I. REACTIONS OF PHTHALOYLAMINO ACIDS; MODIFICATION OF THE CARBOXYL GROUP OF AMINO ACID DERIVATIVES

II. DERIVATIVES OF INDOLE 3-CARBOXYLIC ACID

III. METAL ION COMPLEXES OF AMIDOXIMES; THE INHIBITION OF THE ALPHA-CHYMOTRYPSIN CATALYZED HYDROLYSIS OF NICOTINYL-L-TYROSINE HYDRAZIDE BY L-α-ACETAMIDO-β-PHENYLPROPIONAMIDOXIME

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I would like to express my appreciation to Dr. Donald G. Crosby for his friendly interest in my work during my first year on campus.

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ABSTRACT

Various derivatives of α-phthalimido-β-phenylpropionic acid have been synthesized, starting from phenylalanine. The derivatives have a doubly bound nitrogen or sulfur atom in place of the carbonyl oxygen atom. The goal of the work was to prepare similar derivatives of other acylamino acids for evaluation as substrates in studies of α-chymotrypsin catalyzed hydrolysis. This has been accomplished in the case of the amidoximes, as exemplified by the preparation of L-α-acetamido-β-phenylpropionamidoxime. The preparation of this compound was accomplished by the use of a new method for cleaving the phthaloyl group, discovered during the present study. However, the above compound was not hydrolyzed by α-chymotrypsin at pH values ranging from five to nine.

3-Indolecarbonyl chloride has been prepared by an unusual method and used to acylate α-amino acid derivatives.

The enzyme-inhibitor dissociation constant of α-chymotrypsin and L-α-acetamido-β-phenylpropionamidoxime has been determined using nicotinyl-L-tyrosine hydrazide as the substrate.

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PART I: REACTIONS OF PHTHALOYLAMINO ACIDS; MODIFICATION OF THE CARBOXYL GROUP OF AMINO ACID DERIVATIVES

A. INTRODUCTION

Esters, amides, hydroxamides, and hydrazides of certain α-amino acids and acylated α-amino acids have been used as substrates in studies of the alpha-chymotrypsin catalyzed hydrolysis of amino acid derivatives. Various other derivatives of carboxylic acids such as the nitriles, RCN, amidoximes, $RC(=NOH)NH_2$, imidic esters, $R_1C(=NH)OR_2$, amidines, $RC(=NH)NH_2$, and thioamides, $RCSNH_2$, have long been known. The studies reported in this section grew out of a long range goal of examining the suitability of the above derivatives of the α-amino acids or acylated α-amino acids as substrates in enzymatic hydrolyses.

Examination of the literature revealed that only a few such derivatives of α-amino acids had been prepared, and that the methods used were not suitable for obtaining optically active compounds starting from the optically active α-amino acids. Accordingly a synthetic study was initiated and aimed at developing suitable methods for converting optically active α-amino acids to the desired derivatives.

The investigation was divided into four parts:

(1) the preparation of a few derivatives of the model compound, hydrocinnamic acid

(2) the preparation of derivatives of α-phthalimido acids

(3) the preparation of derivatives of other α-acylamino acids

(4) the extension of the methods to optically active α-amino acids.

During the course of the work it was decided that some of the derivatives of α-phthalimido-β-phenylpropionic acid were of sufficient interest to warrant study not necessarily related to the original goal of achieving new substrates. The results of these studies are included in Part I of this thesis.

It may be mentioned here that the four steps of the investigation outlined above have been successfully completed in the case of the amidoximes, as exemplified by the preparation of L-α-acetamido-β-phenylpropionamidoxime. However, when this compound was tested at pH8 as a substrate for α-chymotrypsin, no hydrolysis was observed. The preparation of a number of other derivatives of the desired type has been carried as far as the second step mentioned above. That is, the phthalimido acid derivatives have been prepared.

B. THE CHOICE OF A SYNTHETIC ROUTE; PRELIMINARY EXPERIMENTS WITH HYDROCINNAMIC ACID

Most of the acid derivatives under consideration have the structure $R_1C(=NR_2)X$ where R_2 may be any of various atoms or radicals, and X is oxygen or nitrogen to which one or two groups, respectively, are attached. In order to limit the scope of the study it was decided to consider primarily derivatives in which hydrogen has replaced all possible alkyl groups. Thus $R_1C(=NH)NH_2$ was considered rather than $R_1(=NR_2)NR_3R_4$. In some cases compounds of the desired type may be prepared by direct addition of certain reagents to the nitrile. Probably the most important route to such compounds, however, is by way of the corresponding imino esters, $R_1C(=NH)OR_2$, which are obtained as the hydrochlorides by treating the nitriles with an alcohol and hydrogen chloride.

