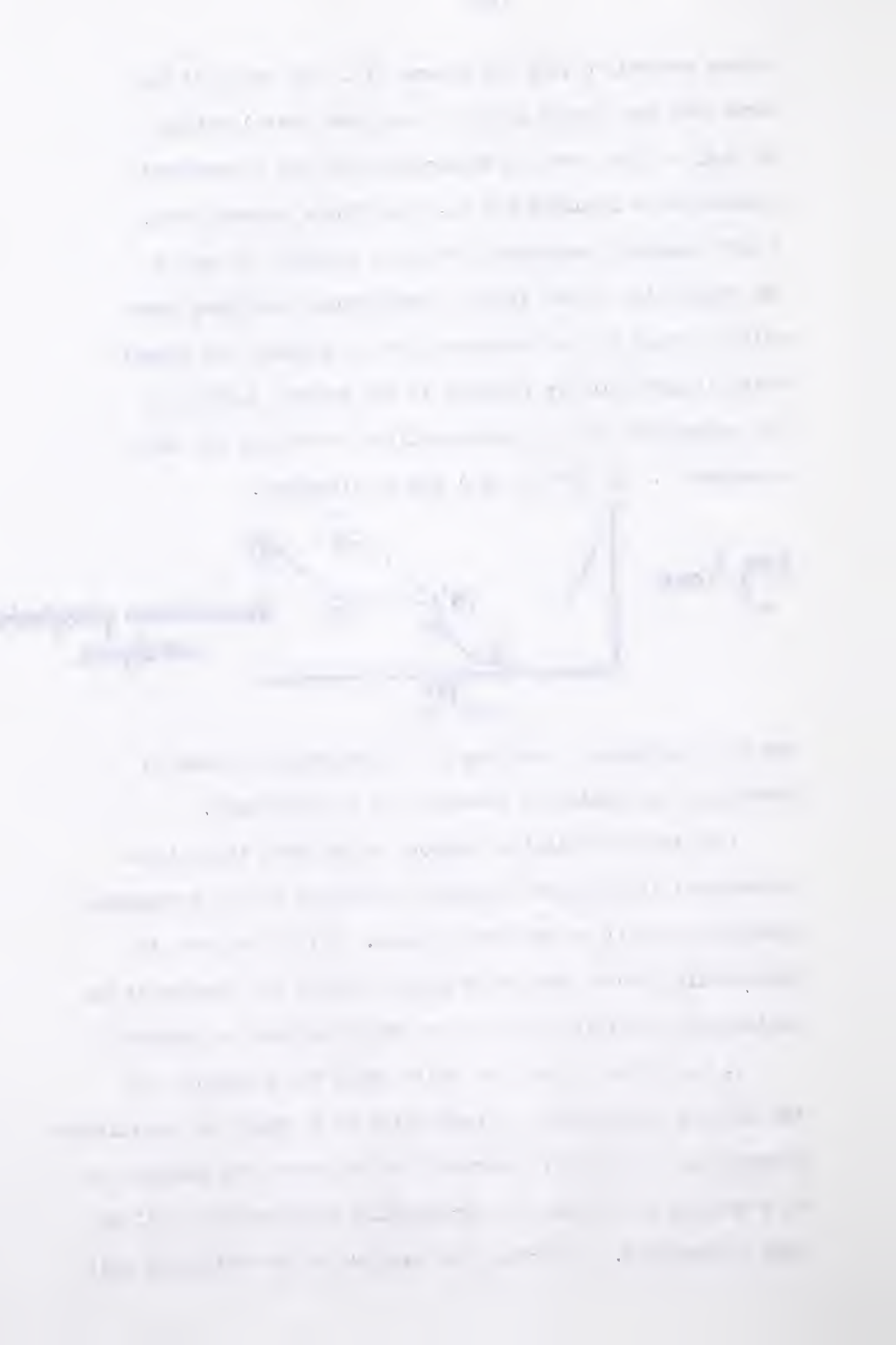


figure 10

For this hydrolysis, occuring by a mechanism analagous to Scheme II, the following features are of importance.

1-In the more alkaline regions of pH where the anionic tetrahedral intermediate prevails, breakdown of the tetrahedral intermediate will be rate determining. This is because the kinetically favored path of breakdown favors the reverse of the nucleophilic addition step and not the breakdown to products.

2- The effect of buffers which favor the breakdown of the neutral intermediate to amine will be to shift the equilibrium between the tetrahedral intermediates and speed the reaction up to a maximum rate where the nucleophilic addition step will be rate determining. The rate under maximum buffer catalysis will



therefore be determined by the rate of the nucleophilic addition step and its prerequisite requirement for hydroxide ion. As such the regions of the curve may be described as follows:

In region D, there is rate limiting breakdown of the anionic tetrahedral intermediate. The overall rate is subject to the pre-equilibrium which supplies that tetrahedral intermediate. As such it is directly dependant on the hydroxide ion concentration since base catalysis determines the concentration of the intermediate present in the steady state.

In region C, the availability of the route of interconversion via the neutral tetrahedral intermediate becomes evident. This pathway becomes significant at a rate corresponding to the diminution of hydroxide ion concentration. The curve thus levels off.

In region B, there is a deceleration in the rapidity with which the pathway via the neutral intermediate becomes available as we decrease the pH, as we are below the pK. The hydroxide ion concentration is so low that the nucleophilic addition step is rate determining.

In region A, the concentration of the neutral tetrahedral intermediate becomes high enough (compared to that of the anionic tetrahedral intermediate) so that the interconversion reaction proceeds primarily through TH. (see figure 8) There is rate limiting nucleophilic addition at a rate described by k_4' (in figure 8) and is independant of pH.

Because of their importance in biochemistry, and because

additional information on the nature of tetrahedral intermediates can be obtained from compounds with more than one product of hydrolysis, imidates have been extensively studied. These studies should seek to answer the questions posed by Schmir[19].

" 1) Do there exist several tetrahedral intermediates in acid base equilibrium with each other?

2) What is the effect of pH variation on the distribution of these species?

3) What is the mode of decomposition of each species?

4) How is the decomposition of each species affected by the presence of general acid-base catalysts? "

Section I

Chapter 3

Iminolactones

Iminolactones are cyclic iminoesters. They are usually generated by attack of the oxygen nucleophile of the ambident amide group on an activated saturated carbon center.

figure 11

Attack by the nitrogen atom under these circumstances leads to pyrrolidones which have often been isolated in attempts to synthesize iminolactones. In comparison to the multitude of other imidate derivatives which have been prepared, relatively few iminolactones have been isolated. Nevertheless, they have been proposed as intermediates in a number of important reactions. This is especially true of reactions involving peptides. A good review on the subject of iminolactones has been prepared by Cunningham[20]. References not directly cited in the following discussion may be found therein.

The importance of iminolactones in peptide chemistry has been their hypothesized intermediacy in a number of specific peptide cleavages. Examples have been the specific cleavage of peptides at points adjacent to tryptophane or tyrosine by N-bromosuccinimide or the cleavage at the carboxyl group of methionine in peptides treated with BrCN. The explanation given for the specificity of these cleavages has been stabilization

of the iminolactone intermediate by adjacent groups. Some of the more successful routes of preparations of iminolactones are probably direct analogies to the proposed mechanism of iminolactone intermediacy in peptide cleavage.

Several synthetic methods have been used to prepare inimolactones successfully. These involve the isolation of iminolactones following fusion of γ-bromoamides[21], or treatment of γ-chloroanilides with $AgBF_4$[1] according to the method of Peter et. al.[22]. Other methods have included reactions of amines with O-ethylbutyrolactonium tetrafluoroborate salts[23], or with 2,2, diethoxytetrahydrofuran[24]. A number of phenyliminotetrahydrofuran derivatives have been prepared by the last mentioned method. Another interesting method which has been reported involves the addition of azides to cyclic enol ethers[25] as shown in figure 12.

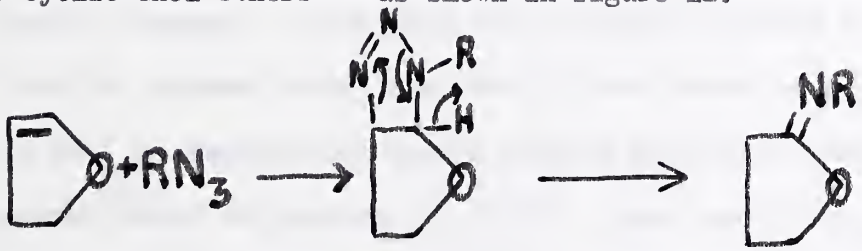


figure 12

In general, prior to the elucidation of the mechanism and products of hydrolysis of the iminolactone, 2-phenylimino-tetrahydrofuran, by Cunningham and Schmir[1], all that was known about the hydrolysis of iminolactones was that they generated amines, when hydrolyzed in acid. In addition to this, one instance of hydroxyanilide generation had been reported, from the decomposition of the methiodide ion of an iminolactone, when it was treated with aqueous potassium carbonate[20].

Other acyl group reactions that had been elucidated for iminolactones were exchanges of amine at elevated temperature. The mechanism worked out for iminolactone hydrolysis on the basis of the work with 2-phenyliminotetrahydrofuran was the basis for many of the conclusions discussed in Chapter 2.

The importance of the extension of the iminolactone investigation is evident from the considerations in this and previous chapters. In terms of the mechanims discussed in Chapter 2, involving acid base dissociations of tetrahedral intermediates, it becomes important to study derivatives of imidates, derivatives of varying structure to assess the effect on kinetics, products, and susceptibility to catalysis of modifications in structure. The p-nitro and p-methoxy derivatives of 2-phenyliminotetrahydrofuran were chosen for several reasons. Aside from their widely different basicities from the present amine, the substitutions would be expected to lead to compounds of higher melting point than that of the parent phenyl derivative (32-33° C)[1]. They would thus probably be easier to handle and purify. Furthermore, the availability of an assay for aniline compounds by a modification of the method of Bratton and Marshall[26, 27], would allow accurate product analysis. Of additional convenience was the yellow color of paranitroaniline, not possessed by p-nitro substituted anilides, and which was adapted for direct assay during kinetics and for product analysis (see Section II, Chapter 1.) Although reports of the preparation of the p-methoxyphenyliminotetrahydrofuran (isolated only as an oil (see appendix))[28], and of 2-p-nitro-

phenyliminotetrahydrofuran[25] were found in the literature at the start of this project, neither of the γ-chloroanilides needed for the proposed route of synthesis nor the desired γ-hydroxyanilides for study had been reported.

Section II

The iminolactone 2-p-nitrophenyliminotetrahydrofuran:

was prepared and purified for the reasons on pages 20-22, and the kinetics of hydrolysis as well as the products of hydrolysis were studied at varying pH's and with varying concentrations of monofunctional and bifunctional buffers.

Section II

Chapter 1

Materials and Methods

A- Materials

I- Solvents and buffers

Acetonitrile- was purified according to method D of Coetzee et. al.[29]. It was first stirred over CaH_2 (10g/l) for 48 hrs, then was distilled from P_2O_5 (5g/l). It was then redistilled.

Benzene- was distilled from sodium.

Tetrahydrofuran- Fisher reagent grade tetrahydrofuran (1 liter) was stored overnight over 50g of KOH and 5g of $KMnO_4$. It was then filtered by gravity and stored overnight over another 50g KOH and 5g $FeSO_4$. It was again filtered and distilled from sodium (bp 65.5°C).

Methylene Chloride- was distilled from P_2O_5 (5g/l).

Pyridine- annhydrous pyridine was stored overnight over BaO (10g/l) and then distilled (bp 115-16°C). The hydrochloride was prepared in ether using HCl gas. It was filtered and as it was hygroscopic, the pyridine hydrochloride was stored under vacuum over P_2O_5 and KOH pellets.

Imidazole- Eastman Kodak Co. imidazole was recrystallized from acetone-petroleum ether (bp 30-60°C fraction).

Tris (hydroxymethylaminomethane)- was purchased from Sigma Chemical Co. and was used without purification.

All other solvents or materials were reagent grade and were not further purified.

II- Synthesized materials and chemicals used in their preparation.

p-Nitroaniline- was recrystallized from hot 95% ethanol and recovered as yellow needles. (mp 147.2-.5°C). Its IR spectrum is shown in figure 13. In the UV, its principle maximum absorption occurs at 380 mμ. The wavelength used for assay of p-nitroaniline was 390 mμ (εmax 13, 800), compared to (εmax 600) for both the hydroxyanilide and the iminolactone, at 390 mμ. A standard curve drawn at 390 mμ is shown in figure 14.

4-chlorobutyroyl chloride- was purchased from Aldrich Chemicals and was distilled under water aspirator vacuum with the main fraction collected at 73-75°C (28mm Hg).

γ-chloro-p-nitrobutyranilide- was synthesized as follows: To 7.18g of 4-chlorobutyroyl chloride (50 mmoles) dissolved in 10 ml of tetrahydrofuran and stirred magnetically at 0°C, a solution of 13.8g (100 mmoles) of p-nitroaniline in 50ml of tetrahydrofuran was added by addition funnel over 10 min. Upon addition, a yellow precipitate was observed. The mixture was stirred for an additional 15 min at 0°C and then for 1 hr at room temperature. The solution was filtered and the precipitate identified by UV spectrum in water as p-nitroaniline hydrochloride. The solvent was removed from the filtrate in vacuo leaving a pale yellow crystalline product which was recrystallized from ethyl acetate-petroleum ether (30-60°) 1:1 with a final volume of 75ml. The product was collected by filtration and dried under vacuum, over P_2O_5 and KOH pellets. mp 98.8-99.0°C. Yield 8.1g (66% yield). The IR spectrum see figure 15

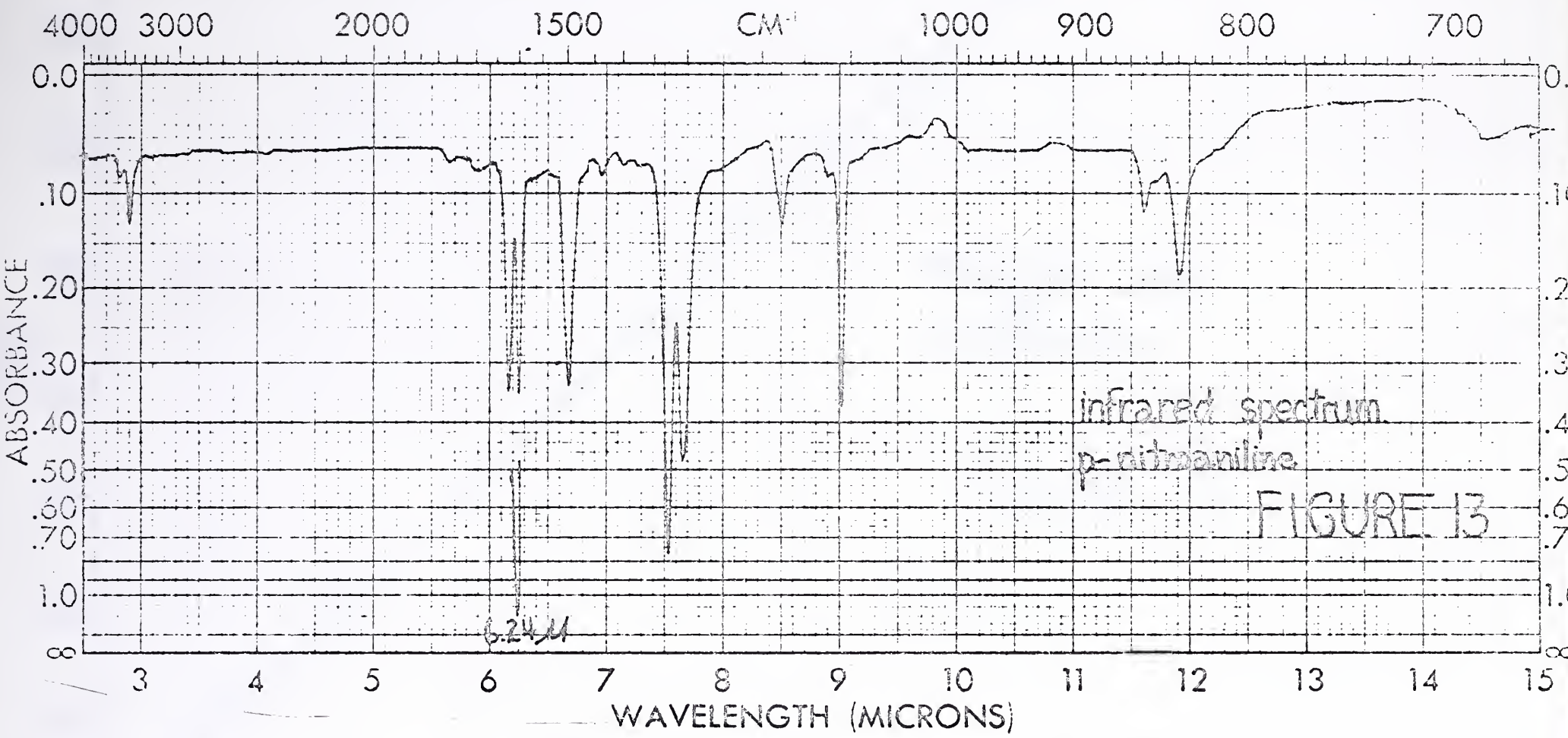

4000 3000 2000 1500 CM⁻¹ 1000 900 800 700
0.0
.10
.20
.30
.40
.50
.60
.70
1.0
∞
ABSORBANCE
6.24μ
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)
infrared spectrum
p-nitroaniline

FIGURE 13

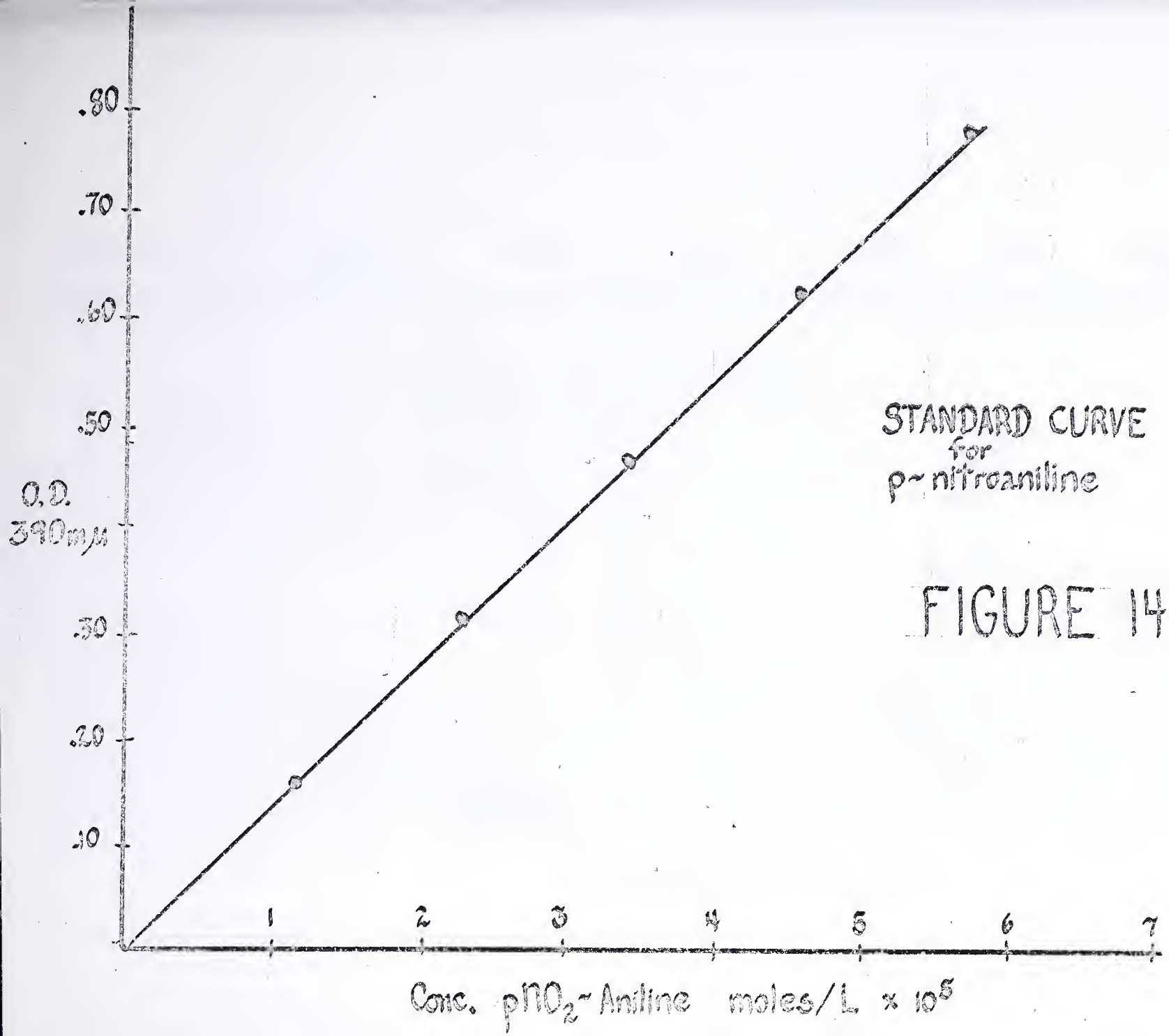
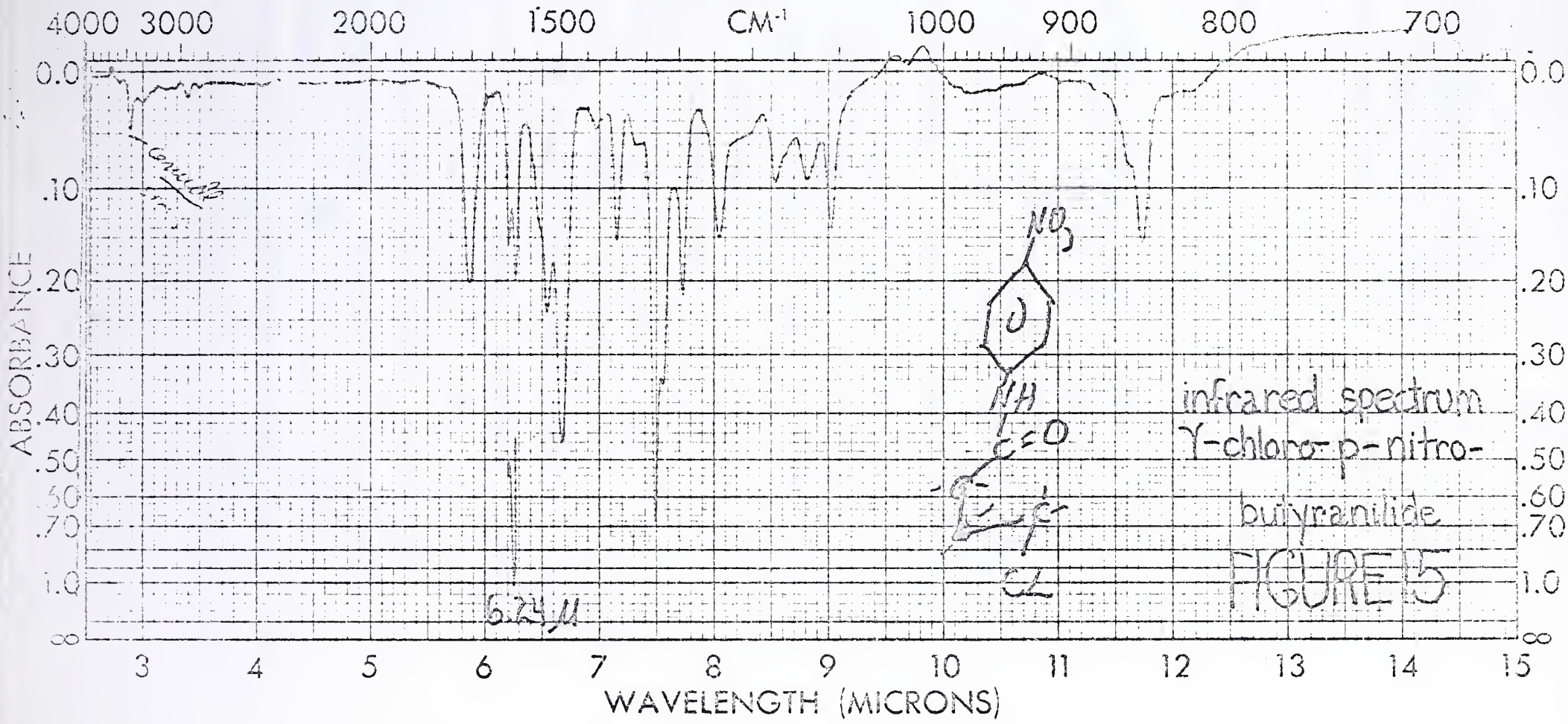

.80
.70
.60
.50
.30
.20
.10
O.D. 390mμ
1
2
3
4
5
6
7
Conc. pNO2-Aniline moles/L. × 10^5
STANDARD CURVE for p-nitroaniline

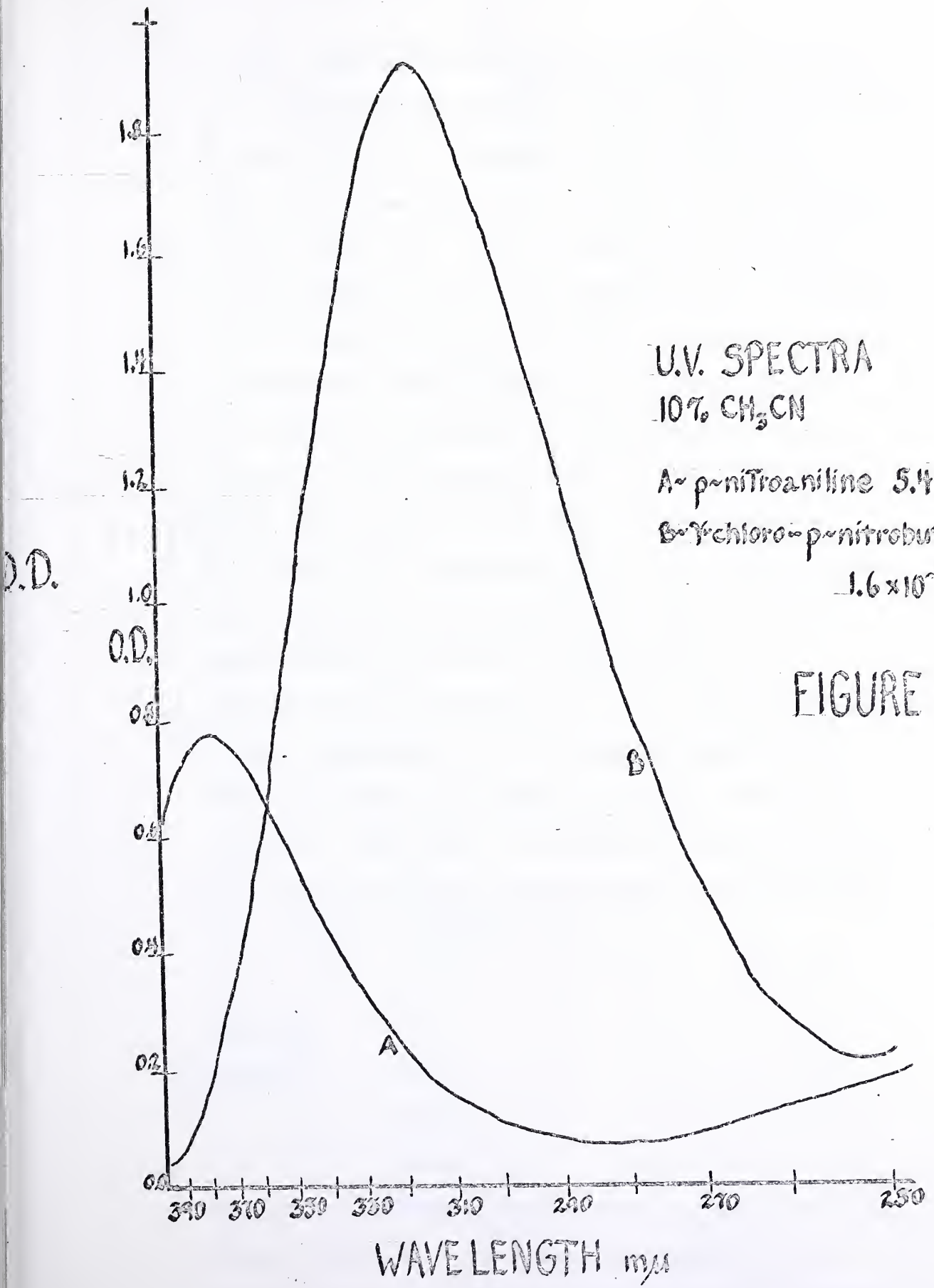
FIGURE 14


4000 3000 2000 1500 CM-1 1000 900 800 700
0.0
.10
.20
.30
.40
.50
.60
.70
1.0
∞
ABSORBANCE
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)
6.24 μ
NO2
NH
C=O
Cl
infrared spectrum
γ-chloro-p-nitro-
butyranilide
FIGURE 5

revealed a carbonyl absorption at 5.85 . The UV spectrum in acetonitrile revealed a maximum at 317 m with a molar extinction coefficient of 14,800. (figure 16).