Accordingly hydrocinnamonitrile was allowed to react with one mole of methanol in the presence of dry hydrogen chloride. A solid slowly separated which was probably the imino ester hydrochloride. Treating some of the above solid with alcoholic ammonia and evaporating most of the solvent gave white prisms which were probably the amidine hydrochloride. These hydrocinnamic acid derivatives were not further characterized because by this time it was found that the corresponding derivatives of α-phthalimido-β-phenylpropionic acid were as easy to handle as the hydrocinnamic acid compounds.

C. AMINO NITRILES

1. The Literature of Amino Nitriles

The extension of the synthetic route mentioned above to the α-amino acid series was accomplished by the use of α-phthalimido-β-phenylpropionitrile as an intermediate. As a basis for comparing this compound with amino nitriles reported in the literature, a summary of the literature of amino nitriles is given here.

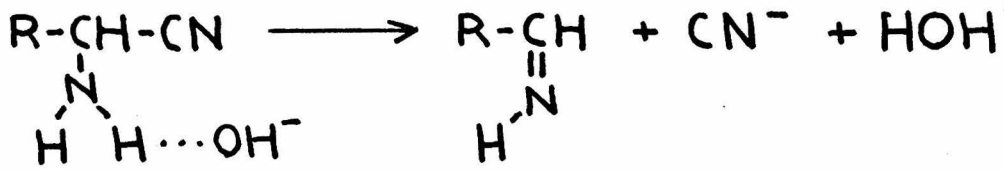
Examination of the literature has revealed few examples of α-amino nitriles or their derivatives except those obtained in the well-known Strecker synthesis of α-amino acids or a modification thereof*:

$$RCHO + NH_3 + HCN \qquad RCH(NH_2)CN$$

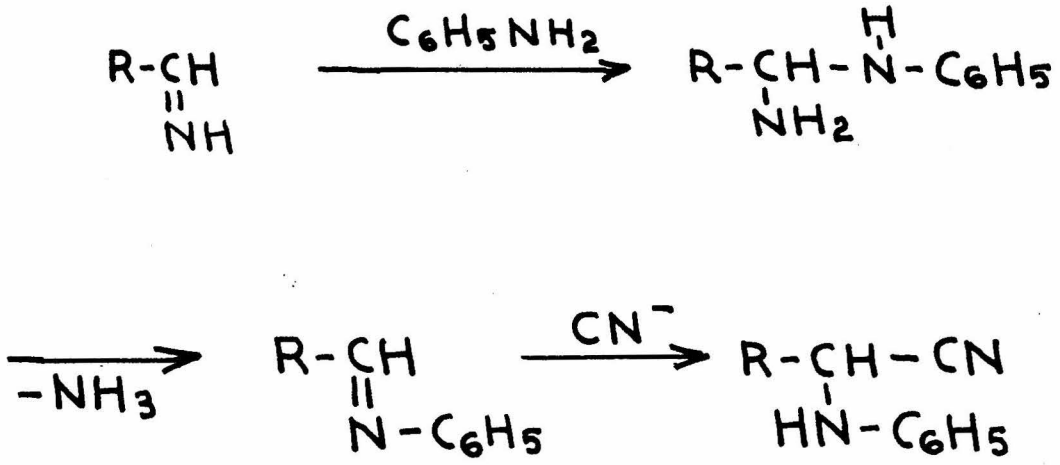
---

*The known exceptions are a patented reaction of halonitriles with ammonia to give amino nitriles (1, 2, 3), a reaction of potassium phthalimide with chloroacetonitrile giving phthalimidoacetonitrile (4, 5), and a reaction of phthalimidoacetamide or α-phthalimidopropionamide with phosphorus pentoxide, giving the corresponding nitrile (6). The last reaction will be discussed later.