Analysis	% C	H	N	Cl
Calculated	49,07	4,53	11,40	14,50
Received	49,58	4,75	11,66	14,32

2-p-nitrophenyliminotetrahydrofuran- was prepared as follows:[1, 22, 25] Silver tetrafluoroborate (Ozark Mahoning Co.), 6g (39 mmoles) was dissolved in 110 ml 1:1 benezene-methylene chloride and filtered through cellite on a vacuum funnel. A potassium thiocyanate solution was standardized by titration against a solution made up with primary standard $AgNO_3$. A ferric alum incicator was used in the titration. Two five ml aliquots of the $AgBF_4$ solution were then standardized against the potassium thiocyanate solution. The unknown solution was .263 N in silver ion and assuming all to be the tetrafluoroborate, 85 ml of the solution (ca. 25 mmoles) was added dropwise to a solution of 4.84 g of γ-chloro-p-nitrobutyranilide dissolved in 100 ml of methylene chloride. During the addition, the mixture was stirred in an ice salt bath at -17°C. The solutions were added over a period of 45 min with the immediate precipitation of AgCl. The mixture was then stirred for an additional 1 hr at 0°. Next, 18g of triethylamine hydrochloride was added cautiously to destroy excess silver tetrafluoroborate. The solution was then filtered to remove



U.V. SPECTRA
10% CH_3CN

A- p-nitroaniline 5.4×10^{-5} M

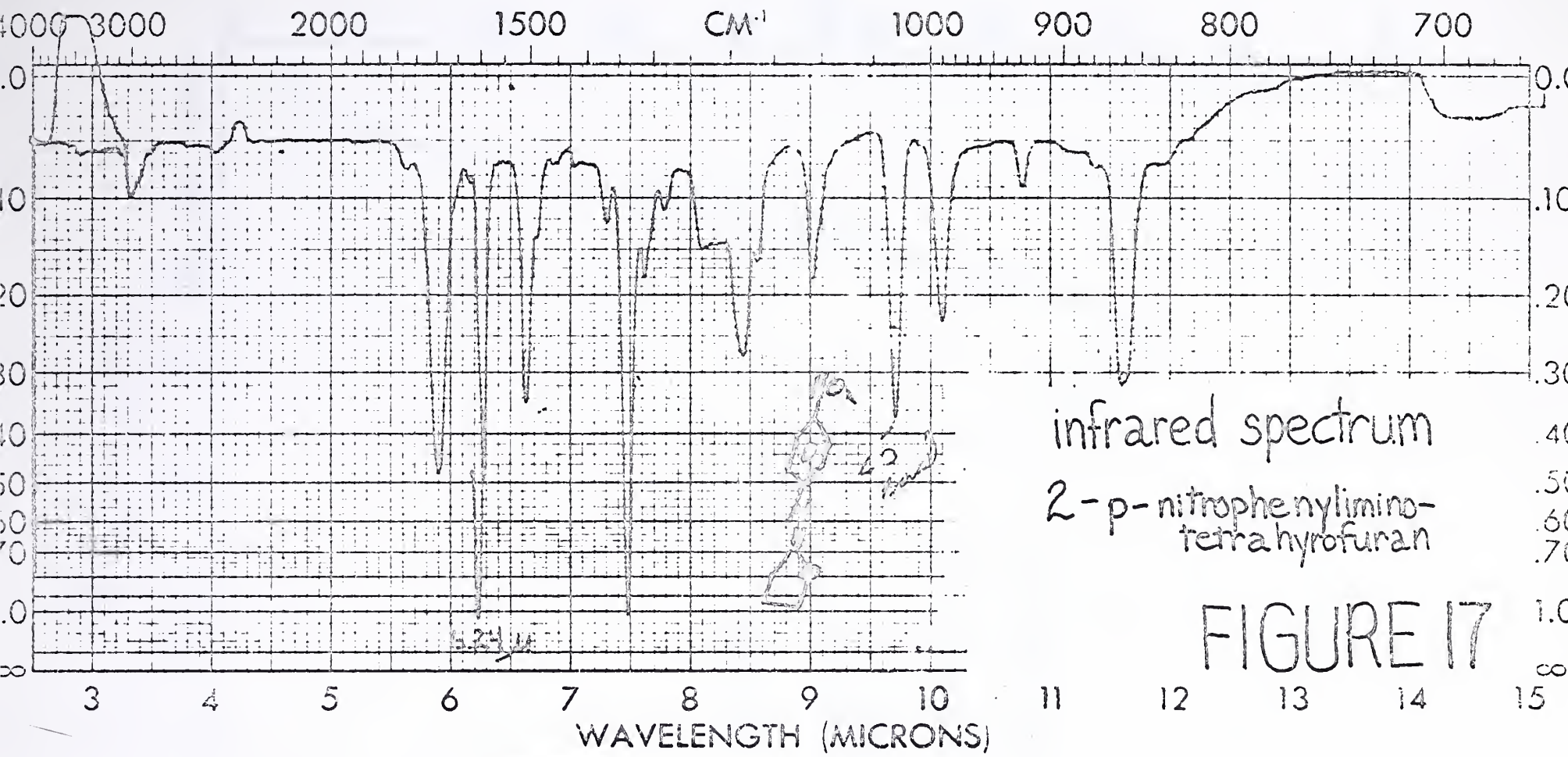
B- γ-chloro-p-nitrobutyranilide
1.6×10^{-4} M

FIGURE 16

the AgCl. The filtrate was extracted with 150 ml portions of 2M sodium carbonate and then saturated NaCl. The solution was then dried for 1 hr over 25 g of annhydrous $MgSO_4$ after which it was evaporated in vacuo on a rotary evaporator to a pale yellow oil. The oily residue was dissolved in 50 ml of annhydrous ether and crystallized upon the addition of 20 ml of petroleum ether (30-60°). It was collected by filtration and recrystallized from 1:1 ether: petroleum ether (30-60°) with recovery of small amounts of p-nitroaniline as an ether insoluble impurity. (mp 147°). The melting point of the recrystallized product was 67-68° C (lit. 62-65° C)[25]. Yield 2.15 g 53% yield. The synthesis was subsequently repeated with similar yield. The IR spectrum of the inimolactone in chloroform revealed a disappearance of the NH bonds ca. 3μ and an imine absorption at 5.92μ (figure 17). The UV spectrum revealed maximum absorbance at 318 m with a molar extinction coefficient of 14,500. (figure 18). A satisfactory elemental analysis was not obtained for this compound from either preparation after repeated recrystallizations.

	% C	H	N
Calculated:	58,25	4,89	13,59
Received:	50,91	5,02	13,82
	55,64	5,40	14,94
	56,01	5,13	10,22

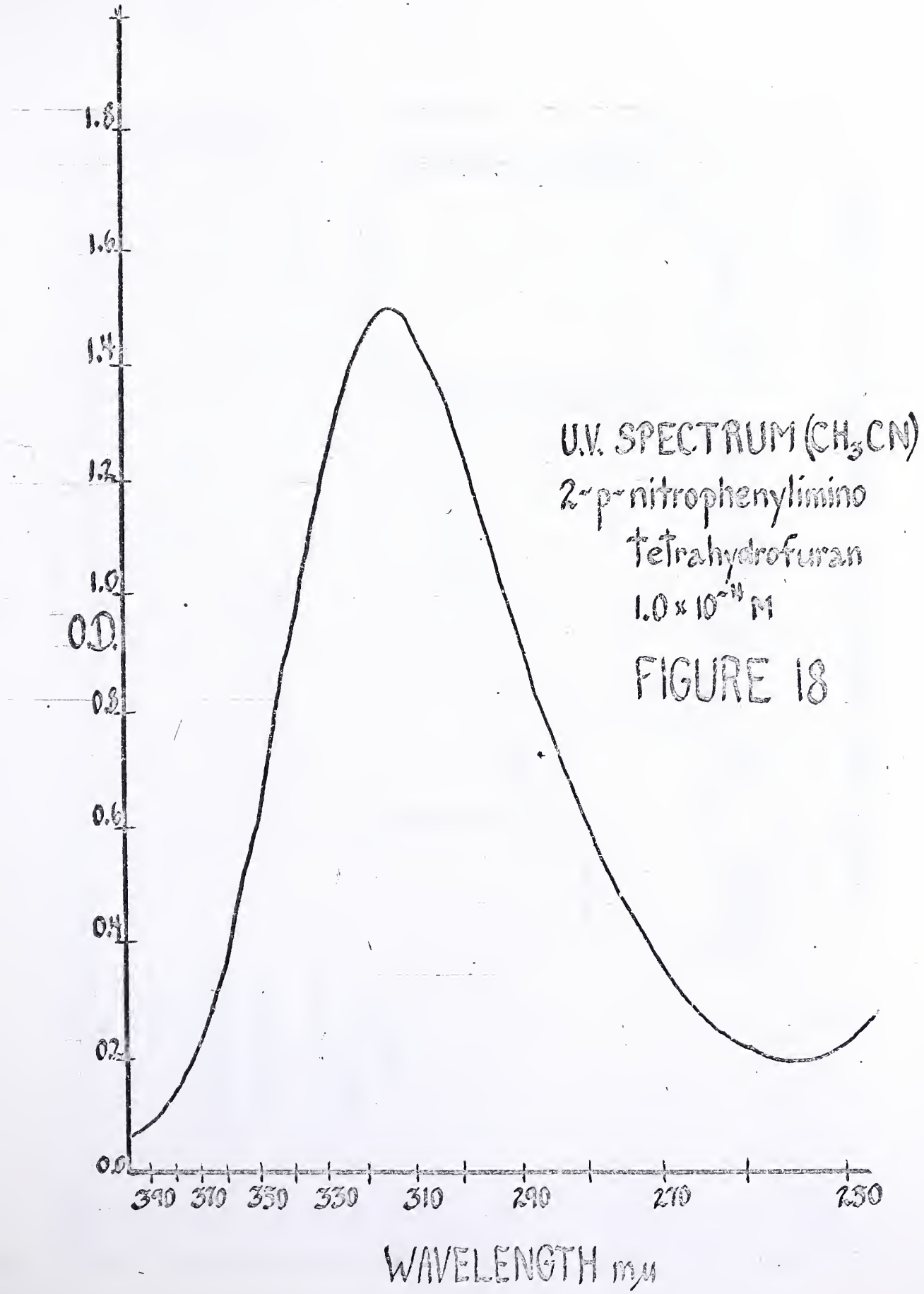
Therefore, for further characterization, an NMR spectrum was obtained (figure 19). The NMR spectrum accounts for all of the protons in the proposed structure and when integrated



infrared spectrum

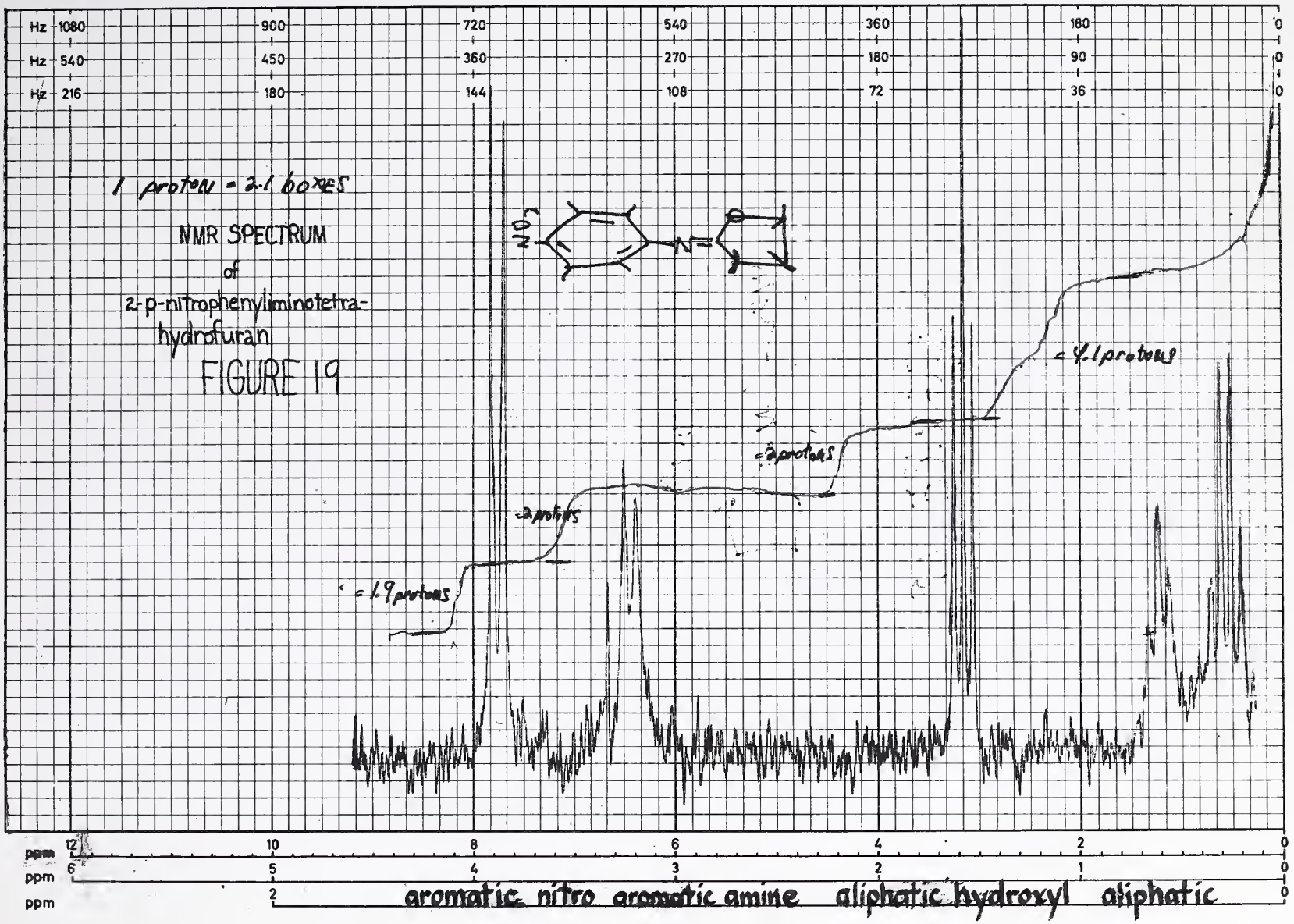
2-p-nitrophenylimino-tetrahyrofuran

FIGURE 17



U.V. SPECTRUM (CH_3CN)
2-p-nitrophenylimino
tetrahydrofuran
1.0×10^{-4} M

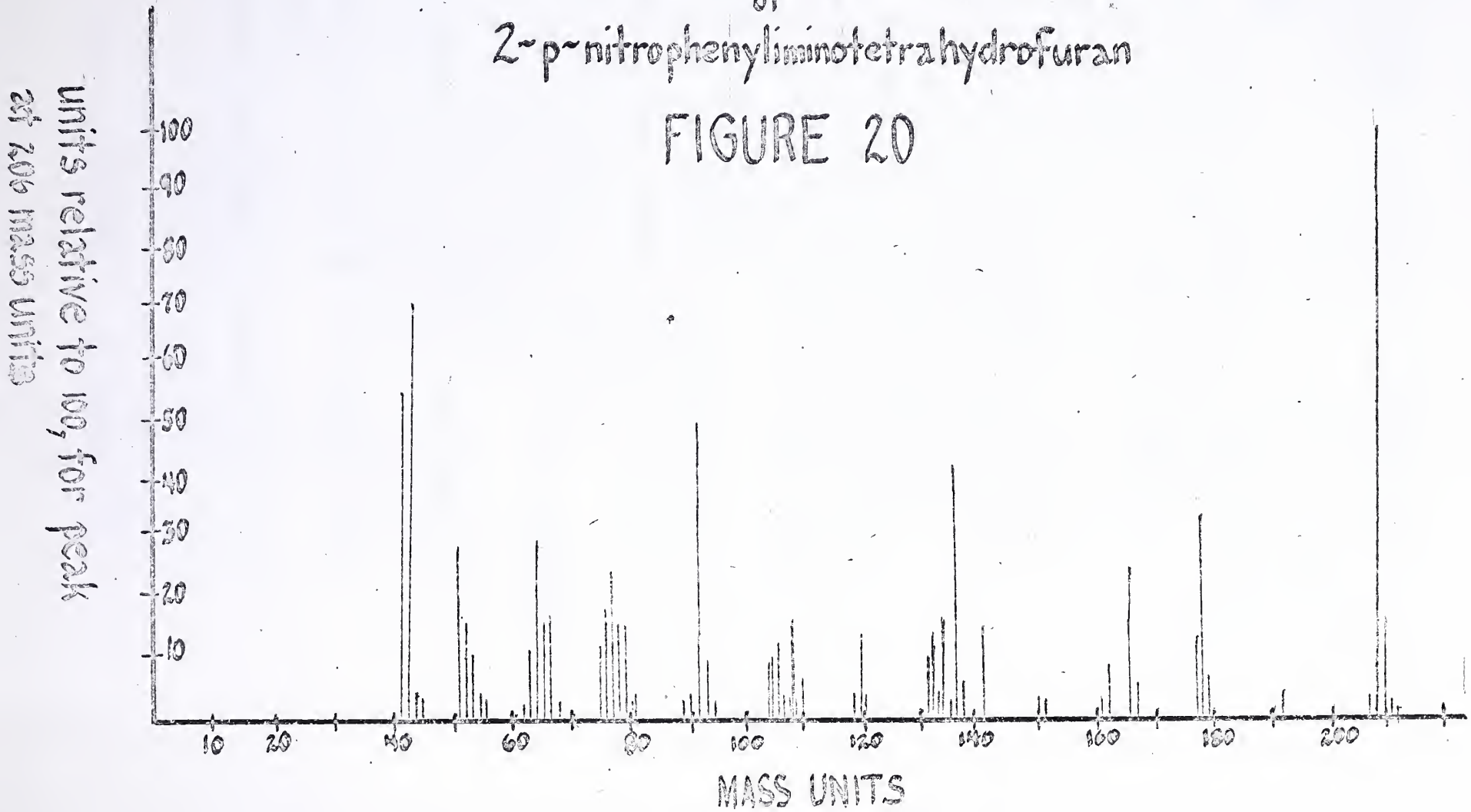
FIGURE 18


1 proton = 2.1 boxes
NMR SPECTRUM
of
2-p-nitrophenyliminotetra-
hydrofuran
FIGURE 19
≈ 1.9 protons
≈ 2 protons
≈ 2 protons
≈ 4.1 protons
aromatic
nitro aromatic
amine
aliphatic
hydroxyl
aliphatic

reveals no contamination with p-nitroaniline. A mass spectrum (figure 20) revealed a principle particle weight of 203, consistent with the proposed structure. Finally, the isolated product, generated a maximum yield of 96% of the expected amount of p-nitroaniline in an UV study and the other 4% of product could be accounted for by the addition of hydroxyanilide (see pages 45-46).

MASS SPECTRUM
of
2-p-nitrophenyliminotetrahydrofuran

FIGURE 20



B- Methods

Melting points- were determined in capillaries and are uncorrected.

Spectra

Infra red spectra were determined in a nujol mull on sodium chloride plates unless other wise specified. Recordings were obtained on a Perkin Elmer 137 sodium chloride spectrophotometer.

Ultraviolet spectra were performed in 1 cm square cuvettes with stoppers and were taken at concentrations ca. $1x10^{-4}$ M in acetonitrile for labile compounds. Otherwise, they were taken in 10% acetonitrile water. Appropriate blank solutions were placed in the reference cuvette. The recordings were obtained on a Perkin Elmer 350 recording spectrophotometer.

NMR spectra were performed in carbondisulfide with a tetramethyldisiloxane reference indicator on a Bruker Hx proton magnetic resonance spectrophotometer.

Mass spectra were performed on an Associated Electronics Industries M-S-9 mass spectrophotometer.

pH measurements- were performed on 3 ml samples of solutions with a Radiometer pHm 4D pH meter which had been standardized at pH 7 and pH 4 with Beckman standard buffer solutions.

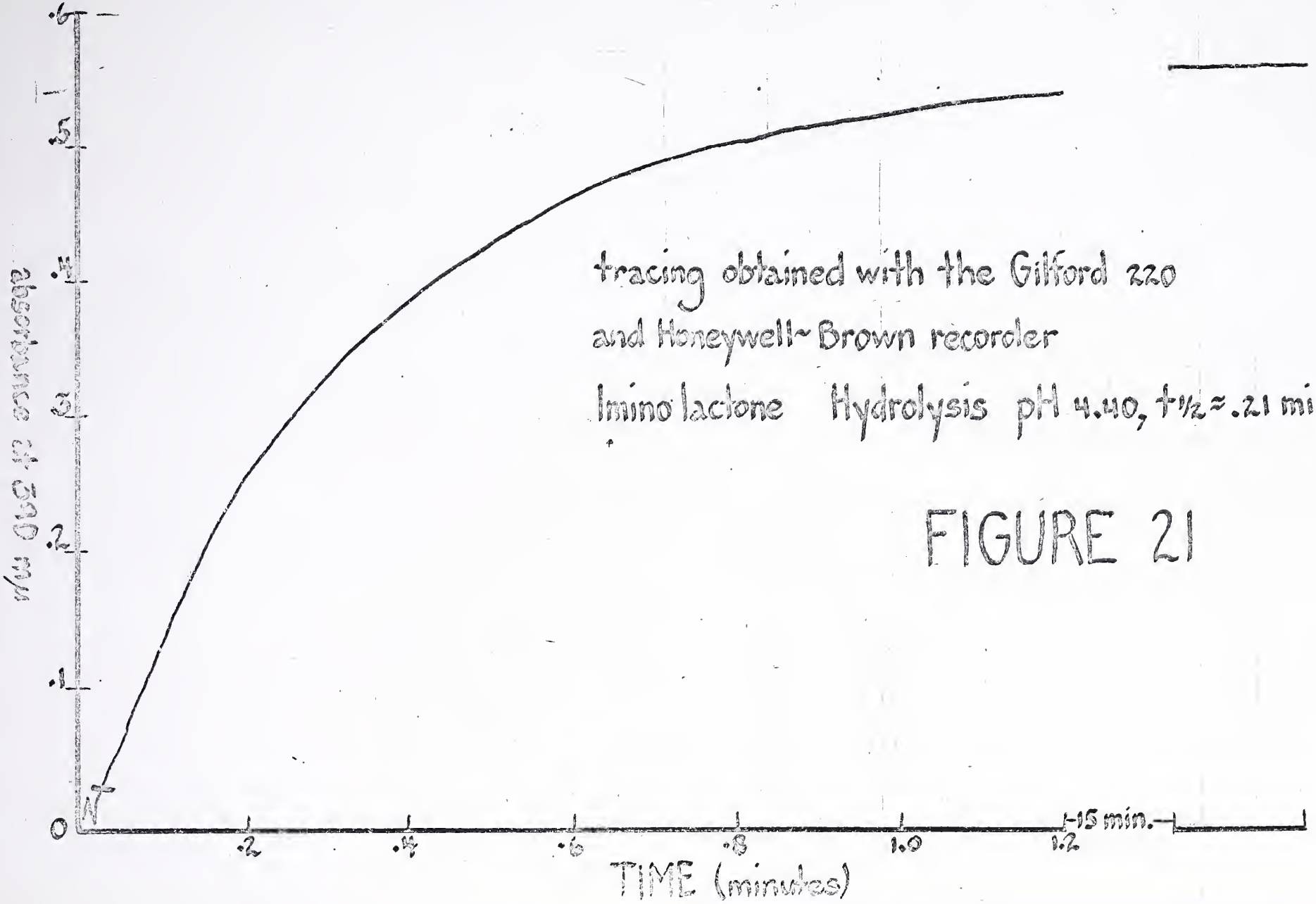
Solutions for reactions- all reactions were carried out with solutions of 10% acetonitrile in water. Concentrations of iminolactone were ca. $1x10^{-4}$ M (see results section for exact concentrations). All solutions were

made up with glass distilled CO_2 free water. For pH's above 7.55 Tris-HCl buffers were used. For pH's 6.1-7.55 Imidazole-HCl buffers were used. For pH's below 6.1, buffers were made with pyridine hydrochloride with the addition of carbonate free NaOH solutions. Phosphate buffers were prepared by mixing measured amounts of analytical grade mono and dibasic potassium phosphate salts in their annhydrous forms. These were dissolved in CO_2 free water and then an imidazole Tris, or pyridine buffer was made up to the same pH. The two solutions were added together and the pH's shown in the results section are those of the final mixtures. All reactions were made up to a final ionic strength of .5 using KCl.