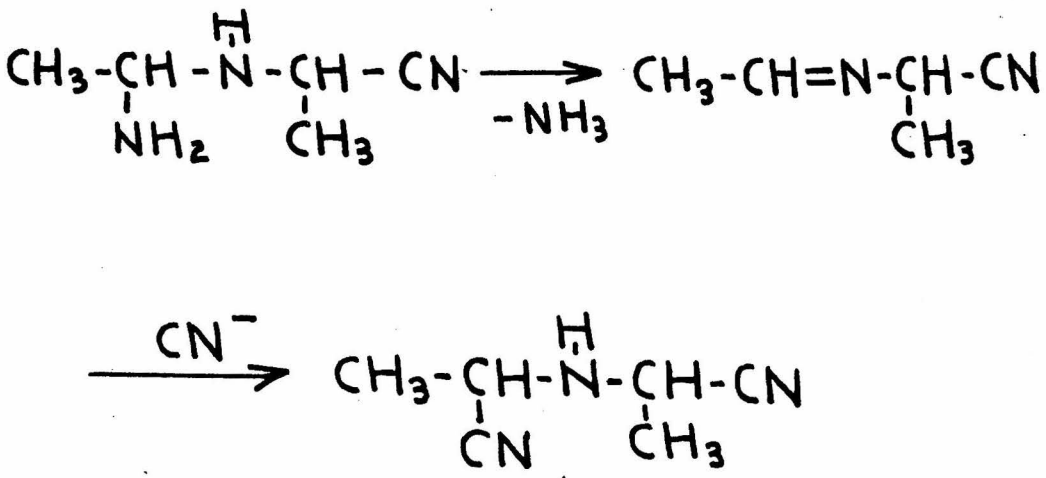
For example, from the reaction of potassium cyanide, ammonium chloride, and formaldehyde, Jay and Curtius (7) obtained crystalline methyleneaminoacetonitrile, which is now known to be a trimer, $C_9H_{12}N_6$, not $CH_2{=}NCH_2CN$ (8, 9). The reaction of this compound with alcoholic hydrogen chloride gave aminoacetonitrile hydrochloride, which was hydrolyzed to glycine by warming with dilute hydrochloric acid. Klages (10) distilled aminoacetonitrile at 15 mm. with partial decomposition. It is of special interest that benzoylation of this nitrile using Schotten-Baumann conditions gave the crystalline nitrile of hippuric acid (benzamidoacetonitrile) (10, see also 11 and 12). Thus acylation occurred faster than the expected base catalyzed decomposition analogous to cyanohydrin decomposition:



Evidence that such an equilibrium does occur is the reported racemization when optically active α-aminopropionitrile is hydrolyzed by sodium hydroxide (12). Further evidence is to be found in the reaction of aminoacetonitrile with aniline to give N-phenylaminoacetonitrile (13), which would logically be formed from the intermediate formaldehyde imine:



A similar intermediate probably occurs in the dimerization of α-aminopropionitrile, prepared from acetaldehyde, ammonia, and hydrocyanic acid. The reaction occurs so readily that this amino nitrile cannot be concentrated in ether solution without the loss of ammonia (14):

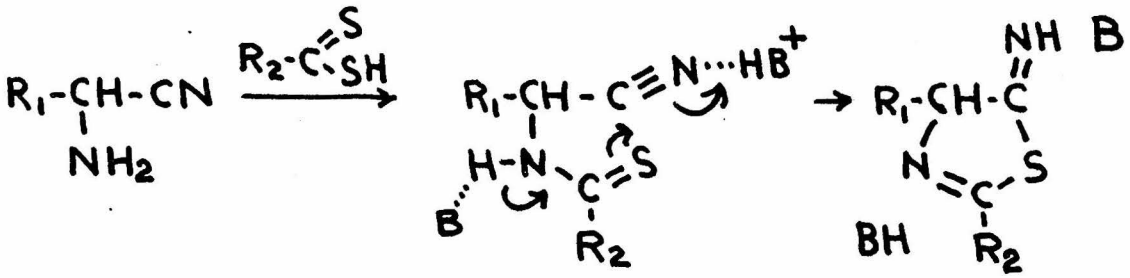


However, the sulfuric acid salt of the nitrile may be isolated.

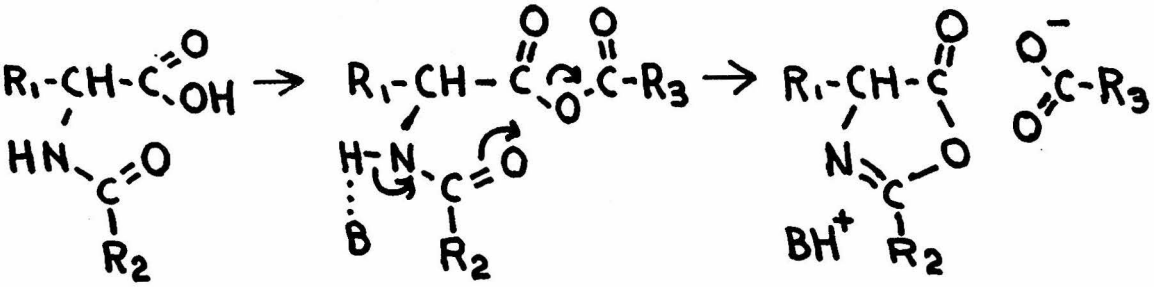
The nitrile corresponding to phenylalanine has been obtained as the hydrochloride, along with some of the dimer, by reaction of phenylacetaldehyde cyanohydrin with alcoholic ammonia (15).