Kinetic Measurements

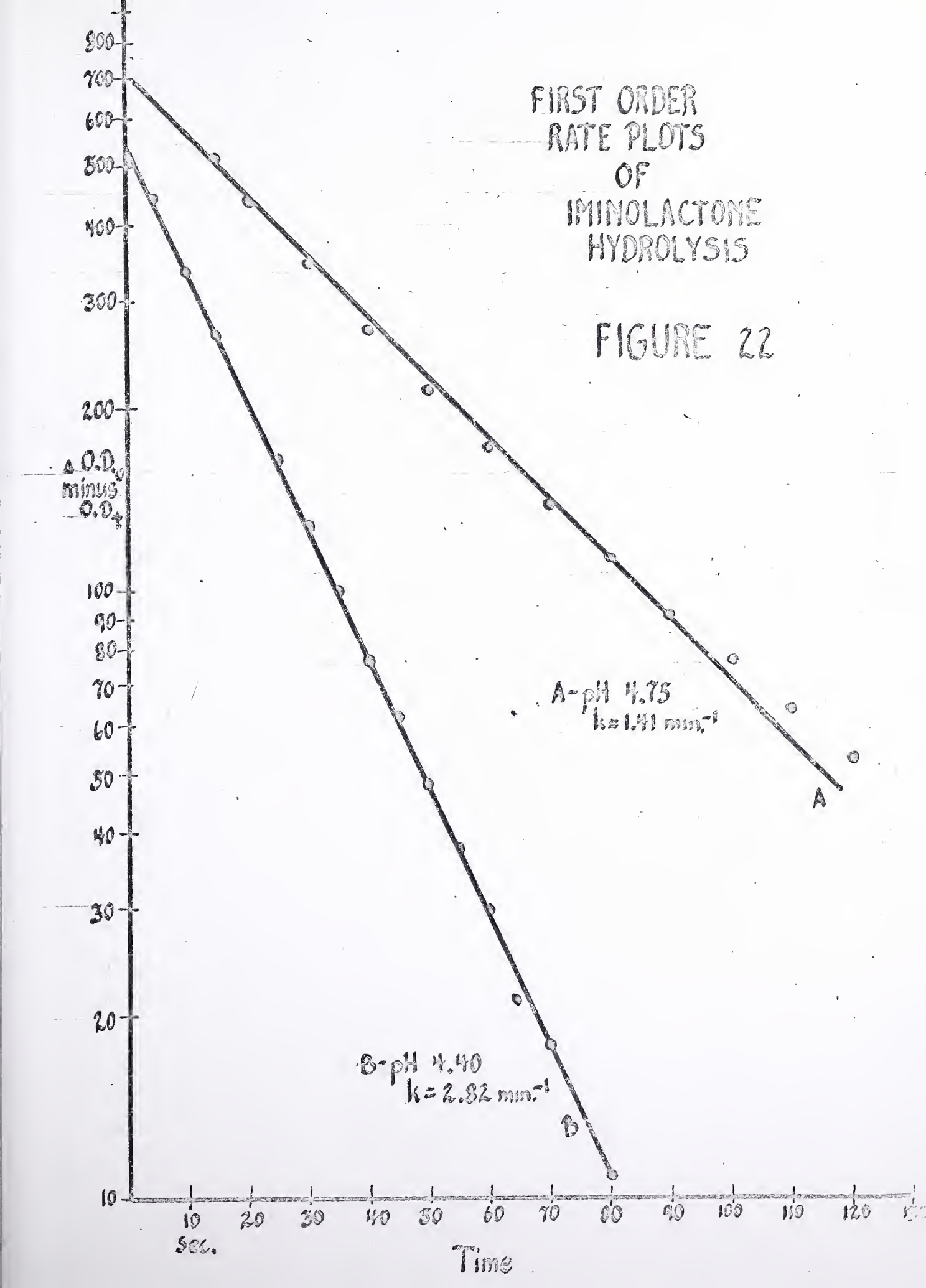
The rate of hydrolysis of the iminolactone was followed by the appearance of absorption of paranitroaniline at 390 mμ. All reactions were carried out at 30° C. Between pH 6.02 and 7.06 reactions were mixed in cuvettes and run on a Zeiss PMQ II spectrophotometer with a constant temperature head supplied with water from a Haake thermoregulator unit. They were followed for 10 half lives. At pH below 6, reaction mixtures were made up in cuvettes and the iminolactone in acetonitrile was added via a blowout pipette while mixing with a stream of air bubbles from a microcatheter. All cuvettes were stoppered. These faster reactions were run in a constant temperature head similarly thermo regulated and attached

to a Beckman DU spectrophotometer equipped with a Guilford 220 adapter for automatic readout and a Honeywell-Brown recorder. In the fastest reactions, the first recordings of OD could not be obtained until the end of the first half life using this technique because of the time necessary to accomplish the mixing and the manipulations required to activate the recording system. A sample recording obtained from the Honeywell-Brown recorder is shown in figure 21. The difference between the OD at completion (10 half lives) and the OD at time T was plotted against time on semi log paper to give plots such as that in figure 22. Rate constants were determined from the slope of the resulting line. Since above pH 6, the % yield of p-nitroaniline decreases rapidly, the concentration of iminolactone in the reaction mixtures had to be increased to up to 7×10^{-4} M to provide significant OD changes at 390 mμ. However, solutions above 1×10^{-3} M in iminolactone in 10% acetonitrile are slightly turbid so that at pH 7.54 where there is only 4.7% p-nitroaniline formed another assay had to be devised. The reaction was run at a concentration of iminolactone of 1×10^{-4} M and assayed using the assumption that whereas unreacted iminolactone in acid reacts rapidly and almost quantitatively to p-nitroaniline, any hydroxyanilide product formed will decompose to aniline at a rate less than one thousanth of that of iminolactone decomposition in acid. Therefore, if the amount of p-nitroaniline formed at pH 7.5 is minimal and if the abosrbance of hydroxyanilide



tracing obtained with the Gilford 220
and Honeywell-Brown recorder
Imino lactone Hydrolysis pH 4.40, $t_{1/2}$ ≈ .21 min.

FIGURE 21


FIRST ORDER
RATE PLOTS
OF
IMINOLACTONE
HYDROLYSIS
FIGURE 22
800
700
600
500
400
300
200
100
90
80
70
60
50
40
30
20
10
Δ O.D. minus O.D.t
A- pH 4.75
k = 1.41 min.-1
A
B- pH 4.40
k = 2.82 min.-1
B
10
20
30
40
50
60
70
80
90
100
110
120
sec.
Time

at 390 mμ is minimal (see page 25), then the amount of p-nitroaniline (absorpance at 390 mμ) generated from the iminolactone after acidification will be a reliable index of how much unreacted iminolactone was left in the reaction mixture at time T. As the major product of hydrolysis in alkali is hydroxyanilide, and as there is no major difference between the UV spectra of the hydroxyanilide and the iminolactone (see figures 18 and 34), the above assay was the only available method of obtaining rate data at pH above 7.1. Therefore, the reaction mixture was mixed in a volume of 50 ml and was incubated in a volumetric flask in a constant temperature bath at 30° C. At various times a 5 ml aliquot of the reaction mixture was pipetted into 2 ml of IM PO_4 at pH 2.23. The final pH was 2.3. The OD at 390 was read 2 min after acidification.

Determination of Products

The determination of products was based on known concentrations of iminolactone of exactly 1.0×10^{-4} M in the reaction mixtures. The concentration of p-nitroaniline was determined at the completion of the reactions (>10 half lives). The runs from which the products were determined were separate from those used for kinetics. The true concentration of p-nitroaniline in the final mixtures was determined by calculation from an equation containing the molar extinction coefficients of both the aniline and the hydroxyanilide products, thus:

$1.380\ x + .060\ y$ = OD read at completion (1)

and $x + y = 1 \times 10^{-4}$ (2)

The final concentration of p-nitroaniline was then used to compute the % p-nitroaniline as product. Reactions were run in 10 ml volume in stoppered test tubes, in a constant temperature bath at 30°C. This volume allowed determination of pH at the beginning and end of each reaction, (these were always within .02 pH units) and duplication of OD measurements. OD's were measured in open cuvettes on a Beckman DU spectrophotometer. A final set of experiments, to identify the second product as hydroxyanilide and establish the purity of the iminolactone, involved the comparison of UV spectra of reaction mixtures, at completion, with synthetic mixtures of p-nitroaniline and γ-hydroxy-p-nitrobutyranilide made up under identical conditions and in proportions calculated from the percent yield of p-nitroaniline, at four pH's. The total concentrations of the two compounds in the synthetic mixture was the same as that of the iminolactone placed in the reaction mixture (1×10^{-4} M). The solutions were made up at volumes of 50 ml. The variance of pH provided product compositions varying from 96% p-nitroaniline to 96% hydroxyanilide and only 4% p-nitroaniline. The results and spectra for comparison are in the next chapter. (figures 25-32, page 45).

Computer Analysis of Data

Computer analysis was performed only on the data

relating % yield of p-nitroaniline to the concentration of phosphate. The data were fitted to a rectangular hyperbola and the constants of the hyperbola were calculated using a least squares program of Bliss and James[30]. We are indebted to Dr. K.R. Hansen of the Conn. Agricultural Experiments station for use of the program. Calculations were done by an IBM 7094 computer.

Section II

Chapter 2

Results

The rate of hydrolysis of the iminolactone 2-p-nitrophenyliminotetrahydrofuran was studied at 30 C in the range of pH 4.1-7.6. Below pH 4.1 the rate of hydrolysis was too rapid for the reaction to be followed with the equipment available. Above pH 7.6, the finding that most of the product was the hydroxyanilide made it necessary to follow the decomposition of the iminolactone by the acidification assay described on page 31-32.

The results obtained from these kinetic studies are presented in Table I and a graphic representation of the data is found in Figure 23.

Table I

Effect of pH on Hydrolysis of Iminolactone

at 30 C

pH	k_{obs} min^{-1}	Buffer	Note
4.16	4.78	Pyridine	0
4.40	2.82	Pyridine	0
4.75	1.41	Pyridine	0
4.95	8.34×10^{-1}	Pyridine	0
5.01	7.22 "	Pyridine	0
5.34	3.25 "	Pyridine	0
5.66	1.68	Pyridine	0
6.02	7.52×10^{-2}	Pyridine	0
6.45	4.44 "	Imidazole	1
6.74	2.63 "	Imidazole	1
7.06	$1.8\text{-}2.0 \times 10^{-2}$	Imidazole	2
7.54	3.76×10^{-3}	Imidazole	3

0 All reactions were carried out in 10% acetonitrile by volume with buffer conc. .03 M, ionic strength .5. These were followed for 10 half lives at 390 m with OD changes from ca. .060-.980 using 6×10^{-5} M iminolactone.

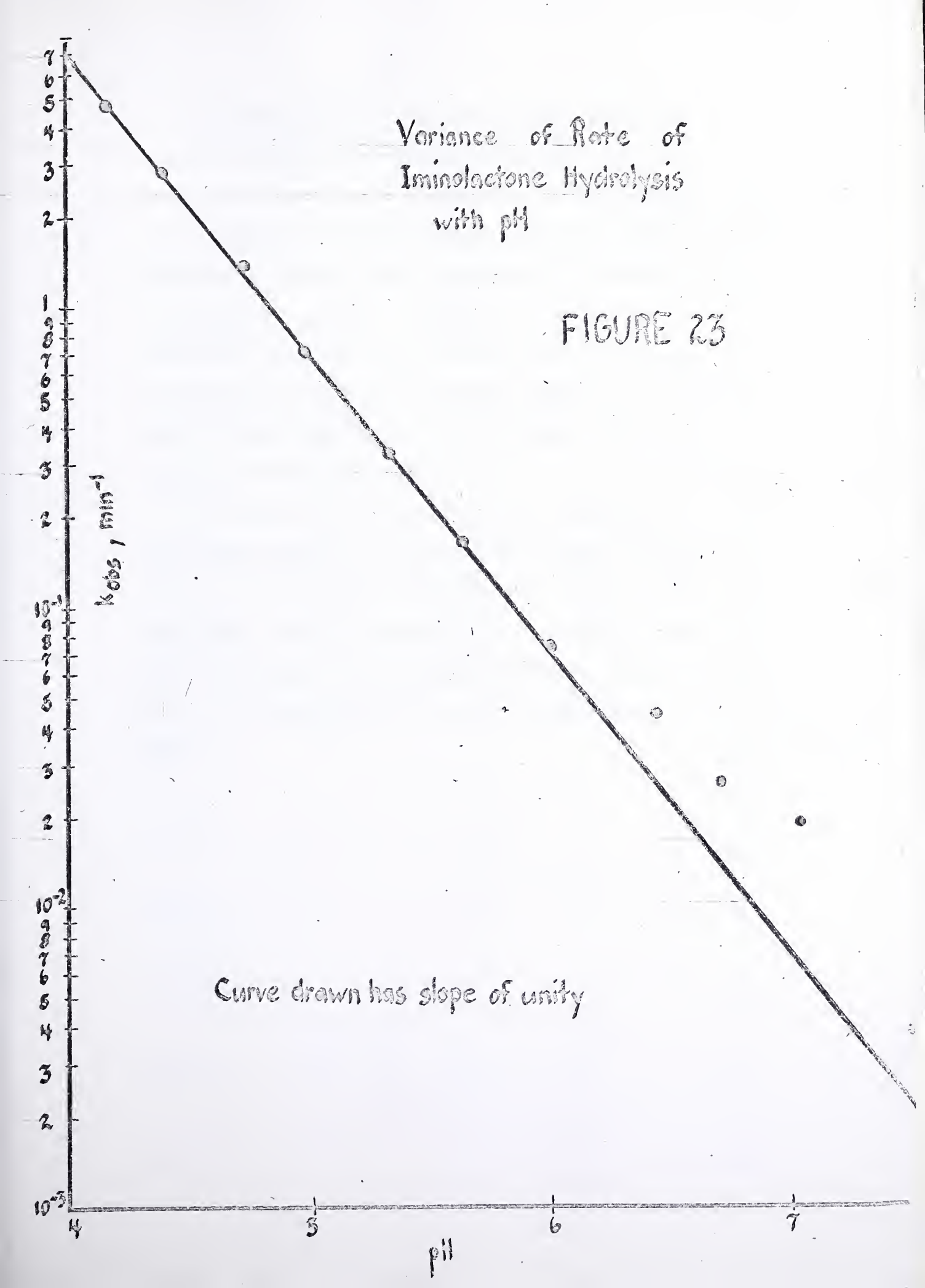
1 Followed for 7 half lives with OD change from .240-.590 at pH 6.45 and from .240-.390 at pH 6.73 with iminolactone conc. 2.5×10^{-4} M.

2 Followed for 7 half lives with OD changes from .720-

Table I (continued)

.950 with 7×10^{-4}M iminolactone.

3 Since at this pH the yield of p-nitroaniline is only 4%, the reaction was run with a concentration of iminolactone of 1×10^{-4}M and was followed by the acidification assay described on page 31 for 7 half lives with OD changes of from .580-.063.


Variance of Rate of Iminolactone Hydrolysis with pH
FIGURE 23
k_{obs}, min^{-1}
Curve drawn has slope of unity
pH
4
5
6
7

The rate of hydrolysis varies linearly with pH with a slope of unity on the semilogarythmic plot. The lack of data below pH 4.1 made us unable to determine whether a bell shaped plot similar to those reported for other imidate compounds (see pages 47-48) could be obtained. It was also not possible to estimate the pK of the iminolactone from the present kinetic data. It can also be noted that the points above pH 6.5 suggest a deviation from the linear relationship just described. This will be further discussed on page 48.

The yield of p-nitroaniline product obtained from the complete hydrolysis of the iminolactone was determined over the same pH range as that where rate data had been accumulated. Data on the variation of % p-nitroaniline yield with changes in concentration of monofunctional buffers was also obtained. This data is presented in Table II.

Table II

Effect of pH on Paranitroaniline Yield

Buffer	Buffer Conc. M	pH	Time of Assay hrs	% p-nitro-aniline
Imidazole	.03	7.55	30	4.8
Imidazole	.02	7.05	7	5.10
Imidazole	.06	7.06	7	4.9
Imidazole	.1	7.06	7	4.8
Imidazole	.02	6.84	7	6.2
Imidazole	.06	6.83	7	5.6
Imidazole	.1	6.83	7	5.5
Imidazole	.02	6.58	3	11.9
Imidazole	.1	6.57	3	10.2
Imidazole	.02	6.35	2.5	24.4
Imidazole	.06	6.34	2.5	18.7
Imidazole	.1	6.33	2.5	16.1
Imidazole	.02	6.08	1.5	58.0
Imidazole	.06	6.06	1.5	53.4
Imidazole	.10	6.05	1.5	50.3
Imidazole	.02	6.03	1.5	60.1
Imidazole	.1	6.02	1.5	50.3
Pyridine	.02	5.82	1	75.8
Pyridine	.06	5.81	1	69.8
Pyridine	.1	5.81	1	65.8
Pyridine	.02	5.67	1	82.5
Pyridine	.06	5.67	1	78.6
Pyridine	.1	5.67	1	74.6
			Time, min	
Pyridine	.02	5.49	40	87.6
Pyridine	.06	5.48	40	84.2
Pyridine	.1	5.48	40	82.8
Pyridine	.02	5.21	40	91.4
Pyridine	.06	5.20	40	89.9
Pyridine	.1	5.20	40	89.0
Pyridine	.02	5.01	10	93.6
Pyridine	.06	4.99	10	92.5
Pyridine	.1	4.98	10	92.1
Pyridine	.02	4.85	10	94.1
Pyridine	.06	4.84	10	93.6
Pyridine	.1	4.83	10	93.5

Table II (continued)

Buffer	Buffer Conc. M	pH	Time of Assay hrs	% p-nitro aniline
Pyridine	.02	4.51	5	94.6
Pyridine	.06	4.50	5	93.8
Pyridine	.1	4.50	5	93.7
Pyridine	.02	4.18	3	96.5
Pyridine	.06	4.18	3	95.4
Pyridine	.1	4.17	3	94.6
Phosphate	.1	2.5	1	96.0
HCL	.0100	2.0	1	96.2

The calculations of % p-nitroaniline yield at varying buffer concentrations are based on optical density measurements. To assure that no change in the extinction coefficient of p-nitroaniline occured with changing buffer concentrations, a control experiment was run. Solutions of 3.6×10^{-4}M p-nitroaniline in 10% acetonitrile were incubated with a pyridine-HCl buffer (ionic strength .5) at 30° for 1 hr (equivalent to the time of incubation of the hydrolysis of iminolactone used to obtain the product data at that pH). The results are expressed in Table III.

Table III

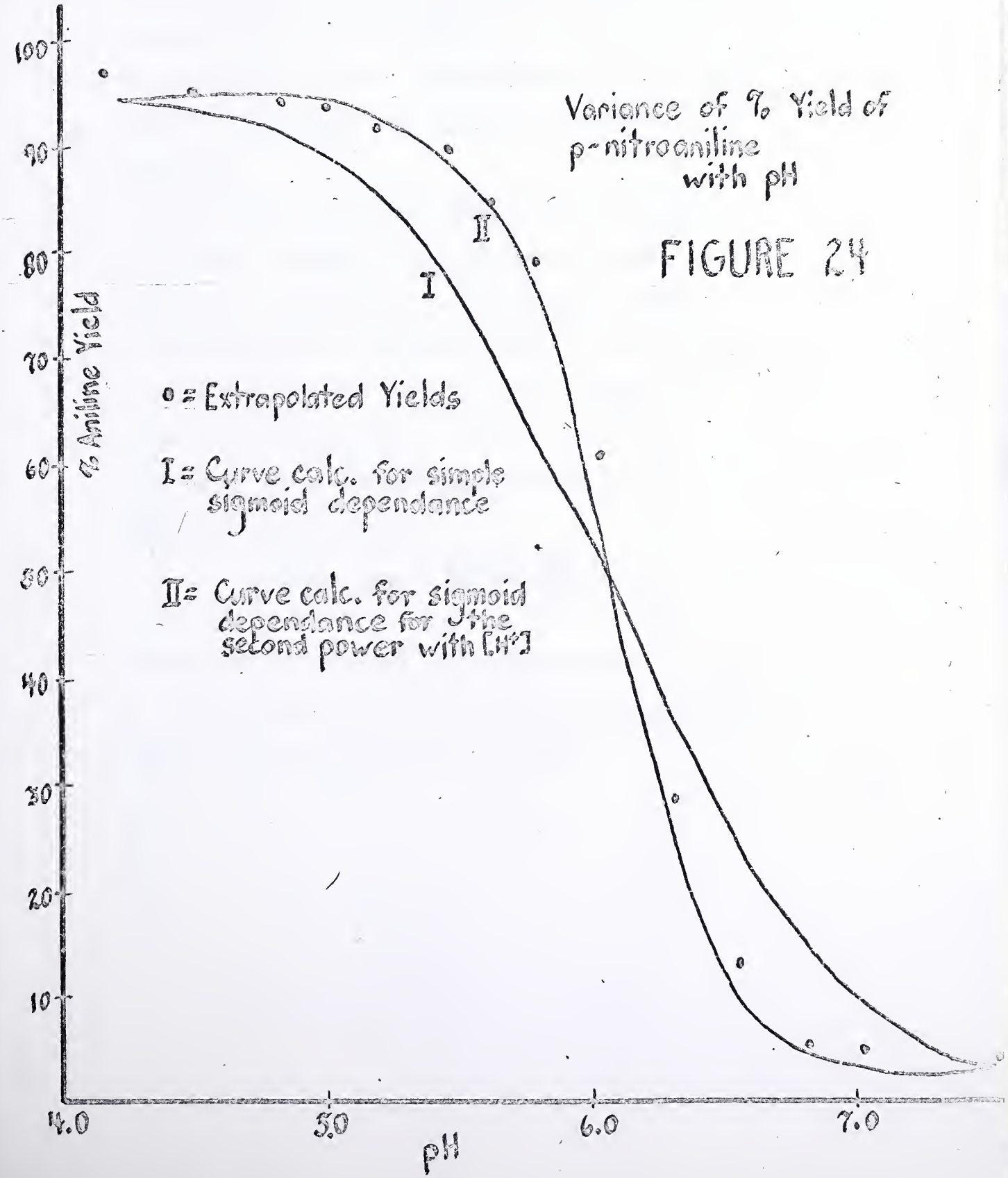
Concentration of Pyridine Buffer	pH	OD at 390 m
.02M	5.67	.488
.06M	5.67	.490
.10M	5.66	.492

Since, at constant pH, the yield of amine depended on buffer concentration, the % yield of p-nitroaniline was extrapolated to zero buffer concentration at each pH. The extrapolated values are found in table IV, and are represented graphically as a function of pH in Figure 24.

Table IV

Effect of pH on Yield of p-Nitroaniline Corrected for Buffer Effects

Buffer	pH	Extrapolated yield of p-nitroaniline %
Imidazole	7.55	4.9
Imidazole	7.05	5.4
Imidazole	6.83	6.7
Imidazole	6.57	13.5
Imidazole	6.34	29.0
Imidazole	6.06	61.0
Pyridine	5.81	79.5
Pyridine	5.67	85.0
Pyridine	5.48	90.0
Pyridine	5.20	92.0
Pyridine	4.99	94.0
Pyridine	4.84	95.2
Pyridine	4.17	97.0


Variance of % Yield of p-nitroaniline with pH
FIGURE 24
% Aniline Yield
100
90
80
70
60
50
40
30
20
10
II
I
o = Extrapolated Yields
I = Curve calc. for simple sigmoid dependance
II = Curve calc. for sigmoid dependance for the second power with [H+]
4.0
5.0
6.0
7.0
pH

The curve describing the dependance of amine yield on pH is generally sigmoidal in shape with a minimum yield of 4.8% and a maximum yield of 97%. The midpoint occurs at pH 6.1. The curve is somewhat steeper than that calculated for the simple sigmoid dependance expected from previous studies with the unsubstituted iminolactone[1] (Curve I, Figure 24). This behavior is further discussed on page 49.

The effect of phosphate buffer on the yield of p-nitroaniline obtained on hydrolysis was determined at constant pH in the presence of .03M imidazole buffer. Low concentrations of phosphate buffer lead to an increase in amine yield (Table V). The curves describing this effect at four pH's (Figures 25-28) are rectangular hyperbolas. The parameters K_{app} and Yield $_{max}$ were calculated from the equation:

$$\frac{\text{Yield}}{\text{Yield}_o} = \frac{[\text{Buffer}]}{[\text{Buffer}] + K_{app}} \qquad (3)$$

using the computer program previously described on page 34. The calculated values for the parameters K_{app} and Yield $_{max}$ for each pH are found in Table V.

Table V

Effect of Phosphate Buffer Concentration on Yield of p-nitroaniline

pH	other Buffer	Conc. Phosphate M	% Yield p-nitroaniline	Time of Assay
6.10	.03 M Imidazole	0	39.8	3 hrs
6.12	"	.001	43.2	
6.13	"	.002	46.6	
6.16	"	.005	52.4	
6.15	"	.01	59.2	
6.16	"	.02	67.2	
6.16	"	.05	74.0	
6.16	"	.12	80.2	
6.17	"	.25	83.7	
	$Yield_{max} = 84.8 \pm .7$ %		$K_{app} = .013 \pm .0007$ M	
6.28	.03 M Imidazole	0	26.1	5 hrs
6.28	"	.001	34.7	
6.28	"	.002	39.9	
6.31	"	.005	45.5	
6.28	"	.01	51.3	
6.29	"	.02	60.4	
6.34	"	.05	69.3	
6.38	"	.12	74.1	
6.39	"	.25	77.6	
	$Yield_{max} = 77.1 \pm 1.8$ %		$K_{app} = .019 \pm .001$ M	
6.74	.02 M Imidazole	0	6.26	6 hrs
6.74	.03 M Imidazole	.002	11.8	
6.74	"	.005	17.5	
6.75	"	.01	28.9	
6.75	"	.02	37.3	
6.75	"	.03	46.2	
6.77	"	.06	57.5	
6.78	"	.23	72.4	
	$Yield_{max} = 78.8 \pm 2.1$ %		$K_{app} = .026 \pm .002$ M	

Table V (continued)

pH	other Buffer	Conc. Phosphate M	% Yield p-nitroaniline	Time of Assay
7.54	.03 M Imidazole	0	4.88	30 hrs
7.54	"	.002	5.70	
7.55	"	.005	6.35	
7.52	"	.01	7.60	
7.53	"	.03	13.5	
7.52	"	.06	18.6	
7.56	"	.12	24.7	
7.55	"	.18	28.8	
7.55	"	.25	32.0	
7.55	"	.50*	38.5	

$Yield_{max} = 46.3 \pm .8$ % $K_{app} = .12 \pm .006$ M

All reactions run at iminolactone 1×10^{-4} M

* Ionic strength greater than .5.

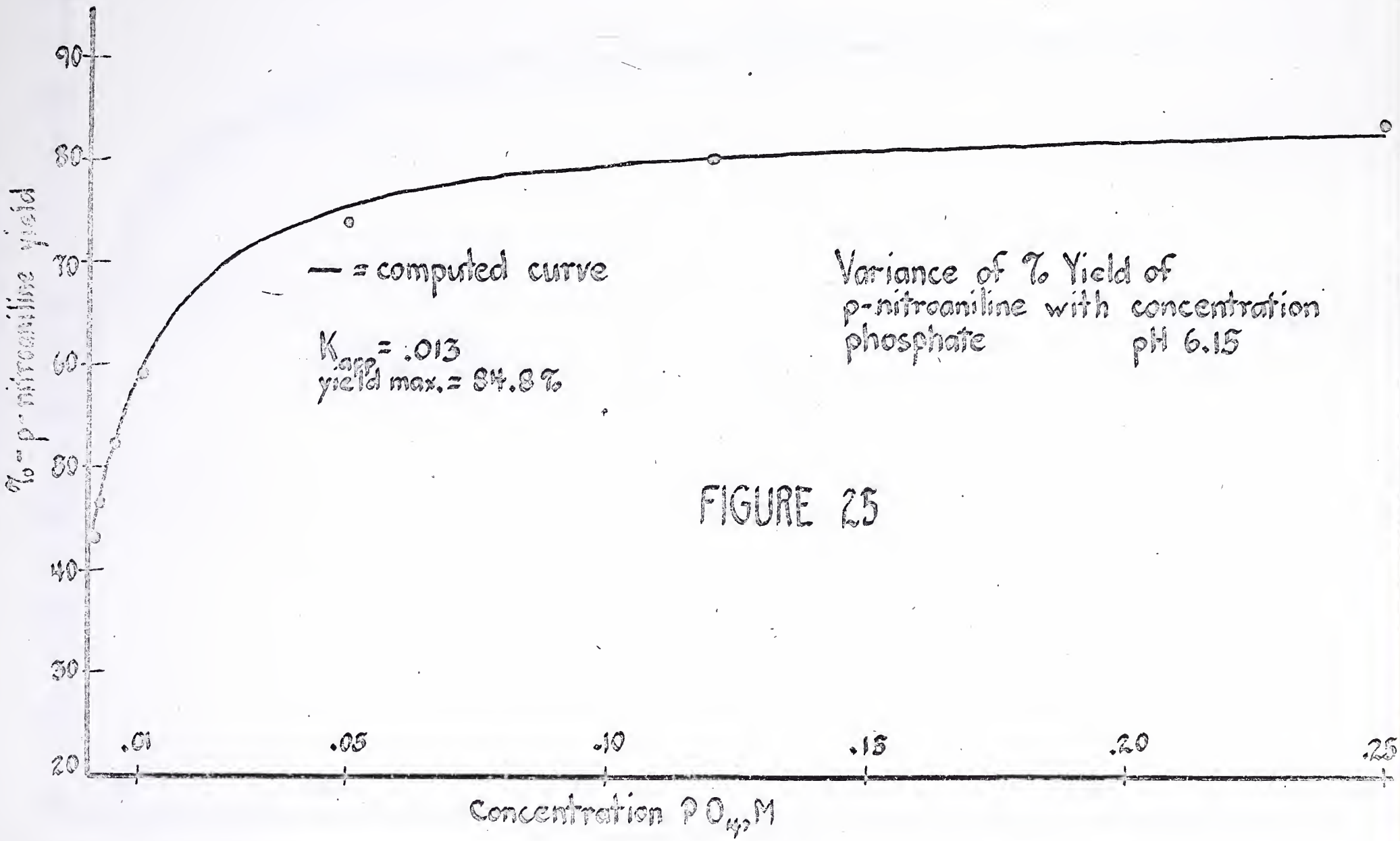

90
80
70
60
50
40
30
20
% p-nitroaniline yield
— = computed curve
K_{app} = .013
yield max. = 84.8 %
Variance of % Yield of
p-nitroaniline with concentration
phosphate pH 6.15
.01
.05
.10
.15
.20
.25
Concentration PO_4, M

FIGURE 25

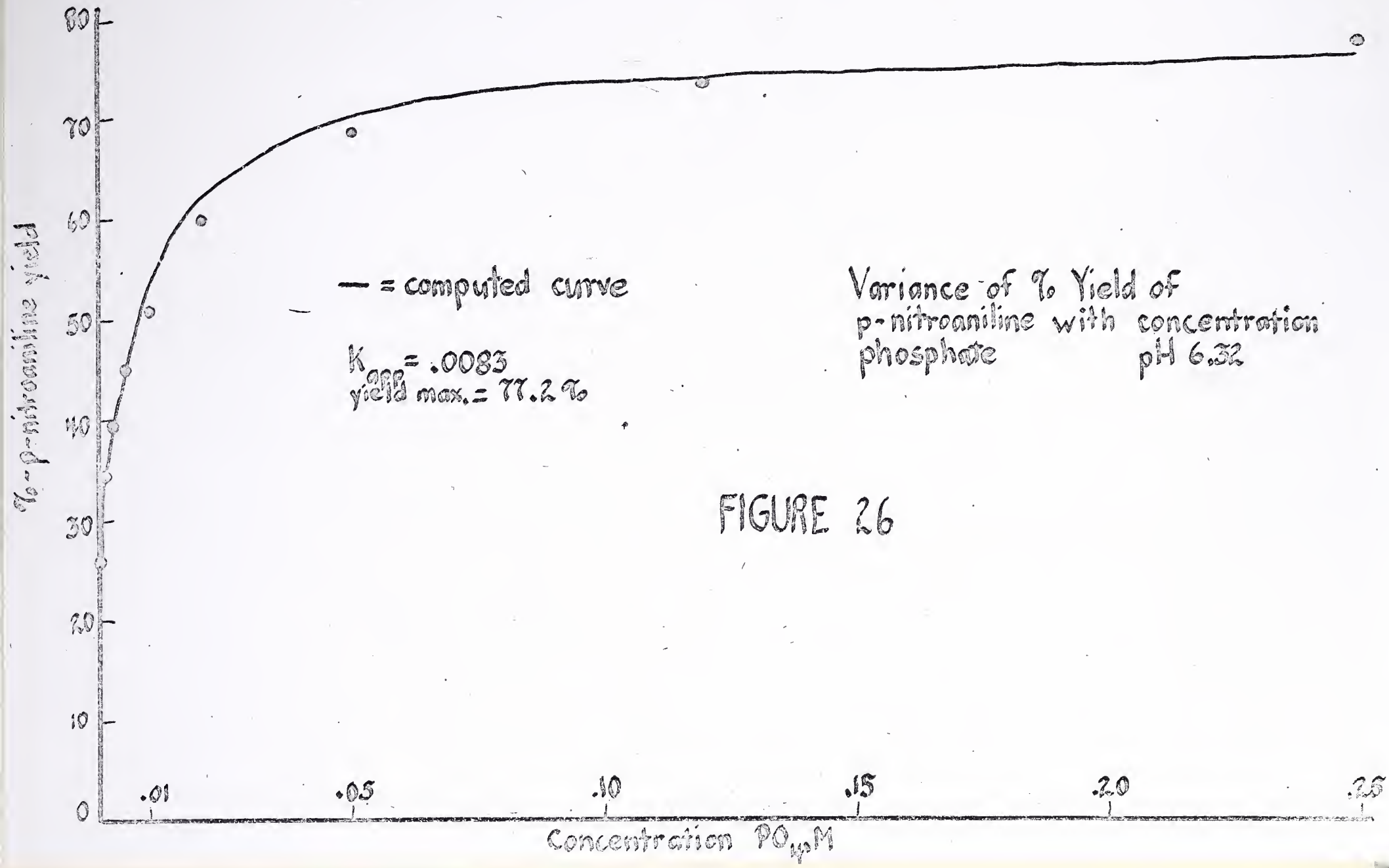

80
70
60
50
40
30
20
10
0
% - p-nitroaniline yield
.01
.05
.10
.15
.20
.25
Concentration $PO_4 \mu M$
— = computed curve
K_{app} = .0083
yield max. = 77.2 %
Variance of % Yield of
p-nitroaniline with concentration
phosphate pH 6.32

FIGURE 26

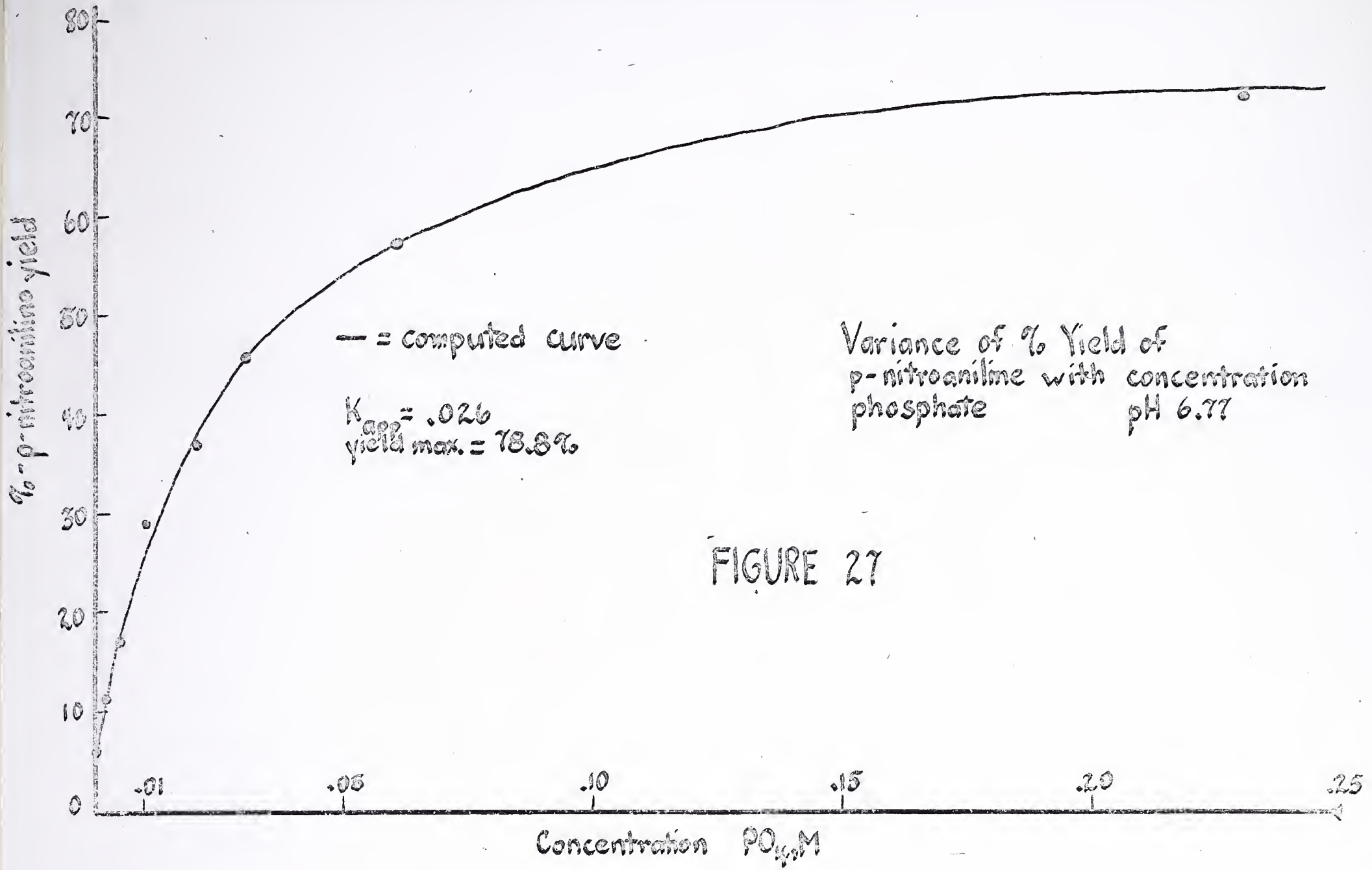
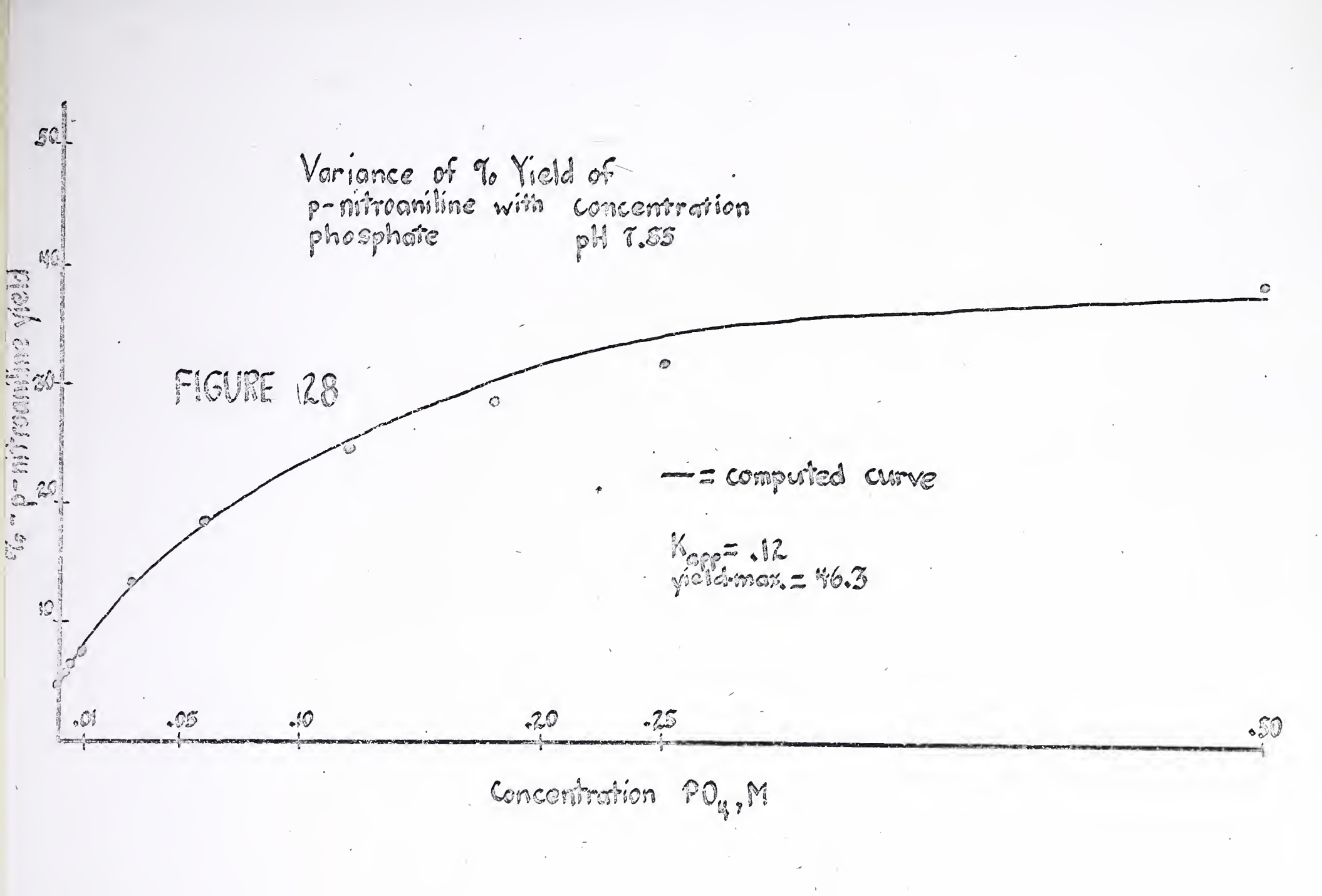

80
70
60
50
40
30
20
10
0
% - p-nitroaniline yield
— = computed curve
K_{app} = .026
yield max. = 78.8%
Variance of % Yield of
p-nitroaniline with concentration
phosphate pH 6.77
.01
.05
.10
.15
.20
.25
Concentration PO_4 M

FIGURE 27


Variance of % Yield of
p-nitroaniline with Concentration
phosphate pH 7.85
FIGURE 128
— = computed curve
K_{app} = .12
yield-max. = 46.3
% p-nitroaniline yield
50
40
30
20
10
.01
.05
.10
.20
.25
.50
Concentration PO_4, M

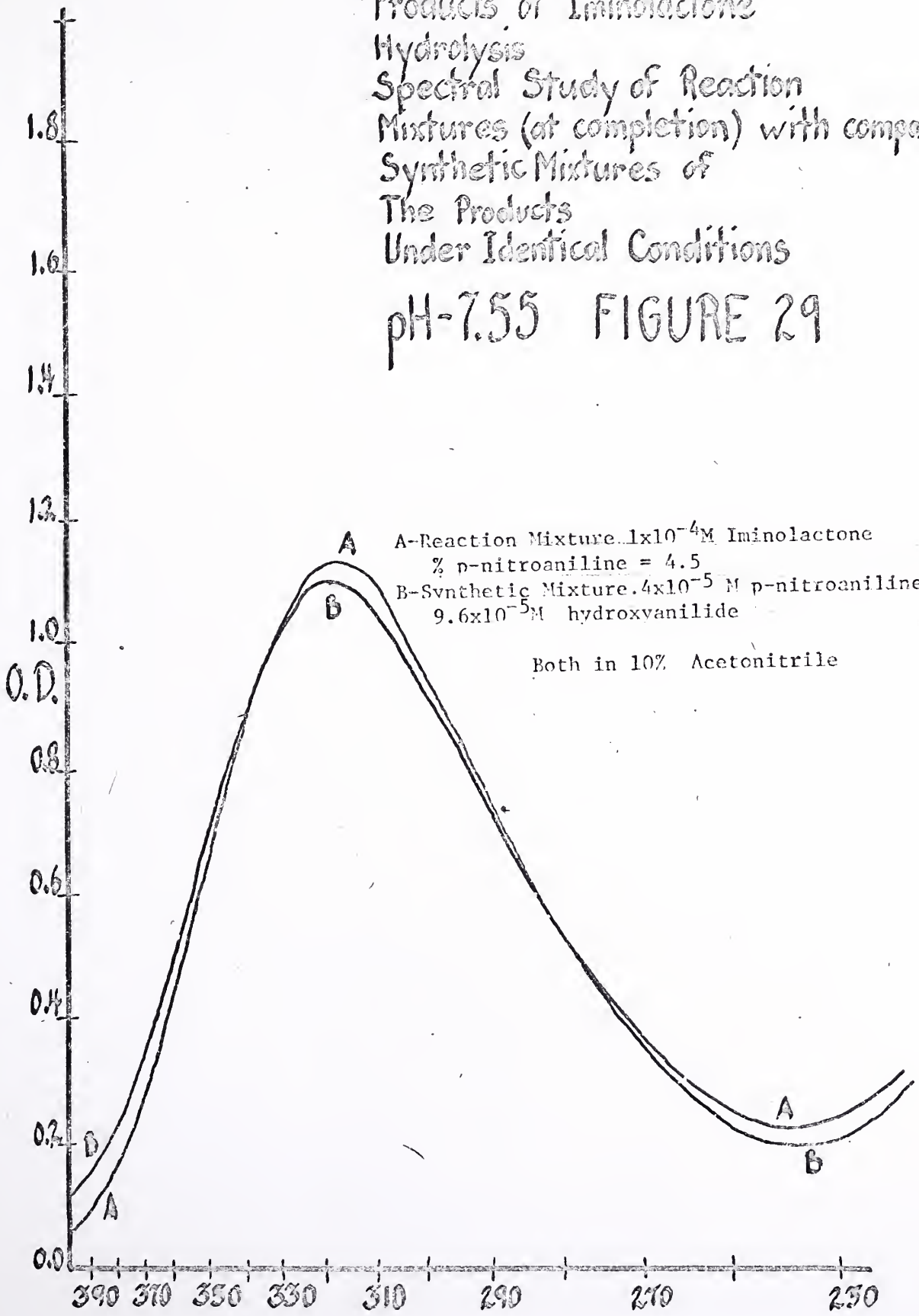
The substantial efficiency of bifunctional buffers to catalyze the hydrolysis of iminolactone in favor of the p-nitroaniline product is demonstrated here as in the studies on the parent unsubstituted iminolactone[2].

Finally, as a check on product determination and the identity of the second product obtained, the UV spectra of reaction mixtures at completion of hydrolysis were compared to those of mixtures made up of p-nitroaniline and γ-hydroxy-p-nitrobutyranilide of the expected composition. It may be seen that the spectra of the reaction mixtures run at pH 7.55, 6.10, and 5.45 (figures 29-31 A) is in good agreement with those of the solutions made up synthetically with the products at the corresponding pH's (figures 29-31 B). The corresponding % aniline yields at these pH's are 4.5%, 35.4%, and 84% respectively.

In figure 32, a reaction mixture at pH 2.5 (1 M phosphate buffer) with p-nitroaniline yield 96%, was examined spectrally in the UV range. (Curve A) The spectrum of the reaction mixture varied slightly from that of p-nitroaniline alone (Curve B) and could be made almost identical only if the synthetic mixture was made up assuming that the remaining 4% of product was the hydroxyanilide (Curve C).

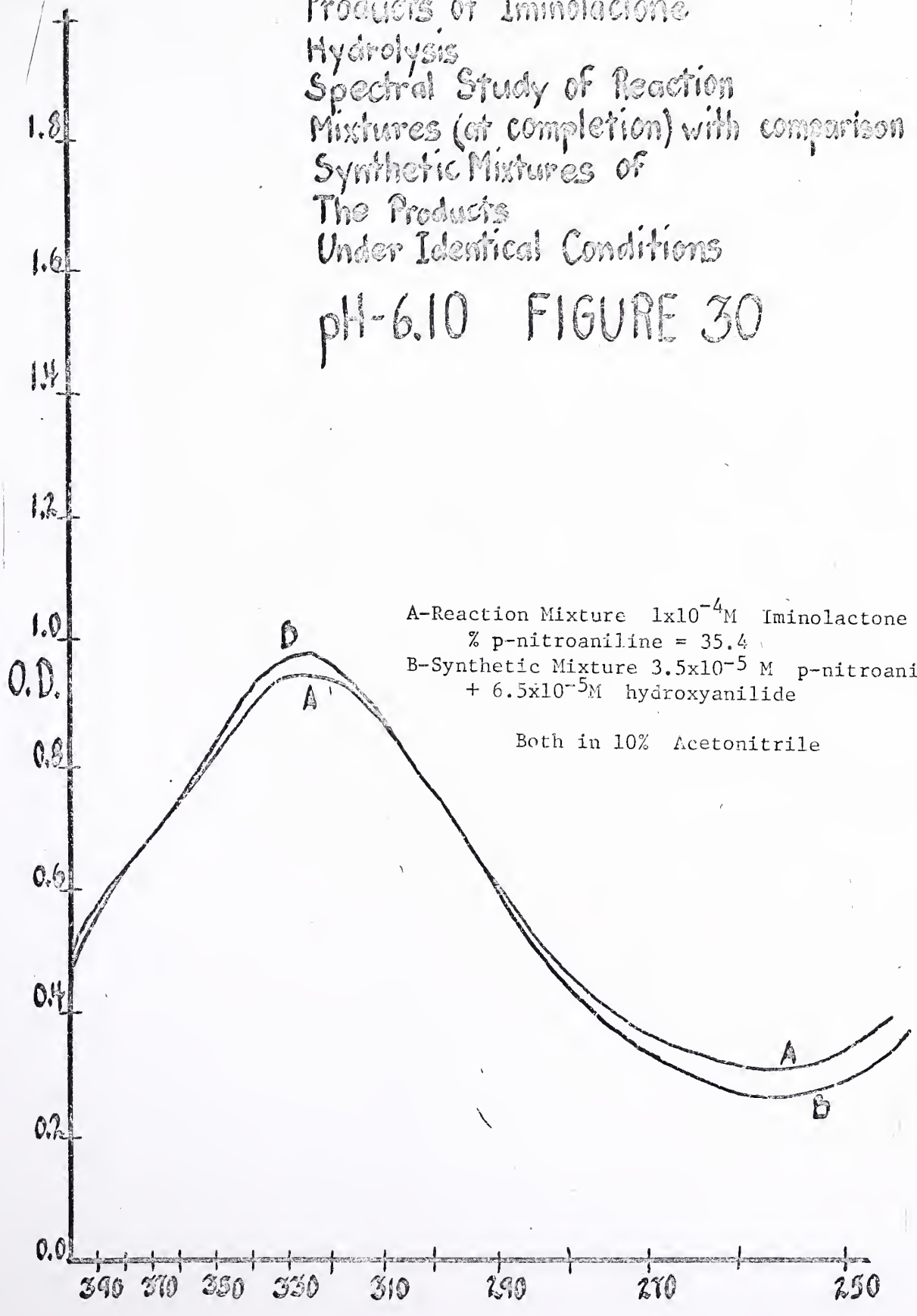
Products of Iminolactone Hydrolysis
Spectral Study of Reaction Mixtures (at completion) with comparison to Synthetic Mixtures of The Products Under Identical Conditions

pH-7.55 FIGURE 29



Products of Iminolactone Hydrolysis
Spectral Study of Reaction Mixtures (at completion) with comparison to Synthetic Mixtures of The Products Under Identical Conditions

pH-6.10 FIGURE 30

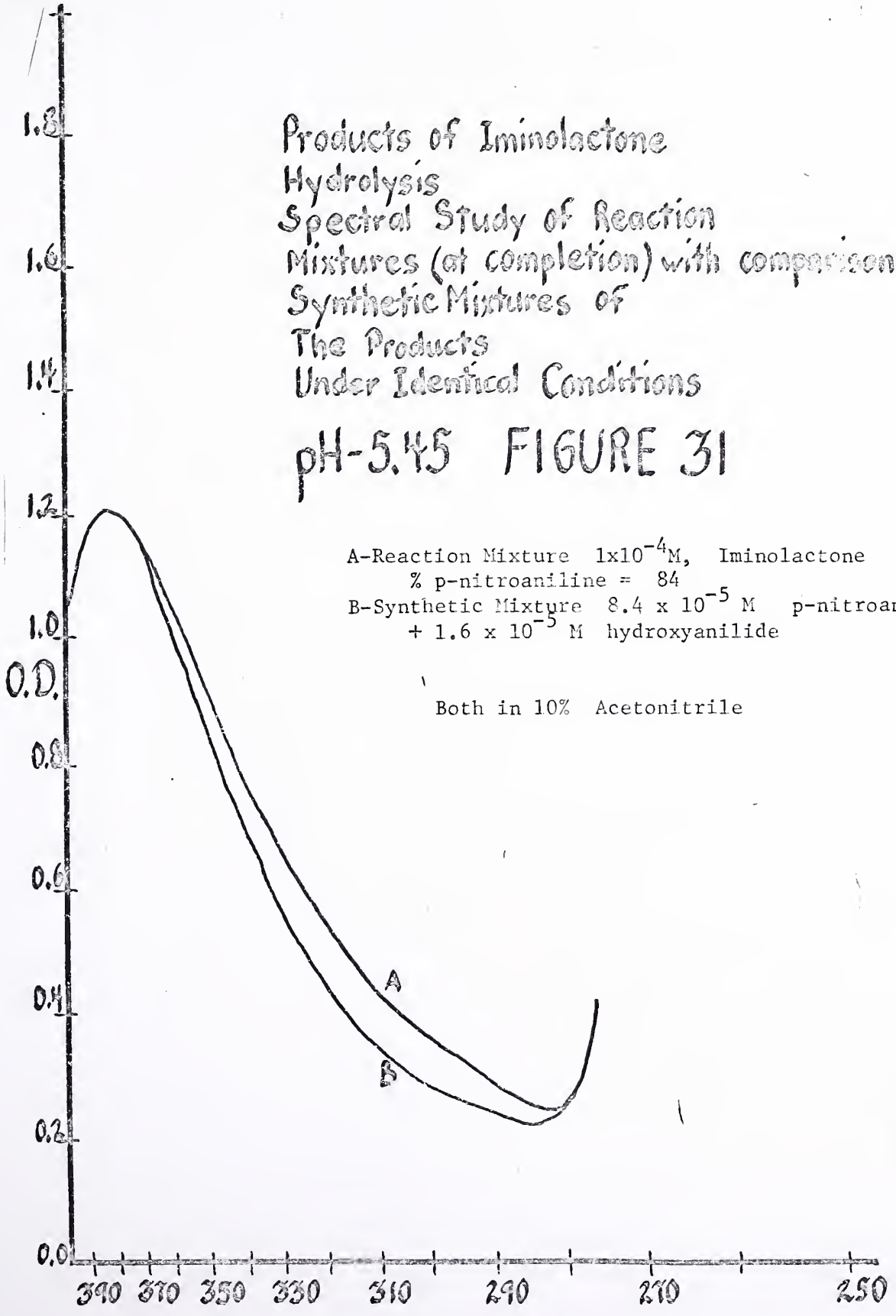


A-Reaction Mixture $1x10^{-4}$M Iminolactone
% p-nitroaniline = 35.4
B-Synthetic Mixture $3.5x10^{-5}$ M p-nitroaniline,
+ $6.5x10^{-5}$M hydroxyanilide

Both in 10% Acetonitrile

Products of Iminolactone Hydrolysis
Spectral Study of Reaction Mixtures (at completion) with comparison to Synthetic Mixtures of The Products Under Identical Conditions

pH-5.45 FIGURE 31

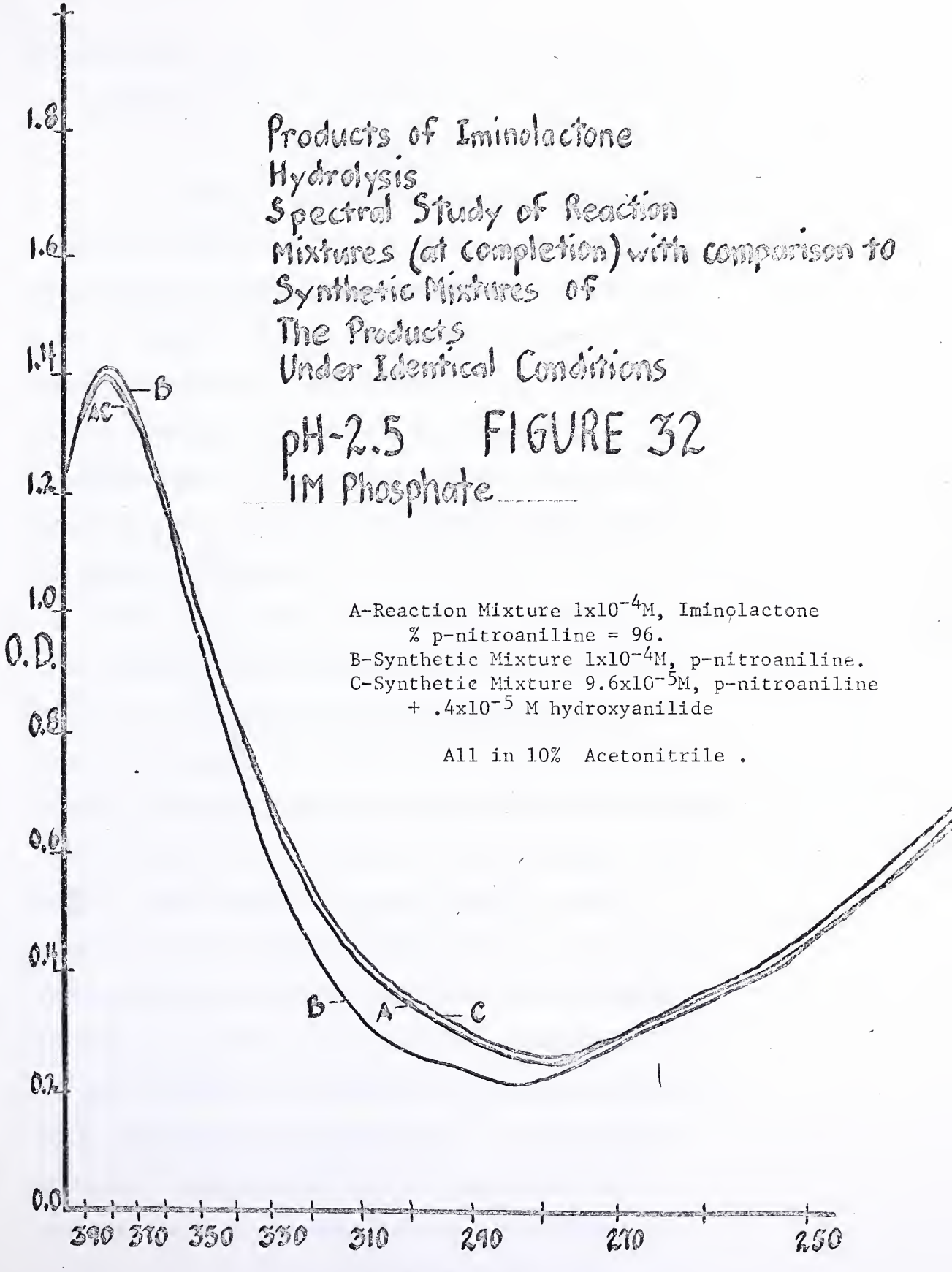


A-Reaction Mixture 1×10^{-4}M, Iminolactone
% p-nitroaniline = 84
B-Synthetic Mixture 8.4×10^{-5} M p-nitroaniline,
+ 1.6×10^{-5} M hydroxyanilide

Both in 10% Acetonitrile

Products of Iminolactone Hydrolysis
Spectral Study of Reaction Mixtures (at completion) with comparison to Synthetic Mixtures of The Products Under Identical Conditions

pH-2.5 FIGURE 32
1M Phosphate



A-Reaction Mixture $1x10^{-4}$M, Iminolactone
% p-nitroaniline = 96.
B-Synthetic Mixture $1x10^{-4}$M, p-nitroaniline.
C-Synthetic Mixture $9.6x10^{-5}$M, p-nitroaniline
+ $.4x10^{-5}$ M hydroxyanilide

All in 10% Acetonitrile .

Section II

Chapter 3

Discussion

In this chapter, specific points concerning the results described in Chapter 2 will be discussed with regard to their similarities or differences from predictions made from, or results obtained from studies of other imidates or the unsubstituted iminolactone [1, 2]. The significance of these findings in terms of a general mechanism for hydrolysis of the entire family of p-nitro substituted compounds (the iminolactone and hydroxyanilide) will be the subject of Section IV.

In the region studied, the kinetics of hydrolysis of 2-p-nitrophenyliminotetrahydrofuran reveal a linear dependance on hydrogen ion concentration (figure 23). As with other imidates, [1, 3, 14, 18] the protonated species of the iminolactone seems to be the active one with respect to hydrolysis via the formation of the tetrahedral intermediate. The formation step (nucleophilic attack) is probably the rate determining step. The rate therefore depends directly on the concentration of the protonated species and therefore on hydrogen ion concentration. At low pH, this linear relationship is interrupted near the pK of the unsubstituted iminolactone [1] as protonation is completed. The pK of the unsubstituted iminolactone derived from this type of curve is 5.1 (see figure 5A). As there is no deviation from linearity (in figure 23)

in the range studied, the pK of the p-nitro substituted iminolactone is probably no higher than 3. In the absence of further kinetic determinations above pH 7.5, it is not certain whether the deviations from linearity seen at pH greater than 6 in Figure 23 result from the contribution of a pH independant reaction (protonated iminolactone reacting with hydroxide or neutral iminolactone reacting with water) to the rate determining step. The deviations could also be explained by the appearance of minor rate enhancing effects of the buffers used. These might become apparent only when the rate is slow enough for the contribution to be significant.

The similarity of the dependance of product on pH to a sigmoid titration curve (figure 24) has been shown for several imidates [1, 17, 18]. It has been assumed to be the result of the derivation of the two products primarily from different forms of the tetrahedral intermediate which differ by 1 proton, in their states of dissociation. The midpoint of amine yield on these curves is given as pK, the pH where the two forms of the tetrahedral intermediate contribute equally to product formation. It is seen that the experimental points in figure 24 are somewhat steeper than that calculated from the equation:

$$\%P_1 = [H]/[H]\ K' \qquad (4)$$

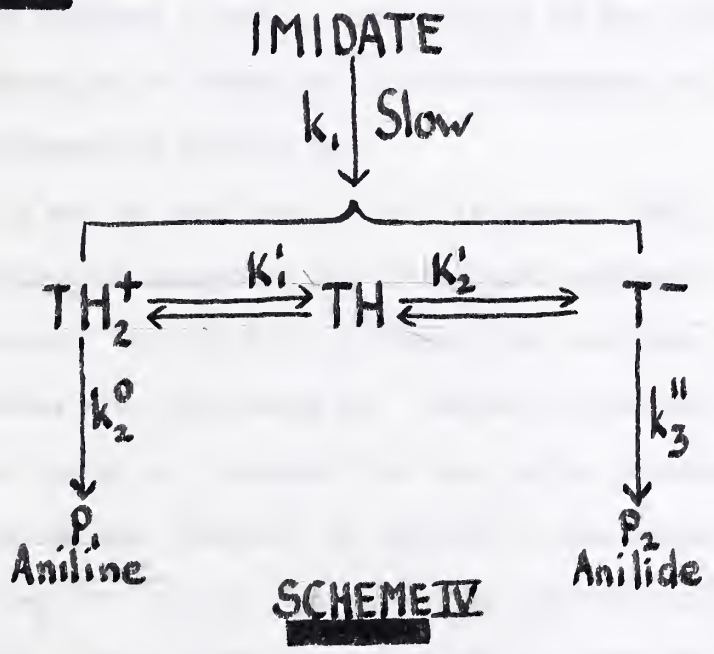
which expresses the above relationship (Curve I-figure 24).

The experimental points, however, closely approximate the curve (curve II, figure 24) calculated from the expression:

$$\%_{P_1} = [H]^2 / [H]^2 + K'' \qquad (5)$$

(a sigmoid relationship to the second power of $[H]$. This equation expresses the relationship expected if the forms of tetrahedral intermediate giving rise to the two products are separated by two protons in their states of dissociation.

[redacted].



SCHEME IV

Here K'' incorporates not only the kinetic considerations expressing how fast these two forms decompose to products but is also a product of the K's for the two dissociation steps separating the intermediates. pK' and pK'' for the two curves (I and II) was assumed to be 6.1 for their calculation. The significance of this finding in terms of the mechanism of hydrolysis of these compounds is discussed in Section IV.

Another significant deviation from findings with other imidates [2, 17, 18] seen in Table II is the apparent effect of monofunctional buffers on the product distribution. These buffers, with increasing concentration, decrease the % yield as p-nitroaniline. In previous studies as with the unsubstituted iminolactone [2], monofunctional buffers were markedly less efficient than bifunctional buffers in catalyzing the generation of amine from the hydrolysis, but their effect was in that direction. This reversal of effect requires a further modification of the scheme for the mechanism of hydrolysis for this compound and this is discussed in Section IV.

It can be seen from Table V (Figures 25-28) that the effect of phosphate (a bifunctional catalyst) is to increase the yield of p-nitroaniline and that the K_{app} increases with increasing pH. Therefore, the efficiency of the buffer as a catalyst in this system decreases with increasing pH. However, in contrast to the results obtained from similar studies of the unsubstituted iminolactone [2], the % maximum yield of aniline obtainable by phosphate catalysis decreases with increasing pH.

An examination of the behavior of the hydroxyanilide γ-hydroxy-p-nitrobutyranilide has further elucidated the unique mechanisms of hydrolysis of the p-nitro substituted compounds. (See Section III).

Section III

In order to further elucidate steps in the mechanism of the hydrolysis of the iminolactone (Section II) which are not rate determining, and with the considerations discussed on page 21 , the hydroxyanilide, γ-hydroxy-p-nitrobutyranilide

was prepared and purified. It was then studied kinetically at varying pH and with varying concentrations of both mono and bifunctional buffers.

Section III

Chapter 1

Materials and Methods

A-Materials

Solvents and Buffers - see page 24

Synthesized Materials

p-nitrophenylsuccinimide - was prepared according to a method for succinimide preparation outlined by Westhead and Morawetz[31].

In a mortar was placed 13.8 g (100 mmoles) of p-nitroaniline. This was ground together with 13.61g (100 mmoles) of potassium hydrogen sulfate and 10g (100 mmoles) of succinic annhydride. The mixture was fused in a crucible and upon formation of a melt, it was allowed to cool to a thick gray mass which was pulverized and then extracted with 700 ml of water. The grey brown solid residue was dissolved in 150 ml of hot glacial acetic acid and was decolorized in solution with norite. After gravity filtration and upon cooling, a pale yellow crystalline product was isolated and recrystallized from hot glacial acetic acid. The product was triturated with 250 ml of water to remove acid contaminant and stored in vacuo over KOH and P_2O_5.

M. p 210[32] Yield 15.7 g, 71% yield.

γ-hydroxy-p-nitrobutyranilide - was prepared from the succinimide in the method after Horii et al[33].

Greater than a seven fold excess of reducing agent, borohydride, was required for complete reduction.

In 45 ml of methanol at 0° was placed 5.4 g (24 mmoles) of p-nitrophenylsuccinimide and two drops of 1N NaOH. The solution was stirred at 4° C in a refrigerated cold room and to it was added 45 ml of a cold methanolic solution containing 2.3 g (60 mmoles) of 99% sodium borohydride and two drops of 1N NaOH. The borohydride solution, though cold, was generating hydrogen before it was added to the succinimide. The addition was accomplished drop-wise over ½ hour in the cold room. During the addition, the methanolic solution became cloudy and the intensity of its yellow color increased. The mixture was stirred for an additional 6 hrs at 4° C during which its color turned to yellow green. It was then stirred overnight at room temperature. Next, an additional 60 mmoles of borohydride was added in 45 ml of methanol as before and the sequence was repeated. The color changes were also similar. After the second overnight stirring, 12 ml of glacial acetic acid in 12 ml of cold methanol was added over 15 min to destroy excess reducing agent. During the addition, the solution became bright green in color. The solution was evaporated to dryness in a rotary evaporator under a vacuum of 10 mm Hg and the solid obtained was triturated with 250 ml of annhydrous ether. The ether phase was then dried for 1 hr over 50 g of annhydrous $MgSO_4$. The addition of 40 ml of petroleum ether (30-60°) to the ether

solution, after removal of the drying agent by filtration, produced green crystals mp 102-107 C which even after overnight storage over P_2O_5 and KOH under vacuum, possessed 2 peaks in the IR region of 5.8μ. In addition, the height of the OH peak in the area of 3 was very small when the spectrum (IR) was taken in chloroform. Therefore, the product was assumed to be a mixture of the γ-hydroxy-anilide and the aldehyde. Therefore, to 1.8 g recovered (6 mmoles) dissolved in 15 ml of cold methanol as before was added 770 mg (20 mmoles) of borohydride in 15 ml of cold methanol. Addition and stirring were as before and with the same color changes. Next, 4 ml of glacial acetic acid in 4 ml of methanol was added before evaporation to dryness and the crystals obtained from the ether layer were recrystallized from ether-petroleum ether 30-60 to yield pale yellow crystals mp 110-111 C. The yield of the preparation was 550 mg 10%. In a subsequent repetition of the synthesis, three reduction steps were carried out without isolation of the product between steps and the yield was 25%.

The IR spectrum (Figure 33) revealed OH band absorption at 2.9 and carbonyl absorption as a single peak at 5.90 . In the UV spectrum, the absorption maximum was at 319 m with a molar extinction coefficient of 13, 900. (Figure 34) The following elemental analysis was obtained:

	C	H	O
% Calculated	53,57	5,40	12,22
Received	53,75	5,45	12,55.

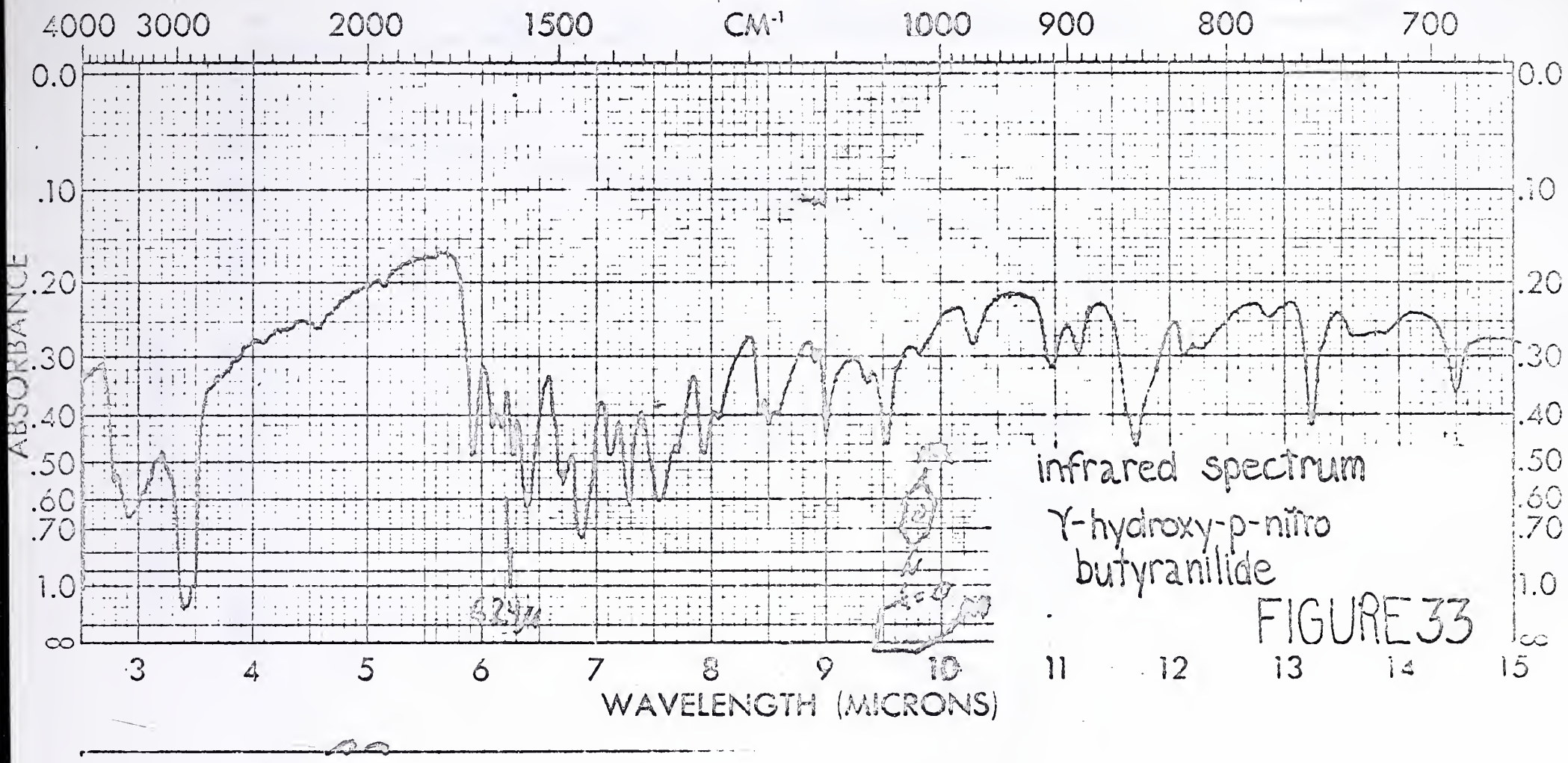
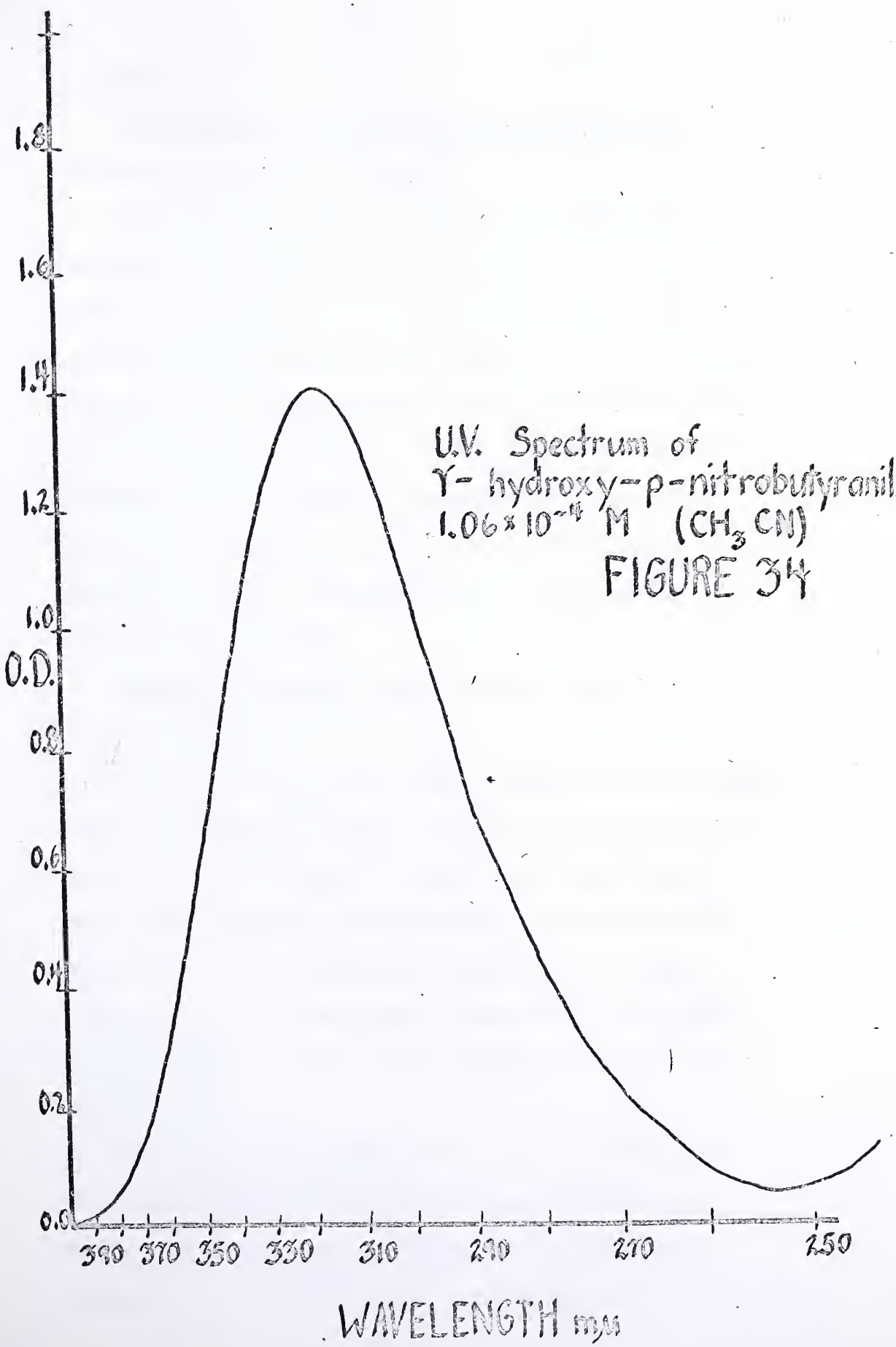

4000 3000 2000 1500 CM-1 1000 900 800 700
0.0
.10
.20
.30
.40
.50
.60
.70
1.0
∞
ABSORBANCE
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)
infrared spectrum
γ-hydroxy-p-nitro
butyranilide

FIGURE 33



U.V. Spectrum of
γ- hydroxy-p-nitrobutyranilide
1.06×10^{-4} M (CH_3CN)

FIGURE 34

B- Methods

Melting points, pH measurements and spectra were performed as described on page 29.

Solutions- were made up as described on page 29 . Reactions, however, were run in 10% EtOH because of its greater stability over the time periods required. NaOH solutions were made up free of carbonate and were standardized against primary standard potassium hydrogen phthalate with a phenophthalein indicator. HCl solutions were standardized similarly against the NaOH. These solutions were stored free of CO_2 under absorbant and were dispensed from a delivering burette. Concentrations of hydroxyanilide were as described below.

Kinetic measurements- Hydrolysis of γ -hydroxy-p-nitrobutyranilide.

All reactions were run at 30°. The more rapid reactions, (those run in NaOH and HCl) were run in stoppered cuvettes and were followed by the OD changes at 390 mμ on a Zeiss PMQ II spectrophotometer with constant temperature head. Reaction constants were determined from graphs of $OD_{max} - OD_t$ vs time on semi log paper. Reactions were followed for 7 half lives. Concentrations of hydroxyanilide were $1x10^{-4}$M.

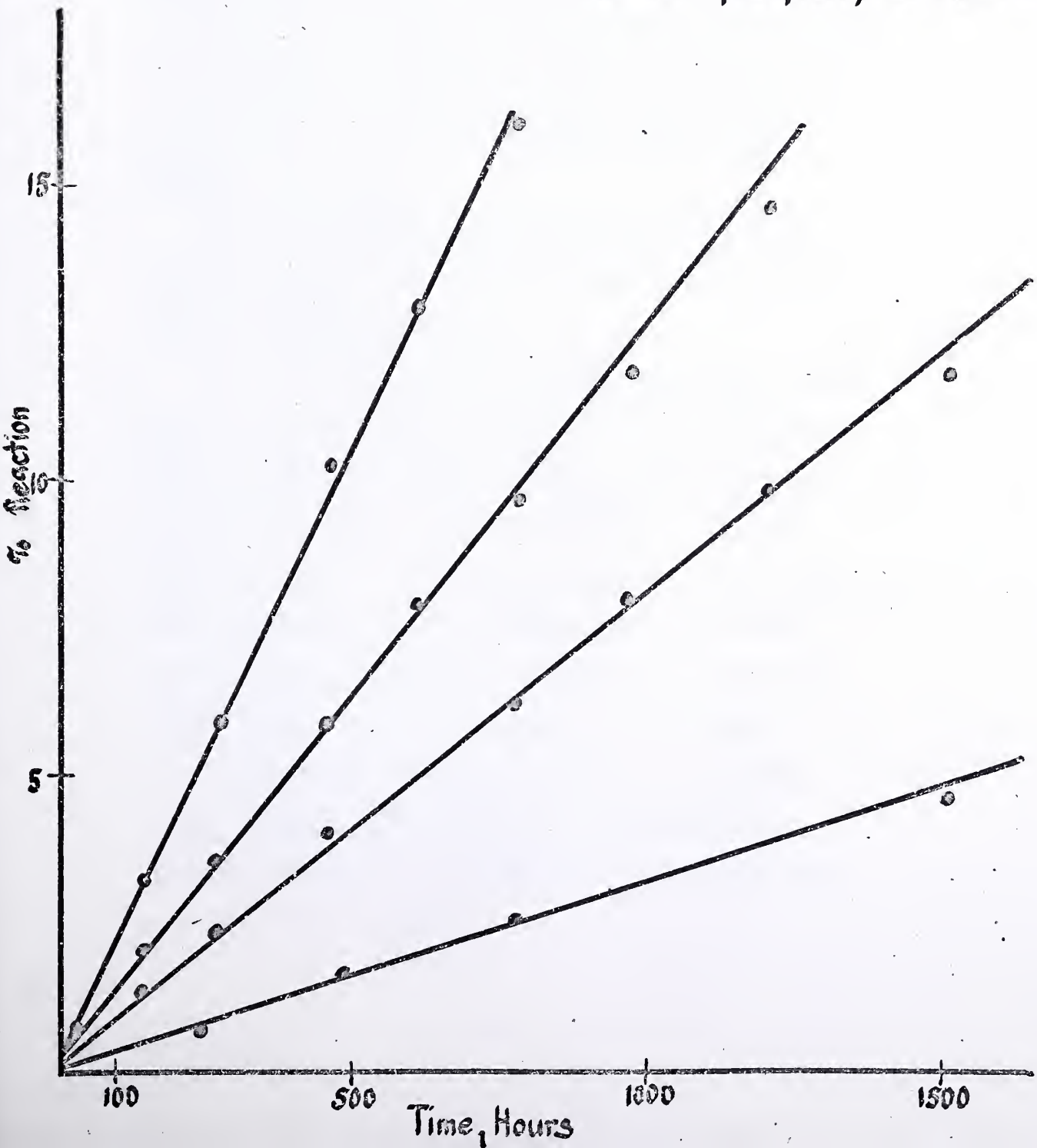
The reactions of hydroxyanilide at intermediate pH were extremely slow. Time did not allow for their being followed to completion. Therefore, it was decided that they would be followed for 20% of completion. In order

to allow this, while obtaining an OD change of at least .800, they were run at a concentration of hydroxyanilide of 4×10^{-4}M. Large samples of hydroxyanilide were weighed out to allow accuracy and 50 ml of each reaction mixture was made up. Samples of 3 ml of reaction mixture were sealed in ampoules and were incubated for the required time at 30° in a constant temperature bath. Ampoules were broken open and were used for one reading, either an OD or a pH reading, at given intervals of time, and were then discarded. Each reaction mixture was accompanied by a control solution of p-nitroaniline under identical conditions to assure its stability over the 3-6 months required to achieve 20% reaction. pH's obtained at the beginning and the termination of each reaction were in good agreement. No decomposition of the p-nitroaniline product could be detected spectrophotometrically. The % reaction (calculated from the OD_{390} at time t, minus the OD at zero time)(that of the hydroxyanilide ε_{max}^{390} = 600) divided by the total expected OD change (calculated from a known conc. of hydroxyanilide using the standard curve for p-nitroaniline) was plotted vs. time. The plots were on linear graph paper (Figure 35). It can be shown that the rate curves for first order reactions approximate linearity for the first 15% reaction with less than 1% error. The reaction rate constants were read from the slope of the straight line obtained. Rate constants were also calculated using the integrated form of the

First Order Rate Plots
Hydrolysis of γ-hydroxy-p-nitrobutyranilide
pH 5.19

FIGURE 35

A- .20 M phosphate, $k = 2.1 \times 10^{-4}$ hr^{-1}
B- .10 M phosphate, $k = 1.3 \times 10^{-4}$ hr^{-1}
C- .05 M phosphate, $k = 8.2 \times 10^{-5}$ hr^{-1}
D- 0 phosphate, $k = 3.2 \times 10^{-5}$ hr^{-1}



first order rate equation. These calculated rate constants were in good agreement with those derived graphically. As a further check on the assumption of the validity of following the reactions for only 20%, one of the phosphate catalyzed reactions which was fast enough to be run to completion was set up in ampoules at pH 6.81 and $1x10^{-4}M$ hydroxyanilide and was followed for five half lives. The data was handled as that for the more rapid reactions (page 55) and the rate constant derived was in good agreement with that determined for the corresponding reaction, followed for only 20%.

The OD measurements for all reactions carried out in sealed ampoules were obtained in open cuvettes on a Beckman DU spectrophotometer.

Due to the ionization of p-nitroaniline (which occurs to any significant degree around pH 1) and the spectral changes which result from it, the OD_{390} differences for the reactions carried out in HCl became less, for the complete reaction. In the extreme case, in .5M HCL where the OD changesat 390 mμ is minimal because the product is extensively protonated, it was elected to follow the course of the reaction at 256 mμ on the Zeiss PMQII. It was otherwise run in the same manner as the other "rapid" hydroxyanilide reactions. Further details on specific reactions may be found in the tables (VI, VIII, and IX) in the next chapter.

Chapter 2

Results

The rates of hydrolysis of γ-hydroxy-p-nitrobutyranilide to p-nitroaniline and butyrolactone were studied to determine the effects of pH, bifunctional and monofunctional buffer catalysis, and to elucidate a mechanism of hydrolysis. Because of the slow rate of hydrolysis of this compound and therefore the length of time necessary to obtain data (even when the reactions were followed to only partial completion) (see page 55-6), only a limited amount of data could be obtained and deviations from expected findings could be studied and elucidated in only a preliminary manner.

The variation of rate of hydrolysis with pH is presented in Table VI. It is graphically represented in Figure 36. The variations of k_{obs} with concentration of OH^- and H^+ are re-plotted in expanded fashion in Figures 37 and 38.

Table VI

Effect of pH Variation on Rate of Hydrolysis of γ-Hydroxy-p-nitrobutyranilide

pH	Buffer	Conc. M	Rate k, hr^{-1}	% Rx followed	Note
13.70	NaOH	.5	4.88		1
13.48	NaOH	.3	3.79		1
13.30	NaOH	.2	2.86		1
13.00	NaOH	.1	1.58		1
12.90	NaOH	.08	1.31		1
12.70	NaOH	.05	.776		1
12.48	NaOH	.03	.476		1
12.00	NaOH	.01	.191		1
8.90-8.78	Tris	.03	11.3×10^{-5}	18%	2
8.27-8.27	Tris	.03	3.6×10^{-5}	10%	2
7.84-7.88	Tris	.03	2.7×10^{-5}	7%	2
7.68-7.56	Tris	.02M	1.3×10^{-5}	7%	2a
7.33-7.40	Tris	.03	1.6×10^{-5}	6%	2
6.65-6.60	Imidazole	.03	2.9×10^{-5}	11%	2
5.97-6.01	Pyridine	.03	6.0×10^{-5}	11%	2
5.12-5.14	Pyridine	.03	2.8×10^{-5}	10%	2
1.30	HCl	.05	5.72×10^{-2}		3a
1.00	HCl	.1	1.04×10^{-1}		3a
0.70	HCl	.2	1.94×10^{-1}		3B
0.30	HCl	.5	5.14×10^{-1}		3c

1
Reactions run at hydroxyanilide conc. 1×10^{-4}M followed for 7 half lives

2
Reactions run at hydroxyanilide conc. 4×10^{-4}M in sealed ampoules, see p. 56. Followed for % indicated. pH represents pH at time 0 and at termination, respectively

2a
Reaction run in .02 M Tris and not represented on Fig 36.

3
Hydroxyanilide conc. 1×10^{-4}M.

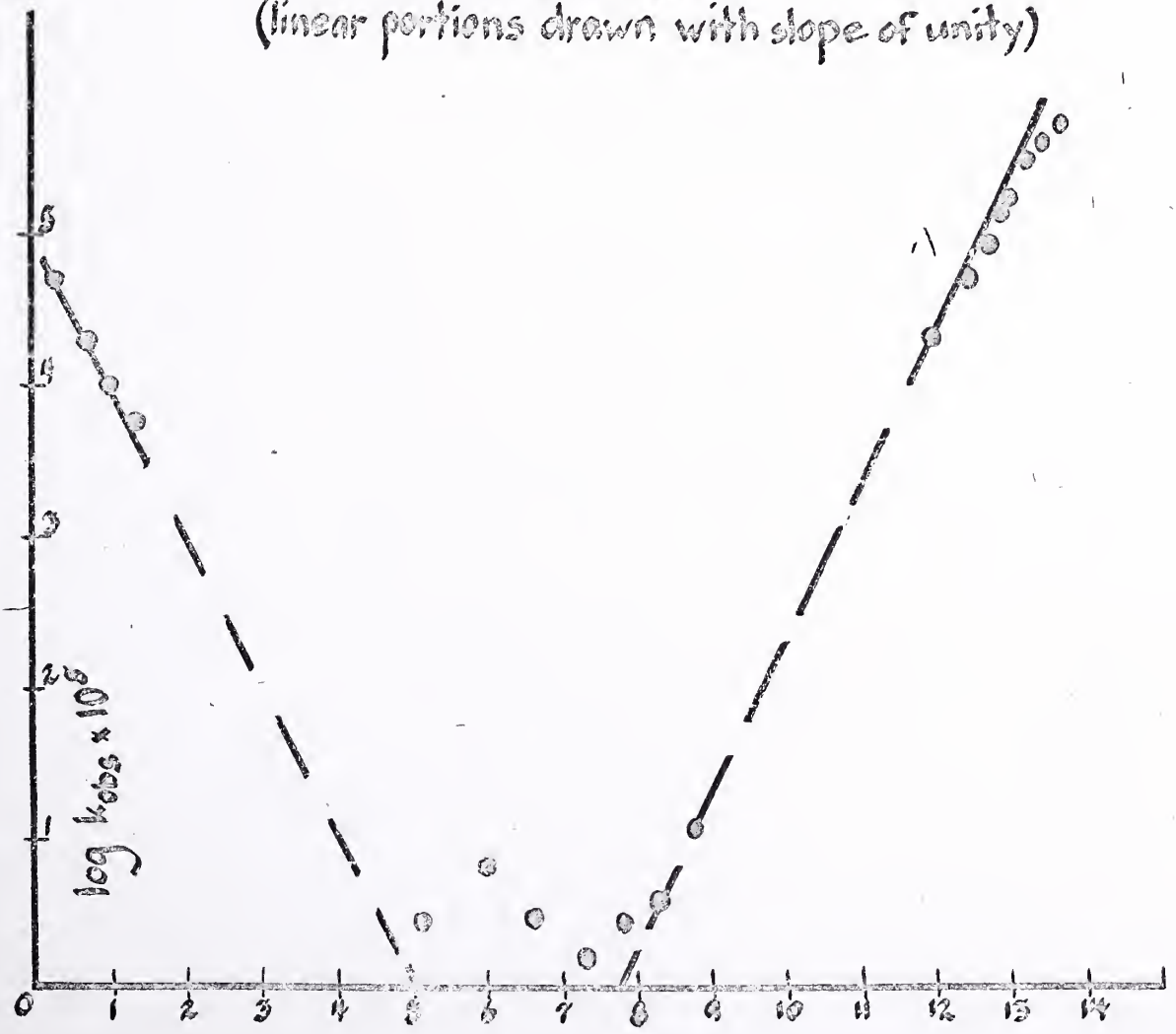
3a
see page 57, followed for 7 half lives with OD changes of .070-.880.

3b
followed for 10 half lives OD changes .070-.500.

3c
followed at 256mμ for 10 half lives OD changes .256-.980 (see page 57).

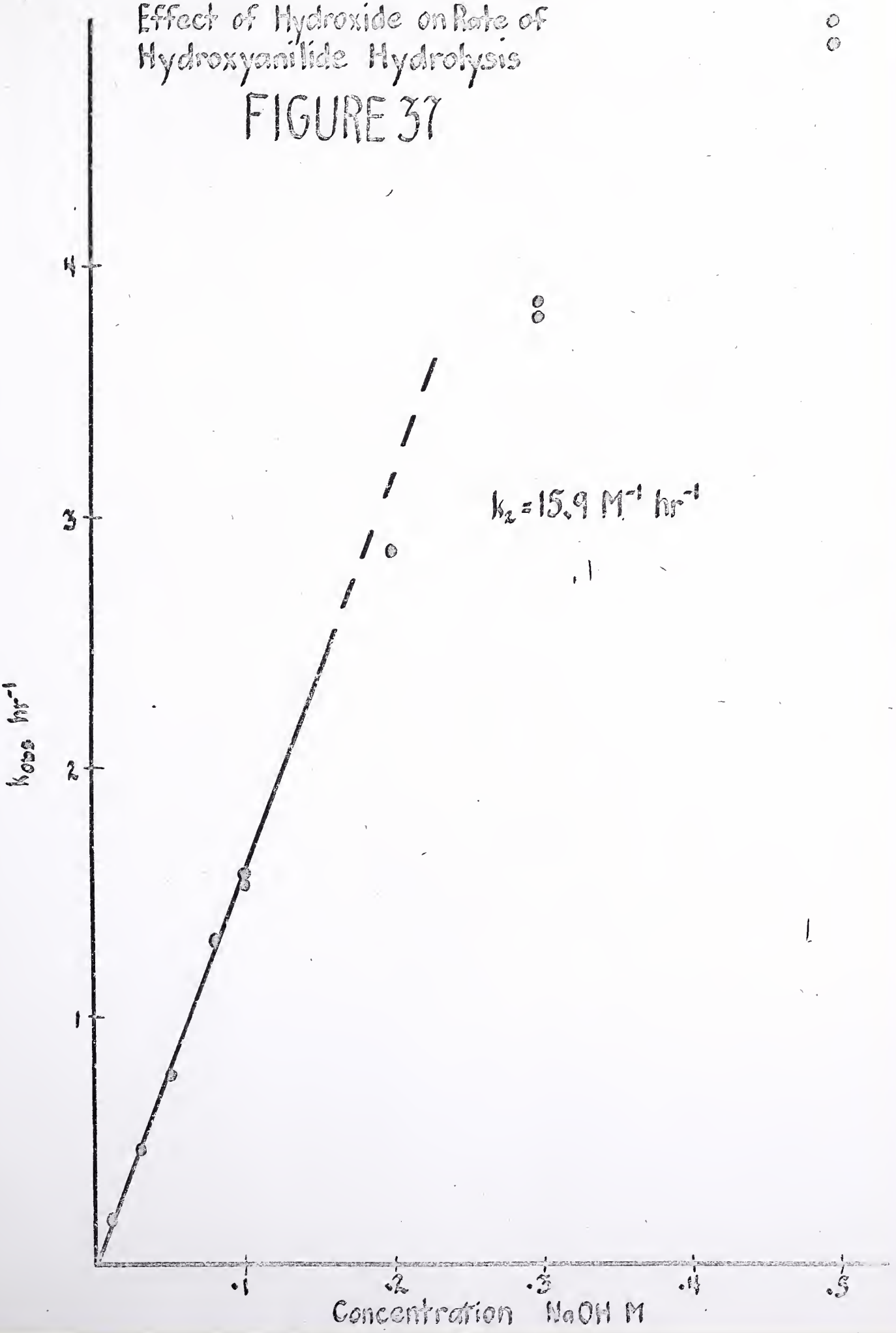
Variation of Rate of
Hydroxyanilide Hydrolysis with pH

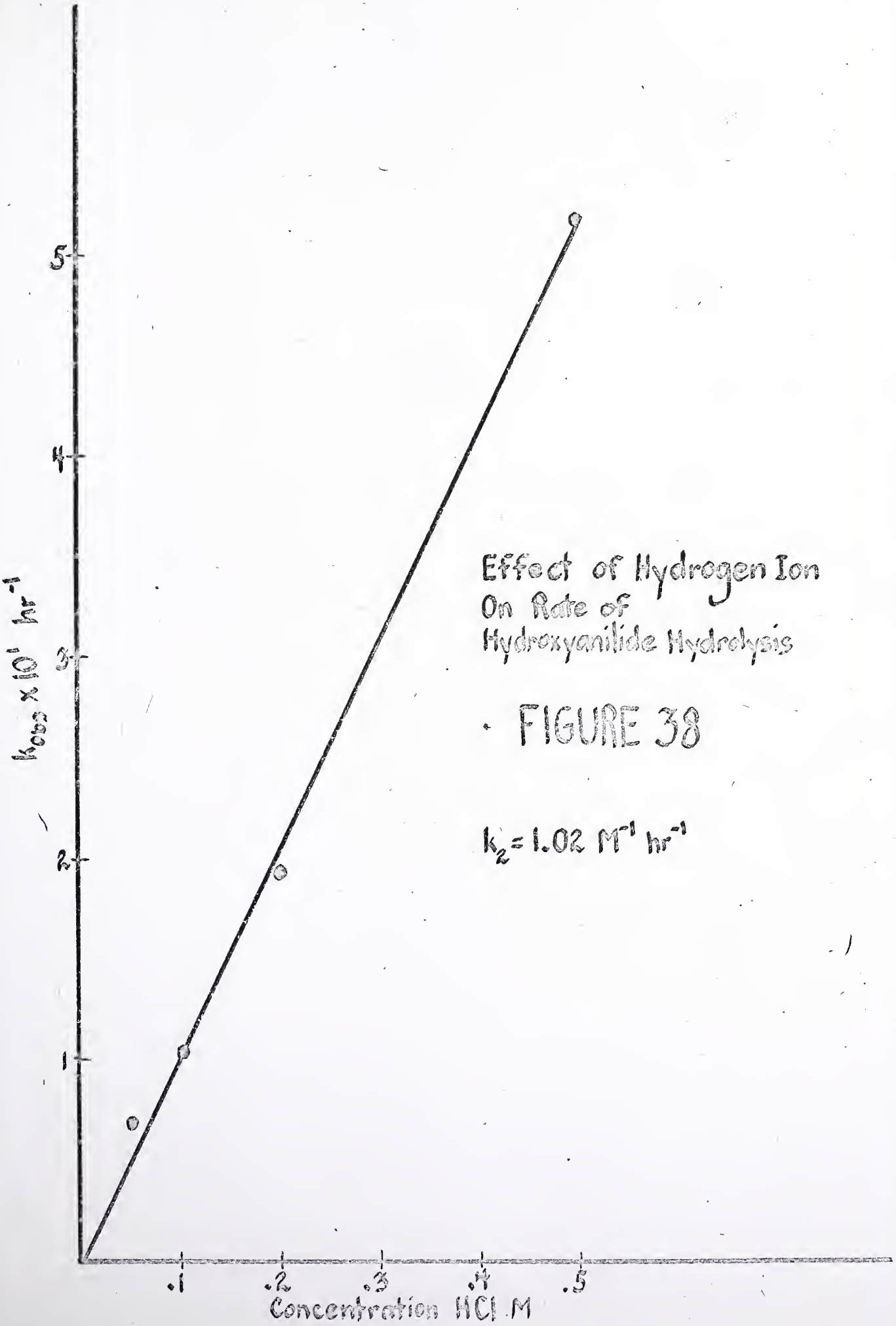
FIGURE 36
(linear portions drawn with slope of unity)



Effect of Hydroxide on Rate of Hydroxyanilide Hydrolysis

FIGURE 37





Effect of Hydrogen Ion On Rate of Hydroxyanilide Hydrolysis

FIGURE 38

$k_2 = 1.02\ M^{-1}\ hr^{-1}$

It may be seen that at the extremes of pH (Figures 36, 37, 38) the rates of hydrolysis vary linearly with concentration of hydroxide and hydronium ions. At alkaline pH, however, there is a suggestion that the curve levels off at high hydroxide ion concentration (fig 37). However, the relationship is generally linear with a second order rate constant of 15.9 $Moles^{-1}$ Hrs^{-1}. In acid medium, the rate varies linearly with $[H^+]$ with a second order rate constant of 1.02 $Moles^{-1}$ Hr^{-1}. At intermediate pH in buffered medium, the relationship of rate and pH is difficult to interpret (Figure 36).

Since the reactions were slow between pH 8.9 and 5.1 and were followed for extended periods of time in sealed vials, and since the kinetic assay was based on the visible absorption of the accumulating p-nitroaniline product, reactions were accompanied by controls (p-nitroaniline conc. 7×10^{-5} M) to assure that it was stabile. Table VII presents sample readings of the controls.

Table VII

Variation of OD_{390} of p-nitroaniline controls with time

pH	Time, days	OD
8.8	0	.930
	30	.925
	101	.920
7.7	0	.930
	3	.925
	14	.930
	89	.955
6.1	0	.925
	13	.922
	81	.920

On the basis of these data (and other data similar but not presented) p-nitroaniline was presumed to be stabile over the necessary time periods.

The variation of rate of hydrolysis of the hydroxyanilide with concentration of phosphate buffer at fixed pH were studied at four pH's. The results are presented in Table VIII and figures 39-42.

Table VIII

Variations of Rate of Hydroxyanilide Hydrolysis with Concentration of Phosphate Buffer

pH	Buffer	Conc. M.	Conc. PO_4M	kx10^5 hr^{-1}	% Reaction followed
7.68-7.56	Tris	.02	0	1.3	7
7.72-7.61	Tris	.02	.05	52	19
7.77-7.67	Tris	.02	.10	93	22
7.82-7.75	Tris	.02	.17	148	30%
6.65-6.60	Imidazole	.03	0	2.9	11
6.72-6.75	Imidazole	.03	.05	65	19
6.76-6.80	Imidazole	.03	.10	95	22%
6.81-6.86	Imidazole	.03	.20	145	28
6.81-6.82	Imidazole	.03	.20	169	°°
5.97-6.01	Pyridine	.03	0	6.0	11
6.10-6.15	Pyridine	.03	.05	21	20
6.15-6.09	Pyridine	.03	.10	32	22%
6.15-6.15	Pyridine	.03	.20	50	25
5.12-5.14	Pyridine	.03	0	3.2	10
5.17-5.14	Pyridine	.03	.05	8.2	22
5.19-5.28	Pyridine	.03	.10	12.9	22
5.21-5.22	Pyridine	.03	.20	21.6	23

All reactions run as described on pages 55-6,;, in ampoules. pH's represent readings at the start and termination of each reaction.

°° Reaction run at conc. of hydroxyanilide 1x10^{-4}M and followed for 5 half lives, see page 55, and page 63.

Effect of Buffers On Rate of Hydroxyanilide Hydrolysis

FIGURE 39
pH-7.6

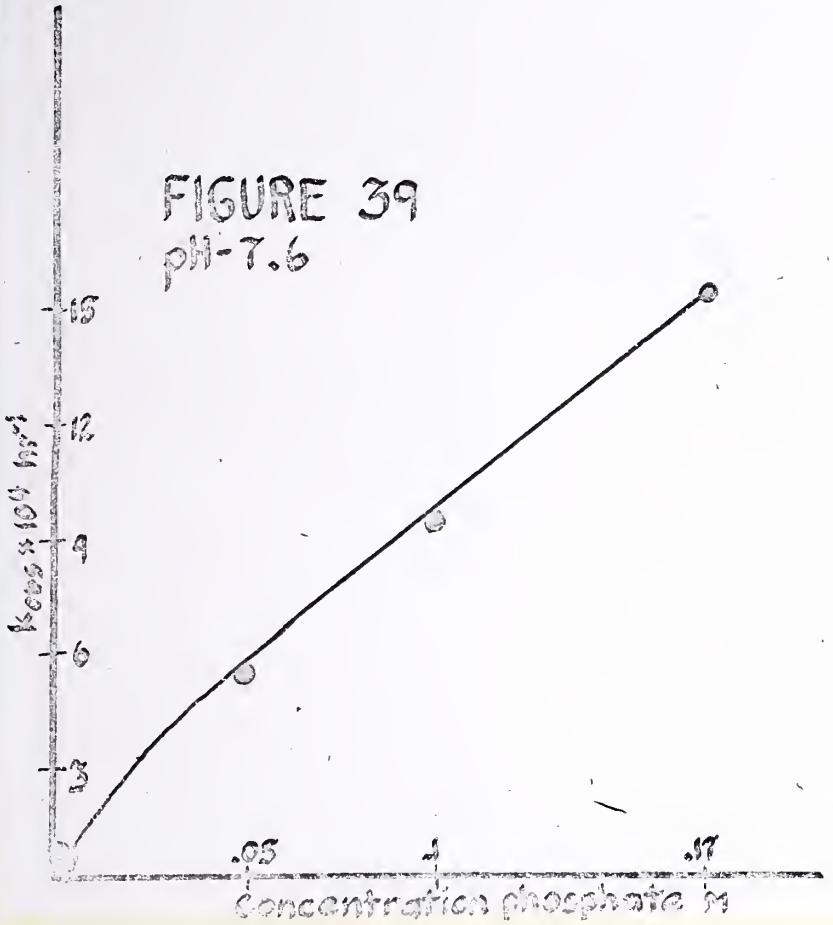
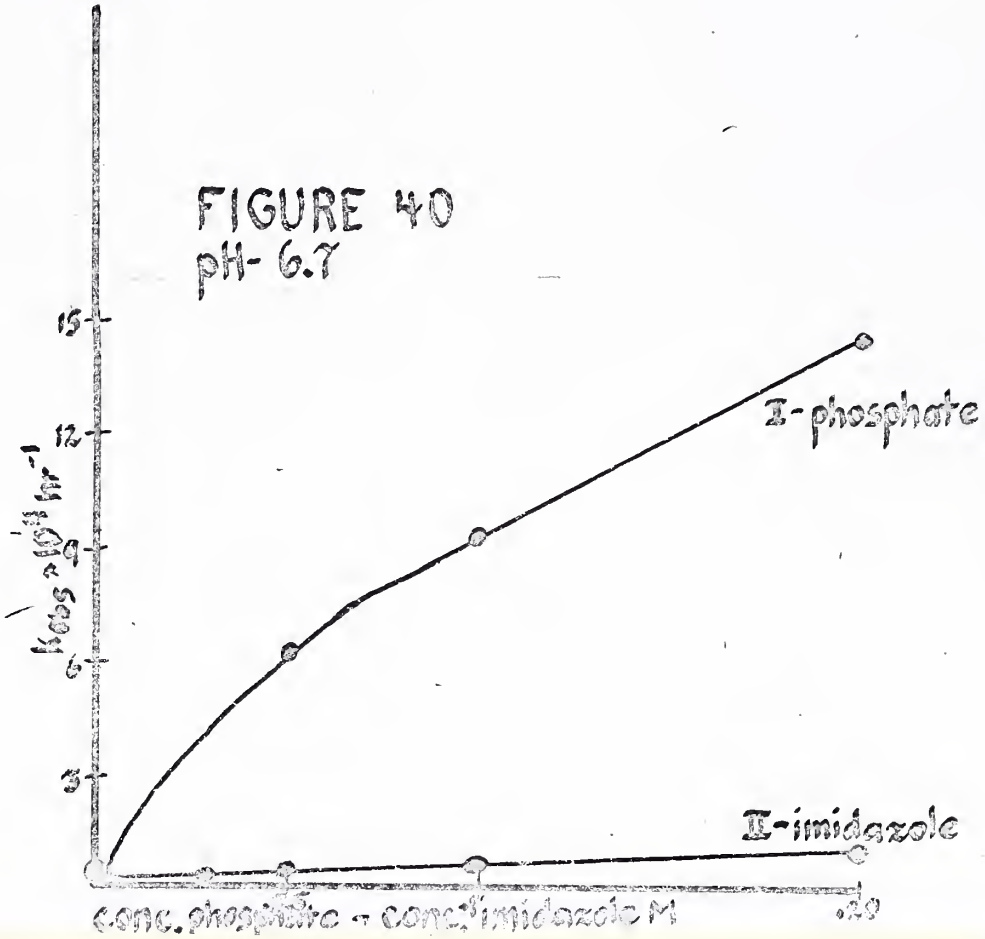


FIGURE 40
pH- 6.7



Effect of Phosphate On Rate Of Hydroxyanilide Hydrolysis

FIGURE 41
pH - 6.1

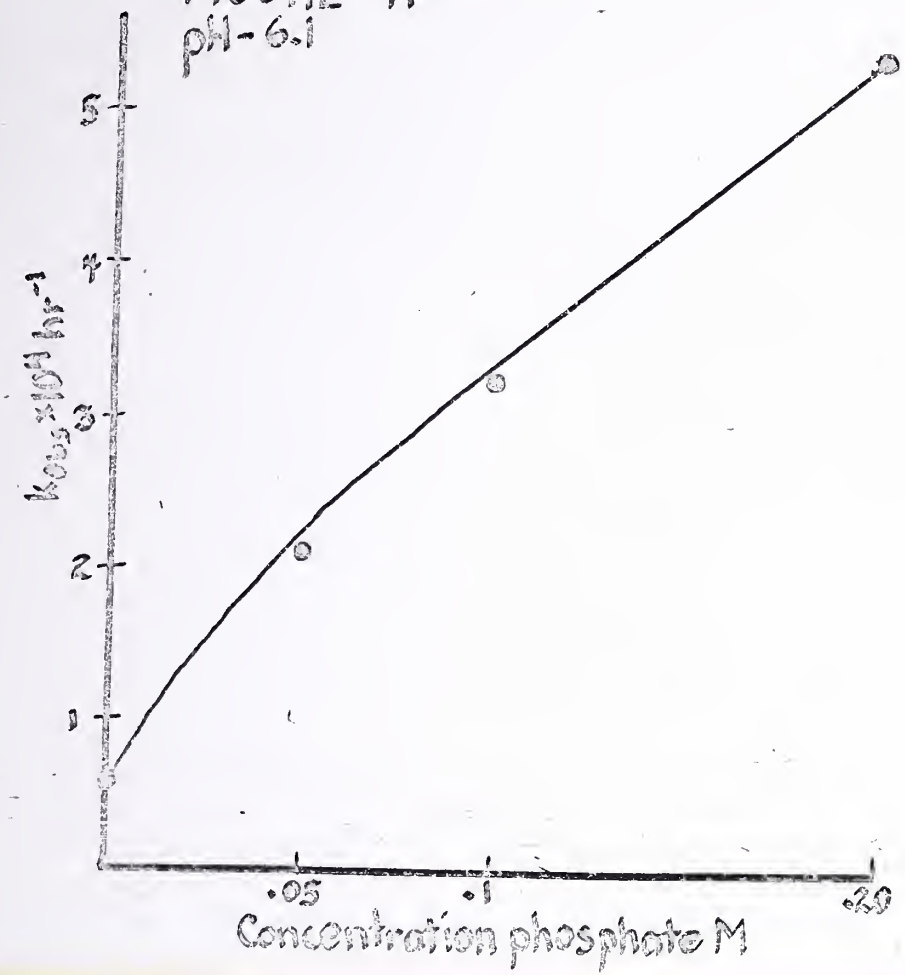
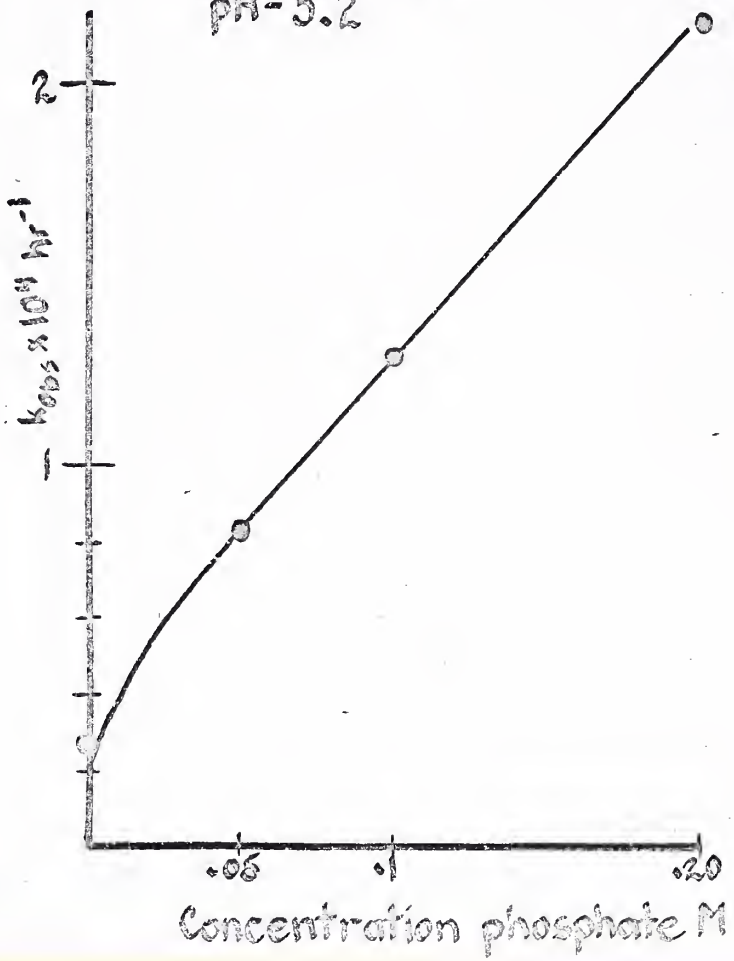


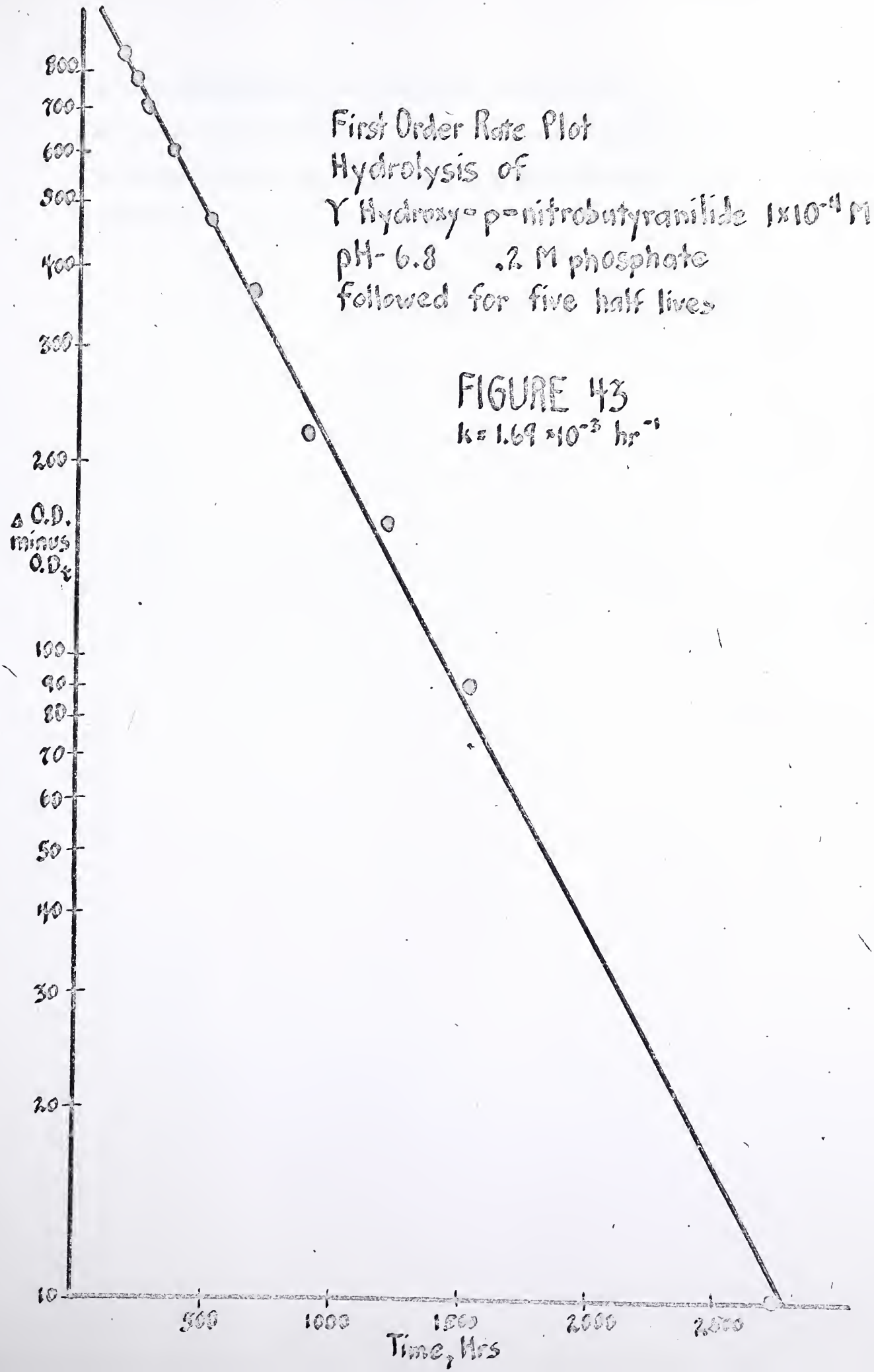
FIGURE 42
pH - 5.2



It may be seen that a significant increase in the rate of hydrolysis occurs in the presence of phosphate, a bifunctional buffer. (Table VIII) Although the number of points used to determine the curves represented in figures 39-42 are few, it was felt that the data could not be approximated by hyperbolic relationships such as those obtained for similar rate vs. phosphate data for the unsubstituted hydroxyanilide[4]. In addition, the data in figures 39, 40, and 41 did not fit a straight line relationship. The curves represented are biphasic and their upper segments are linear. The significance of this assumption is discussed further in Section IV. It is to be regretted that time did not allow the accumulation of additional data.

In order to check the validity of obtaining data on these reactions while only following them for 10-20% of completion, one of the more rapid "phosphate catalyzed reactions" was run at pH 6.81 in the presence of .20M phosphate and with a concentration of hydroxyanilide of $1x10^{-4}$M. It was followed for 5 half lives. The rate constant calculated for this reaction (see page 55) of $169x10^{-5}$ hr^{-1} was in adequate agreement with that calculated from the reaction followed only for 28% and treated as described on page 56. This latter rate constant was 145×10^{-5} hr^{1-}. The first order plot of the reaction followed to completion is shown in figure 43 and is remarkably good for a reaction followed for over 2500 hrs.

The effect of imidazole buffer on the rate of hydrolysis



First Order Rate Plot
Hydrolysis of
γ Hydroxy-p-nitrobutyranilide 1×10^{-4} M
pH- 6.8 .2 M phosphate
followed for five half lives

FIGURE 43
$k = 1.69 \times 10^{-3}$ hr^{-1}

of the hydroxyanilide was studied at a single pH. The data are presented in Table IX and represented graphically in Figure 40 curve II for comparison to the effect of phosphate at similar concentrations.

Table IX

Effect of Imidazole on the Rate of Hydrolysis of γ-hydroxy-p-nitrobutyranilide

pH	Buffer	Concentration M	k x 10^5 hr^{-1}	% Reaction followed
6.65-6.60	Imidazole	.03	2.9	11
6.81-6.83	"	.05	3.7	8
6.80-6.81	"	.10	4.4	10
6.80-6.78	"	.20	5.3	12

Reactions were carried out in sealed ampoules and treated as described on page 56 . pH's represent readings at zero time and at termination of reaction.

It can be seen that imidazole has, compared to phosphate, only a slight rate enhancing effect.

Section III

Chapter 3

Discussion

Several salient features of the results obtained from the studies of the kinetics of hydrolysis of γ-hydroxy-p-nitrobutyranilide will be discussed herein in terms of their compatibility with results obtained previously with the unsubstituted hydroxyanilide[4] and their support for the inclusive mechanism of hydrolysis for these compounds to be proposed in Section IV.

The curve presented in Chapter 2 (Figure 36) of this Section bears some resemblance to that obtained from studies of rate of hydrolysis with varying pH for the unsubstituted compound (See figure 10). At the extremes of pH, the rate of hydrolysis varies linearly with the concentration of hydroxide and hydrogen ions respectively. In addition, with decreasing pH, there appears to be a levelling off (in figure 36) below pH 8. The configuration of the curve below this pH, vaguely resembling a sine curve, is otherwise difficult to describe. The descriptions of the regions of the pH-rate profile found for the unsubstituted compound may be reviewed on pages 16-17. The data obtained in the present study suggest that as before, in alkaline pH, where hydroxyanilide is the favored product of imino-lactone hydrolysis, the acyl transfer reaction involved in the hydrolysis of the hydroxyanilide has, as its rate limiting step, the breakdown of an intermediate whose

rate of decomposition to p-nitroaniline is slow compared to its tendency to decompose by the reverse of the hydrolysis step. The levelling off, in the pH rate profile, as we decrease pH from alkaline regions, represents the contribution of another breakdown pathway, to the rate of hydrolysis. This additional pathway probably involves another intermediate, in another state of ionization, which is formed at lower pH, and the decomposition of which is more favored in the direction of p-nitroaniline. If the assumptions made by Schmir [19, 4] (for the unsubstituted hydroxyanilide, pages 16-17) were in effect for the p-nitro substituted compound, it would be expected that a further decrease in rate would be seen in the region of the midpoint of the product vs pH curve for the iminolactone (pH 6.1, see figure 24, p. 43). This is not seen in figure 36. The significance of this observation will be discussed in Section IV.

In considering the linear dependance of the rate of hydrolysis on the concentration of hydroxide (Figure 37), at equilibrium, the rate of hydrolysis depends on the speed of the rate limiting step: the breakdown of the tetrahedral intermediate. Therefore, it will also depend on the amount of tetrahedral intermediate present. The amount of tetrahedral intermediate present, at equilibrium, depends on the concentration of hydroxide. This is because of the requirement by the reaction mechanism, of base catalysis of the nucleophilic addition

step forming the tetrahedral intermediate. This activation occurs by ionization of the hydroxyl group (Pathway I in Figure 44) with a dissociation constant K_1. The rate of reaction should thus vary linearly with hydroxide and then level off at the completion of the dissociation. This levelling off should therefore begin to occur in the rate vs OH plot in the region around pK_1. The pK's of such hydroxyl groups are very high (> 15) and no levelling off was detected in the case of the unsubstituted hydroxyanilide[4]. However, in the curve in figure 37, a definite levelling off trend may be seen. The pK of the hydroxyl group may be presumed to be the same as that for the unsubstituted compound. However, the amide nitrogen, in the p-nitro compound, under the effect of the electron withdrawing p-nitro group, becomes a stronger acid and its dissociation along Pathway II (Figure 44) might be seen in the alkaline region. The resulting intermediate might be expected to be inactive with respect to hydrolysis of the amide group, because it is no longer electrophilic.

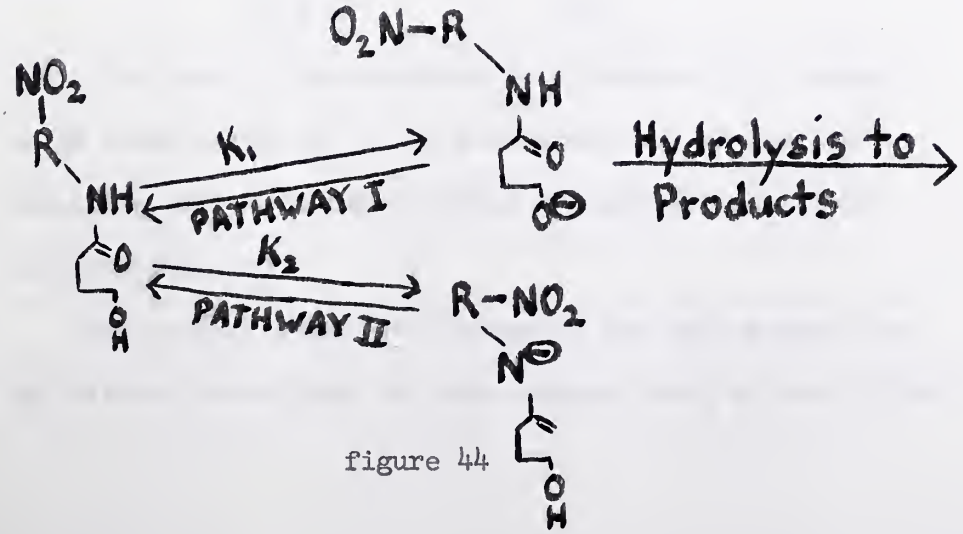


figure 44

The simple pH dependance of the rate of hydrolysis of compounds reacting along Pathway I is expressed by the equation:

$$k_{obs} = \frac{k\ K_1}{K_1 + H} \quad (6) \text{ and if } H \gg K_1 \quad k_{obs} = \frac{k\ K_1}{H} \quad (7)$$

It can be shown that for compounds where the rate of hydrolysis depends on the balance of equilibria such as those in Pathway I and Pathway II that:

$$k_{obs} = \frac{k\ K_1}{H + (K_1\ K_2)} \quad (8)$$

At slightly alkaline pH where H is much greater than K_1 K_2, the above equation (8) reduces to equation (7) and the rate is linear with hydroxide ion concentration. However, as pH becomes more alkaline and since under the influence of the p-nitro group H is small with respect to K_2, the observed rate could be expressed by the equation:

$$k_{obs} = \frac{k\ K_1}{K_1 + K_2} \quad (9)$$

Thus, the rate of the reaction when plotted with respect to pH would level off to be independent of pH and the levelling off would occur around an apparent pK which is lower than pK_1.

The second order rate constants for the variance of the rate of hydrolysis of this compound with pH are $15.9M^{-1}$

Hr^{-1} with respect to hydroxide ion in the alkaline region compared to $9.6 \times 10^{-2} M^{-1} Hr^{-1}$ for the unsubstituted compound [4] and $1.02\ M^{-1} Hr^{-1}$ with respect to hydrogen ion in the acid region compared to $21.2 \times 10^{-2}\ M^{-1} Hr^{-1}$ for the unsubstituted compound.

The second order rate constant for the p-nitro substituted compound in alkali is 167 times as great as that for the unsubstituted compound. The effect of the p-nitro group might be viewed as not only favoring the equilibrium for the addition of alkoxide to the carbonyl group by electron withdrawl, but, in addition its electron attracting effects should favor the expulsion of the p-nitroaniline nucleus in the rate limiting breakdown step by making it a better leaving group. Thus, in alkali the rate of hydrolysis would be increased substantially by the presence of the p-nitro group.

The second order rate constant in acid, is only 4.7 times as great for the p-nitro substituted compound when it is compared to the unsubstituted compound. The mechanism of hydrolysis of the hydroxyanilide in acid (where the tetrahedral intermediate formed preferentially breaks down to the aniline) probably involves pre-equilibrium protonation of the hydroxyanilide to activate the carbonyl, then rate limiting addition of the alcohol group, closing the ring. This is followed by rapid decomposition of the intermediate to butyrolactone and p-nitroaniline.

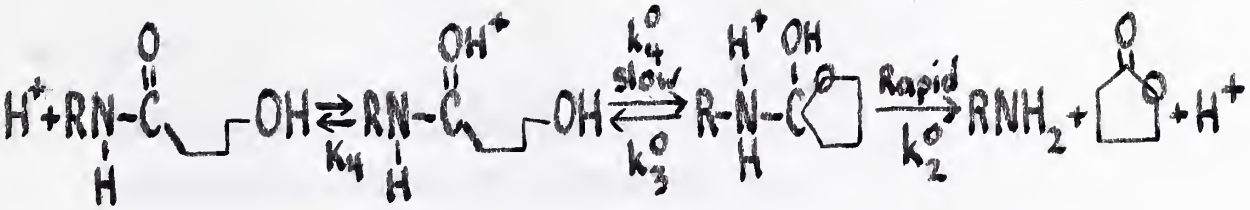


Figure 45

The electron withdrawing effect of the p-nitro group would tend to activate the carbonyl for addition in the rate limiting step. However, the substitution probably results in a lower pK for the hydroxyanilide protonation step and therefore less activation by hydrogen ion.

With regards to the dependance of the rate of hydrolysis of the hydroxyanilide on the concentration of certain buffer species, as previously [4], the catalytic effectivity of phosphate, a bifunctional buffer is much greater than that of imidazole, a monofunctional buffer. However, the relationship of rate to concentration of phosphate buffer does not seem to be as described for the unsubstituted hydroxyanilide [4]. (Figures 39, 40, 41, 42, Imidazole data on Figure 40). The curves are not hyperbolic with the achievemment of a maximum rate. The phosphate curves are biphasic, suggesting two effects of phosphate. The precise role of buffers in the mechanism of hydrolysis will be discussed in Section IV.

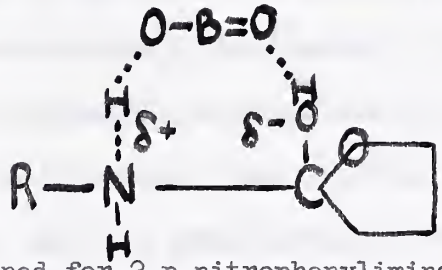
Section IV

Conclusions: The Mechanism of Hydrolysis of p-Nitrophenyl Substituted Iminolactone and -Hydroxyanilide Derivatives.

The results presented and discussed in Sections II and III differ from results previously obtained with the unsubstituted family of compounds [1, 2, 4]. Clearly, some modifications of the previously proposed mechanism of hydrolysis is required for the substituted compounds.

Previously, with the unsubstituted iminolactone [1], the kinetics were interpreted as follows: In weak acid, the rate limiting step is the attack of water on the protonate iminolactone to form a neutral carbinolamine intermediate. Above pH 7 the rate limiting step is probably the attack of hydroxide on the protonated iminolactone and the rates are pH independent. In addition, the reaction proceeds increasingly through an anionic carbinolamine intermediate. The changing of products with pH has been interpreted as follows: the neutral tetrahedral intermediate yields primarily aniline and the anionic intermediate decomposes mainly to hydroxyanilide. Further, bifunctional catalysts have little effect on the rate of reaction and their marked effects on products probably results from interaction after the rate determining formation of the tetrahedral intermediate. It has been proposed [2] that the bifunctional buffers interact with the neutral intermediate in such a fashion

that the electron distribution of the intermediate closely resembles a zwitterion which rapidly expells aniline.



The kinetics obtained for 2-p-nitrophenyliminotetrahydrofuran are compatible with rate limiting attack of water on the protonated form of the iminolactone. The expected lowering of the pK by the substitution of the p-nitro group is compatible with the absence of a change in the slope of the pH rate profile occuring in the region of approach to full protonation at low pH. It will be necessary to use special instrumentation to follow extremely rapid reactions in order to kinetically determine this pK. As discussed in Section II (P. 48), there is no real evidence for the existence of a significant rate limiting attack of hydroxide on the protonated iminolactone since the suggestion of levelling off of the pH rate profile (Figure 23) could also be interpreted as the contribution of catalytic buffer effects.

The steep sigmoid dependance of the product pH relationship (Figure 24) is suggestive of a dependance on hydrogen ion to the second power (Curve II). This would occur if the intermediate species that generates the p-nitroaniline and butyrolactone products on the one hand and that which generates the hydroxyanilide, were separated by two charges and thus two protons. It

may be proposed that a family of three different tetrahedral intermediates is formed in the course of these reactions: a cationic protonated intermediate in acid media, a neutral intermediate in weakly acid media, and a dissociated anionic tetrahedral intermediate in weakly alkaline media. (See figure 46). It appears that the contriubtion of the neutral intermediate to product formation is negligible. This would transform the product pH relationship from one which resembles the titration curve of a bifunctional acid with base, to one where the plateau between the pK's is not seen and the curve resembles a steep sigmoid relationship, dependant on hydrogen ion to the second power. Product-pH dependancies resembling titration curves with two dissociations have recently been obtained for imidates [34].

The effects of bifunctional buffers such as phosphate on the product distribution obtained for the hydrolysis of the p-nitro substituted iminolactone are to significantly increase the % yield of p-nitroaniline. Although no kinetic data were obtained, it would be expected that no significant increase in rate of hydrolysis would be found in the presence of phosphate if its influence is exerted upon the tetrahedral intermediate after the rate determining formation step. There is insufficient data to determine clearly which species of phosphate acts on which species of intermediate; however, some conclusions about the latter may be drawn. It can be seen in the data in Table V that the effectiveness of phosphate in producing product change, decreases with

with increasing pH (and therefore the K_{app} calculated increases). In view of the previous interpretation of the action of bifunctional catalysts on the unsubstituted iminolactone hydrolysis, a reasonable interpretation of the present data would suggest that phosphate acts bifunctionally on the neutral tetrahedral intermediate, the concentration of which, is decreasing with increasing pH. The buffer species might be viewed as influencing the electron distribution about the neutral intermediate so that it resembles a zwitterion. Its structure as a zwitterion would closely resemble the cationic tetrahedral intermediate and it would be more efficient at expelling the aniline nucleus. In addition, however, the finding that the maximum yields of p-nitroaniline obtainable in the presence of phosphate are less than 100% suggests that phosphate can act to catalyze the decomposition of some species to the formation of the hydroxyanilide. The decreasing maximum yield of p-nitroaniline obtainable with increasing pH suggests that phosphate has this second action upon the anionic intermediate, the concentration of which is increasing with increasing pH. Phosphate may act on this anionic tetrahedral intermediate as a monofunctional general acid catalyst enhancing the breakdown of this intermediate to the anilide.

Additional support for this view may be obtained from an interpretation of the ability of monofunctional amine buffers to increase the yield of hydroxyanilide as shown

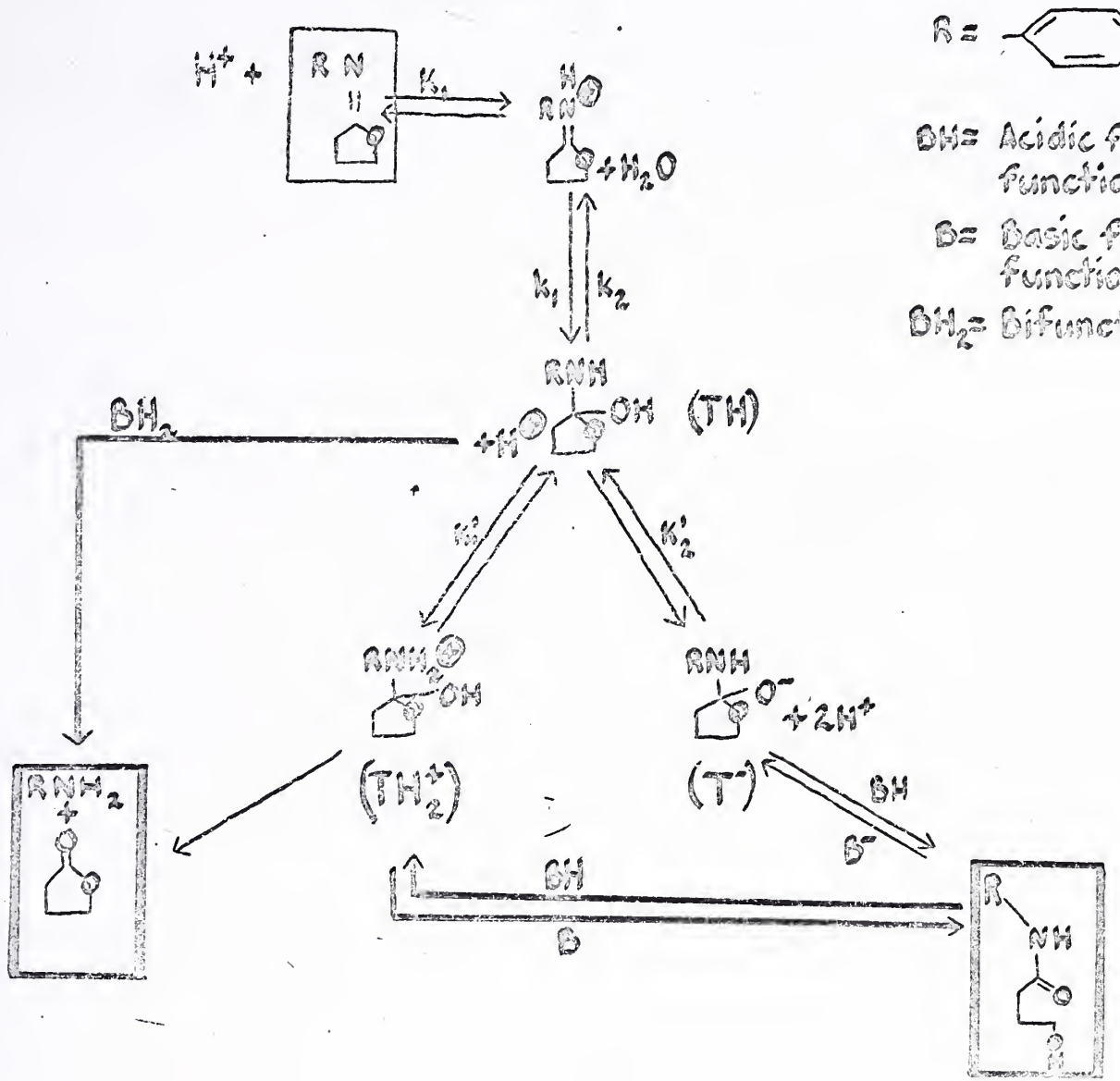
in table II. The action of these may be viewed as identical to the second action of phosphate, i.e., general acid catalysis of the breakdown of the anionic tetrahedral intermediate. Although more data is needed, the general effectiveness of these amine buffers is relatively fixed and small with varying pH. In the mechanism proposed, the increasing presence of the anionic intermediate with increasing pH, would be offset by the decreasing concentration of any one buffer species in its acid form as pH increases. No real variance of buffer ability with pH would be expected.

The modifications of mechanism are summarized in Figure 46.

The assumption of the proposed mechanism (Figure 46) leads to several predictions concerning the hydrolysis of γ-hydroxy-p-nitrobutyranilide. As discussed in Section I (pps. 15-17) and with the data from Section II, the following conclusions may be drawn. Above pH 6.1, the rate limiting step in the acyl transfer reaction is the breakdown of the tetrahedral intermediate. The attack, via ring closure is rate limiting below this pH. The effect of the addition of phosphate is the enhancement of the breakdown of the tetrahedral intermediate. As discussed in Section III, the rate vs conc. phosphate curves (Figs 39-42) are clearly biphasic as drawn. The

Scheme for Mechanism of Hydrolysis of 2-p-nitrophenyliminotetrahydrofuran and γ-Hydroxy-p-nitro-butyramilide

FIGURE 46



lower segment of each curve, before the break in slope, represents the result of bifunctional catalysis on the breakdown step. The changes of rate show greater sensitivity to increasing concentrations of phosphate than do the upper segments. Over the range of pH studied there is a slight decrease in the magnitude of rate changes encompassed within the lower segments and therefore probably a decrease in the effectiveness of phosphate as a bifunctional catalyst as pH decreases. This would seem in contrast to the data presented in Table V and discussed on page 74 where the increase in k_{app} (decrease in effectiveness) of phosphate with increasing pH was cited as a reason why it might be viewed as acting on the neutral intermediate. However, as shown in figure 10, previous work with the unsubstituted hydroxyanilide revealed a maximum level of phosphate catalysis obtainable which decreased with decreasing pH. This maximum occurred after phosphate catalysis had so speeded the breakdown step, that alkoxide attack became rate limiting. The rate of the alkoxide attack and therefore the maximum phosphate catalyzed rate abtaiable was dependant on the presence of hydroxide ion needed to generate the alkoxide [4]. The decrease in the magnitude of the rate changes included within the lower segments of curves 39-42 with decreasing pH is analagous to the behavior of the maximum rates obtaiable with the addition of phosphate in the unsubstituted hydroxyanilide. It is thus still consistent with the action of phosphate as a bifunctional catalyst upon the neutral

intermediate as inferred from the product studies of the iminolactone. The lower segment of the curves represent the bifunctional action of phosphate. The fact that the curves do not level off but change slope and continue with a linear upper portion is compatible with the view that, as shown by its effects on the products of iminolactone hydrolysis, phosphate has a second action. Once as a result of phosphate catalysis of the breakdown step, the addition step becomes rate limiting, the effect of phosphate on this step is revealed. The further increase in rate after the break in slope is the result of general base catalysis of the addition step by the conjugate base of the phosphate anion. This second action of phosphate is analagous to the action of imidazole on the hydrolysis of the hydroxyanilide (Table IX Figure 40 II). As shown, the effect of imidazole is slight. This is because, at the pH that it was studied, breakdown is the rate limiting step, and the action of imidazole as a general base catalyst of the addition step leads to little rate enhancement. A test of the role of imidazole as a general base catalyst and of phosphate as having two catalysis roles would be to elevate the rate of hydrolysis of the hydroxyanilide to the point at which the break in slope occurs, at a single pH, with phosphate, and then view the effect of adding imidazole. It should then have the same effect as phosphate on the second segment of the rate vs buffer concentration curve. Finally,

between pH 6 and 5.1, where the attack step is rate limiting, the effect of phosphate as a bifunctional catalyst and therefore the lower segment of the curve (Figure 42) is minimal. The demonstration that the upper segment of the curve retains a significantly positive slope despite the low pH is compatible with the interpretation that phosphate as a monoanion might act as a pure general acid catalyst in the generation of the cationic tetrahedral intermediate (TH_2) by a direct route as shown at the bottom of Figure 46. Its action at these lower pH's should also be paralleled by monofunctional amine buffers like imidazole, acting as general acids. Both the general acid and general base mechanisms of catalysis of the hydroxyanilide hydrolysis are viewed as being bidirectional by the principle of microscopic reversibility. The reverse of the general base catalysis, i.e., general acid catalysis of the breakdown of the anionic intermediate to hydroxyanilide has already been discussed in this section (page 75) with regards to the action of monofunctional buffers on the products of iminolactone hydrolysis. The existance of the reverse step of the general acid catalysis directly from the hydroxyanilide to TH_2 , a general base catalyzed step is not incompatible with the data. All pathways and catalytic actions may be reviewed in Figure 46.

In summary, the many differences between the behavior of the p-nitro compounds and the unsubstituted compounds may be accounted for by the proposed mechanism. One of

the major differences is the increased importance of the cationic tetrahedral intermediate in the generation of the aniline product. In viewing the kinetic profile of the hydroxyanilide hydrolysis as a function of pH, there are no major differences of alkaline pH. However, as shown in figure 10, for the unsubstituted compound, with decreasing pH the curve which had levelled off because of the contribution of the breakdown through the neutral intermediate falls off again when the protonation of the anionic intermediate is complete (region B) because of the presence of a second dissociation step, in the mechanism of hydrolysis of the p-nitro substituted hydroxy-anilide, this second change of slope in the positive direction (decrease in rate with decreasing not. being seen again) (Figure 36). The exact configuration of the curve around pH 6.1, the point of transition in rate determining step is difficult to explain, mechanistically.

The lessened importance of the neutral tetrahedral intermediate in the generation of aniline is consistent with the change in structure brought about by the substitution of a p-nitro group. The electron withdrawl effect, decreasing the basicity of the N-H group of the aniline nucleus decreases the ability of the neutral tetrahedral intermediate to form a zwitterion capable of expelling aniline but favors the demonstration of a mechanism wherein external protonation (the formation of TH_2) is required for the breakdown to p-nitroaniline and butyro-lactone.

The further elucidation of the mechanism proposed for the decomposition of the p-nitrosubstituted hydroxyanilide and iminolactone will require many more experiments especially with the slowly hydrolyzing hydroxyanilide. However, the questions which need answering and the assumptions that await verification are spelled out in this thesis.

It is most significant that compounds of close derivation behave so differently. In gross effect the shift of pK between aniline and p-nitroaniline from ca. 5 to ca. 1, results in a difference in the pK' for the tetrahedral intermediate families of only from pH 7 to pH 6. However, the substitution of the p-nitro group in this family of compounds drastically alters the pathways of breakdown and the mechanisms of and susceptibility to catalysis.

Appendix

Other related Synthetic and Assay Techniques

The preparation of 2-p-methoxyphenyliminotetrahydrofuran and γ-hydroxy-p-methoxybutyranilide was undertaken. However, the time necessary for the completion of their preparation and purification for study was not available.

Assay for p-methoxyaniline (p-anisidine) - The prospect of a need for accurate product analysis of these compounds made an assay for p-anisidine necessary because, unlike p-nitroaniline, it had no distinctive absorption. (Figure 47)

p-anisidine- was recrystallized from ethanol-water 3:1 mp 58°C

A Bratton Marshall assay[26, 27] with further modification of the method used by Cunningham[1] was employed.

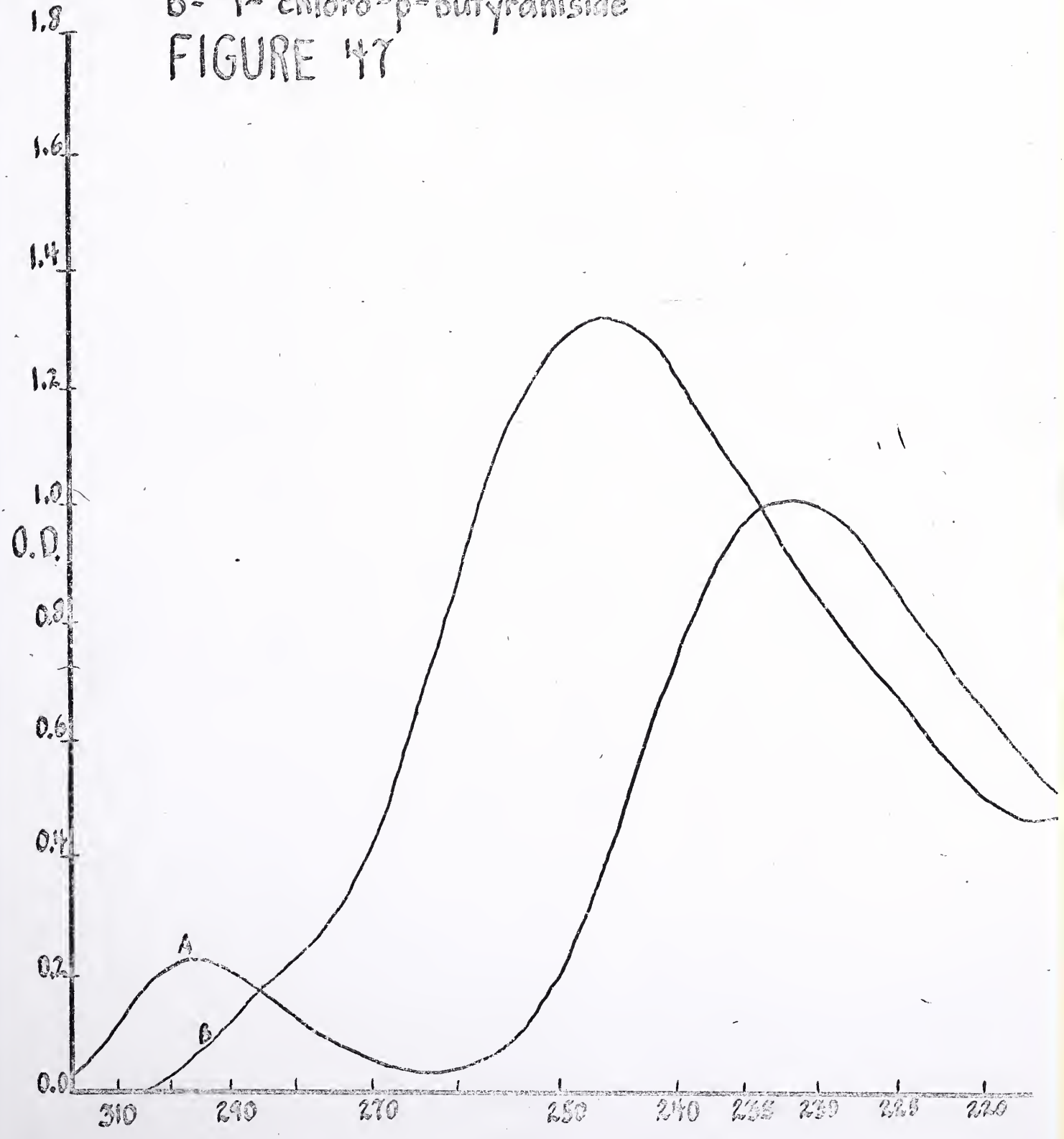
Unknown samples of 2 ml in 10% acetonitrile were added to 3.5 ml of 2M sodium phosphate adjusted to pH 1.7, with HCl. Then .5 ml of .1% (g/v) was added and the mixture was incubated at room temp for 5 min. Excess nitrite was destroyed by the addition of .5 ml of .5% acqueous amonium sulfate. Three minutes later .5 ml of .1% N-(1-naphthyl) ethylenediamine dihydrochloride was added. The reaction mixtures were then incubated for 24 hrs at 30°C. They were read at 570 mμ and the standard curve in Figure 48 was prepared.

γ-chloro-p-methoxybutyranilide (γ-chlorobutyraniside (para))- To 2.81 g (20 mmoles) of 4-chlrobutyroyl chloride in 20 ml of ether stirred at 0° in an ice bath was added 40

U.V. Spectra (CH_3CN)

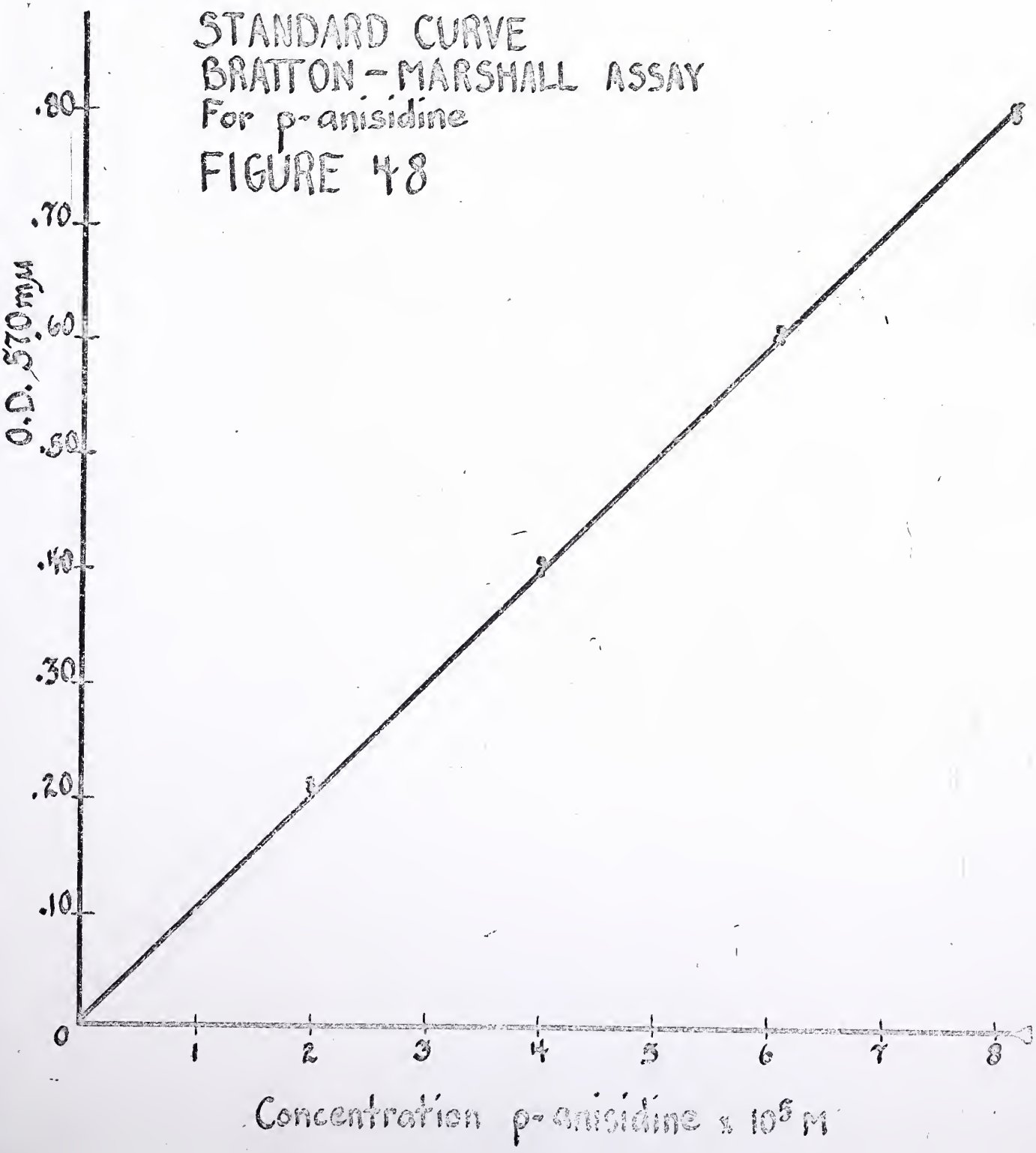
A- p- anisidine 1.3×10^{-4} M
B- γ- chloro-p-butyranisidide

FIGURE 47



STANDARD CURVE
BRATTON-MARSHALL ASSAY
For p-anisidine

FIGURE 48



ml of a solution containing 4.19g (40 mmoles) of p-anisidine in ether. The mixture was then stirred at room temp for 1 hr. A voluminous white precipitate of p-anisidine hydrochloride resulted during the stirring. The mixture was filtered by vacuum and the supernatant liquid when evaporated in vacuo yielded a solid which was recrystallized from 50 ml of 1:1 ether-petroleum ether (30-60°). mp 83°C IR spectrum fig 49 UV spectrum Figure 47 B Yield 1.3g 29%.

Analysis

	% C	H	N	Cl
Calculated	58,01	6,19	6,15	15,58
Received	57,94	6,22	6,24	15,66

2-p-methoxyphenyliminotetrahydrofuran- was prepared after the method of Peter et al[22].

In an ice salt bath at -17 C 1.5 g of γ-chlrobutyraniside (6.6 mmoles) in 35 ml of methylene chloride were stirred. To this solution was added 27 ml of a solution of silver tetrafluoroborate 1.28 N in silver (7.3 mmoles) standardized by the method on page . The addition was accomplished over 15 min and the solution was subsequently stirred at -17°C for 30 min during which a white precipitate formed. The reaction mixture was then stirred for 1 hr at 0°C. It was then warmed to room temperature and 300 mg of triethylamine hydrochloride was added with the formation of additional precipitate.

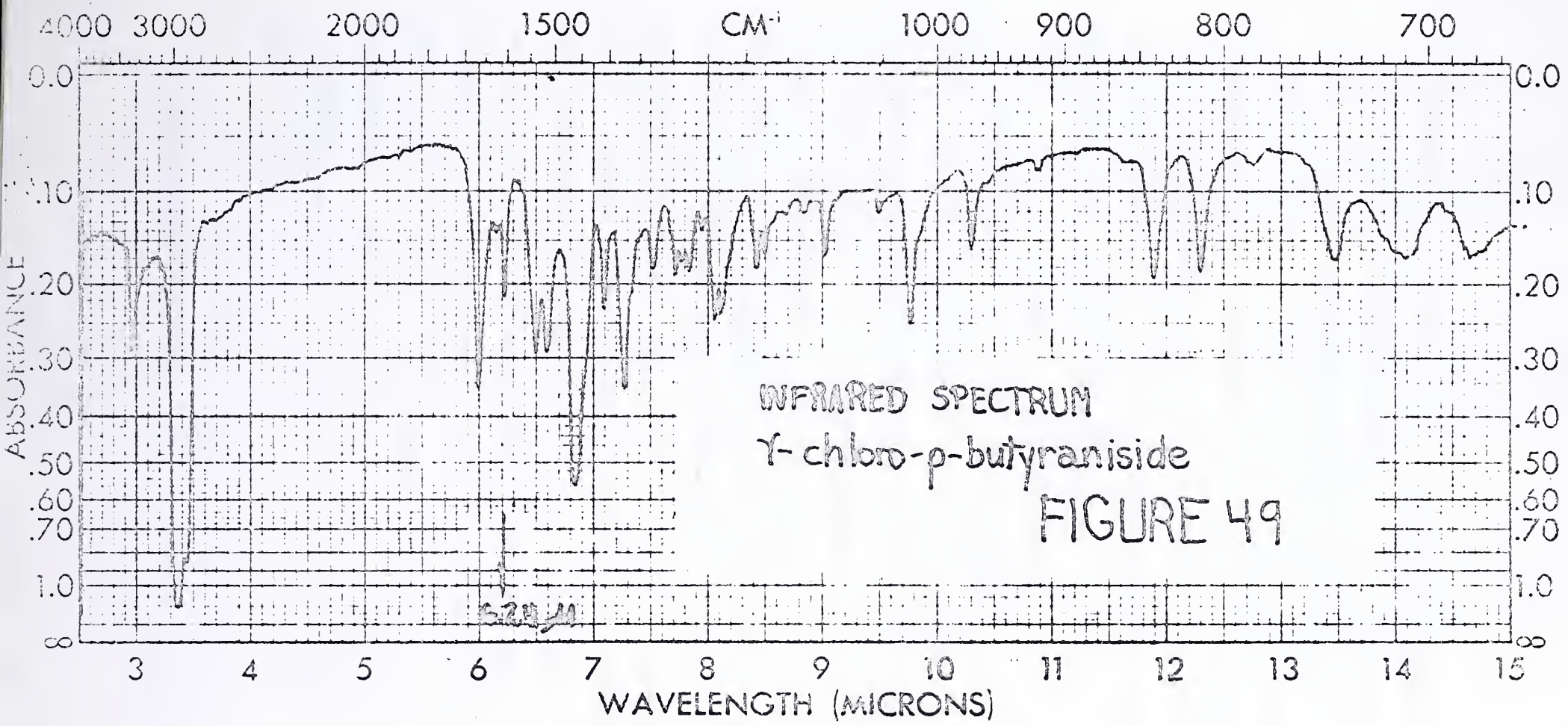
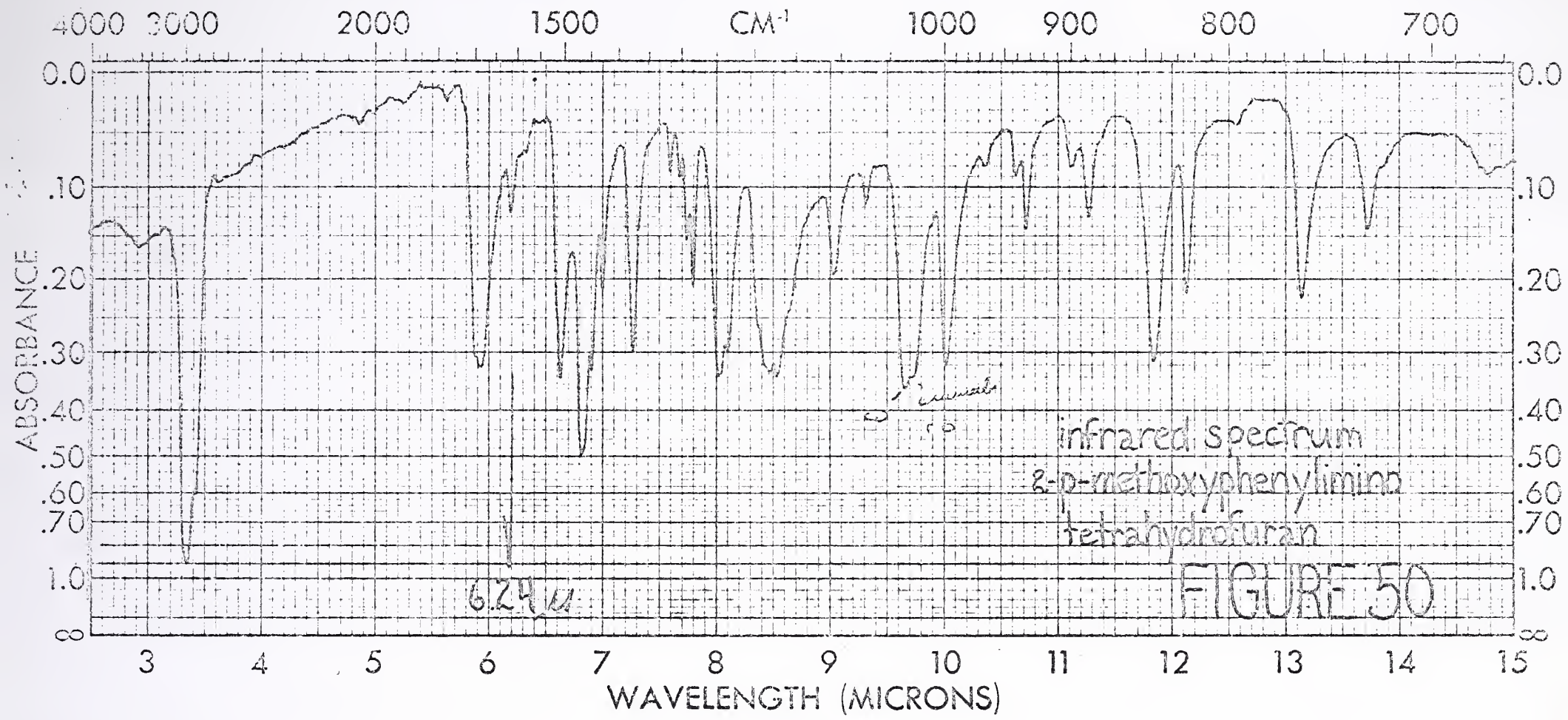

4000 3000 2000 1500 CM⁻¹ 1000 900 800 700
0.0
.10
.20
.30
.40
.50
.60
.70
1.0
∞
ABSORBANCE
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)
INFRARED SPECTRUM
γ-chloro-p-butyraniside

FIGURE 49

The mixture was then filtered by suction and the precipitate washed with benzene-methylene chloride 1:1. The mother liquor was washed by extraction with 50 ml of 2M sodium carbonate and 50 ml of saturated NaCl. The mother liquor was then stored for 1.5 hrs over 15 g of annhydrous $MgSO_4$. The solution was filtered and the mother liquor evaporated in vacuo leaving an oil. The oil crystallized under cold petroleum ether to give a crude crystalline product mp 47-48 . IR spectrum fig 50. Hydrolysis studies in .001 HCl revealed complete conversion of the product Figure 51A.to p-anisidine hydrochloride 51C. The family of spectra for the hydrolysis are shown in figure 51B. The half life was 12 min at 25 . The crystals were soluble in ether, benzene, acetone, and ethyl acetate. However, they could not be precipitated from the above solutions by cooling or the addition of small amounts of petroleum ether 30-60 as a solid. The materal was isolable only as an oil. However, the oil could be crystallized by scratching under petroleum ether when cooled in a dry ice acetone bath at -73 C. The crystals obtained did not melt when rewarmed. No further attempts at purification were made. Chromatography will probably be necessary to obtain a re-crystallizable product. This compound has been reported by Mukaiyama and Sato[29], as an oil with bp 155° at 2 mm Hg.

Yield 500 mg

p-methoxyphenylsuccinimide- prepared after the method

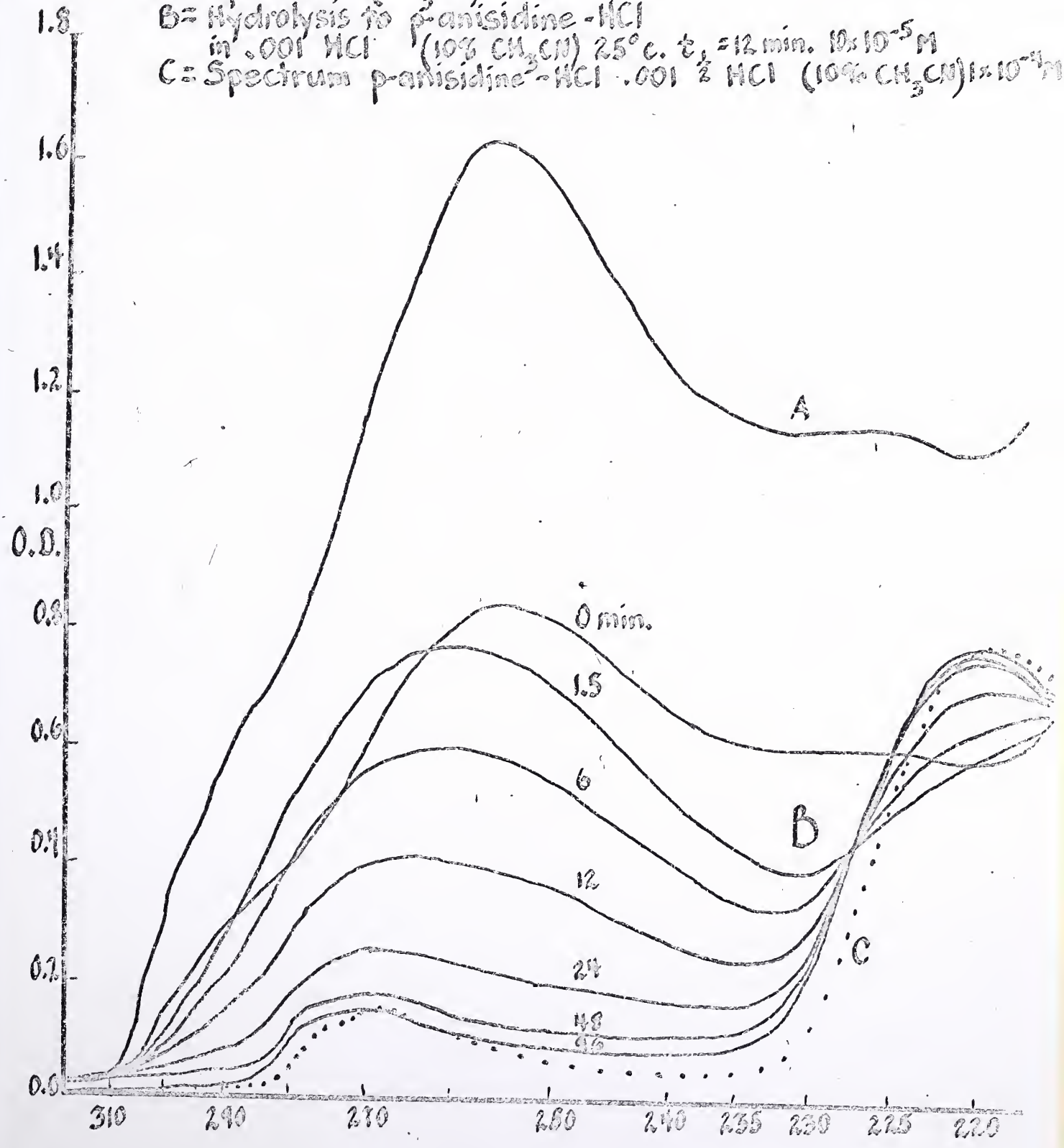


infrared spectrum
8-p-methoxyphenylimino
tetrahydrofuran

FIGURE 50

U.V. SPECTRAL STUDIES OF
2-p-methoxyphenyliminotetrahydrofuran
FIGURE 51

A = Spectrum (CH_3CN) 2×10^{-4} M
B = Hydrolysis to p-anisidine-HCl in .001 HCl (10% CH_3CN) 25° c. $t_{\frac{1}{2}}$ = 12 min. 10×10^{-5} M
C = Spectrum p-anisidine-HCl .001 N HCl (10% CH_3CN) 1×10^{-4} M



of Weasthead and Morawetz[31].

In a mortar were ground 10g of succinic annhydride (100 mmoles), 12.4 g of p-anisidine (100 mmoles) and 13.6 g of potassium hydrogen sulfate (100 mmoles). The mixture was then transferred to a crucible and fused to a solid which was pulverized and triturated with 140 ml of water. The filtered solid was dried in a 115 °C oven for 15 min. It was then dissolved and recrystallized with norite decolorization from 550 ml of boiling ethyl alcohol (absolute) to yield a white solid mp 102-103 °. Yield 11g 40%.

Time did not allow for attempts at borohydride reduction of this compound to the γ-hydroxyanilide as attention was turned towards studies of the hydrolyses of the p-nitro substituted compounds which had been more completely purified.

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