In the present investigation a route other than the Strecker method was desired for synthesis of α-amino nitriles because the amino nitriles and their acylated derivatives obtained by this method are optically inactive. An isolated example of resolution of such a compound, i.e., α-aminopropionitrile, with D-tartaric acid has been reported (12). The resolved compound was benzoylated with retention of optical activity. This is interesting both because of the suggested alternate route to other active α-amino nitrile derivatives and because it emphasizes again the fact that the dissociation of the nitrile during acylation does not occur rapidly. Racemization would occur if the amino nitrile were in equilibrium with the aldehyde imine.

If amino nitriles are acylated by thio acids, a cyclic product may be isolated (16). The reaction, along with a possible mechanism, is as follows:



This reaction is analogous to the formation of azlactones from acylamino acids, which may proceed by way of the mixed anhydride.



A single report of the conversion of α-amino acids to α-amino nitrile derivatives was discovered in the literature after similar reactions had been carried out in this investigation. In 1932 Radde (6) obtained phthalimidoacetonitrile and α-phthalimidopropionitrile by distilling the amides from phosphorus pentoxide at 15 mm. pressure. Radde was unable to prepare the imino ester hydrochloride from phthalimidoacetonitrile. It is interesting that if this report had been found earlier, the present work might never have been carried out, since it was planned to use the imino ester as an important intermediate. After Radde's paper was found, phthalimidoacetonitrile was reprepared by a modified procedure. It was found to be readily convertible to an imino ester hydrochloride, as predicted from a similar behavior which had been observed in the phenylalanine series.

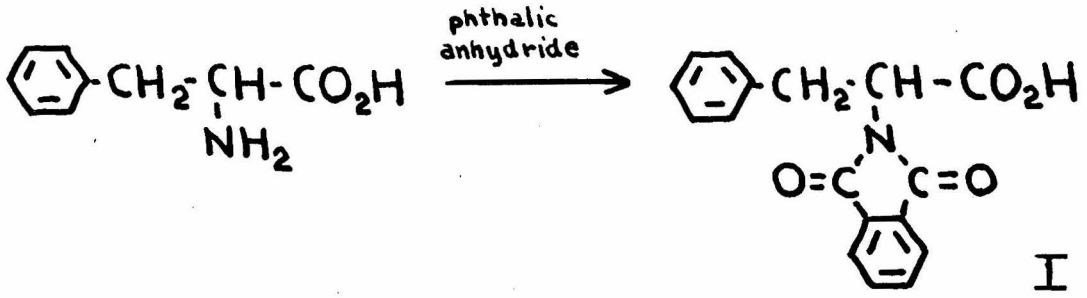
2. The Preparation of α-amino Nitrile Derivatives from α-amino Acids

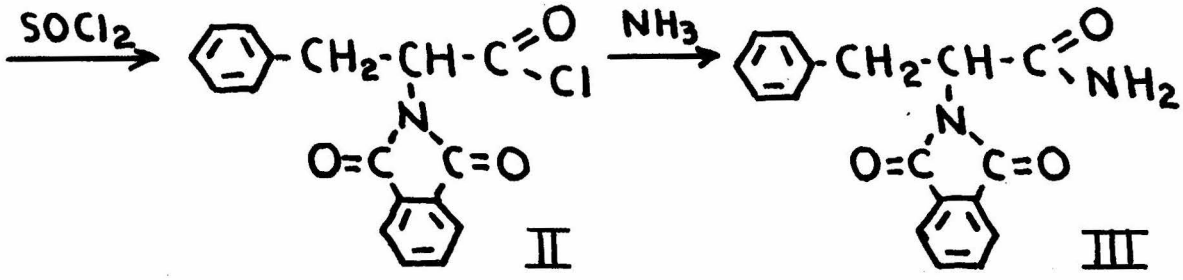
In this investigation attention was first given to the

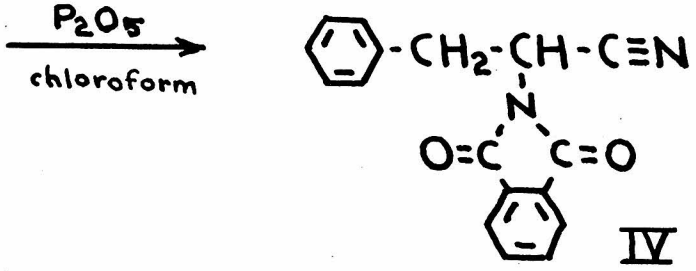
preparation of derivatives of the α-phthalimido acids (α-phthaloyl-amino acids), prepared by fusing the corresponding α-amino acids with phthalic anhydride. This reaction has long been known (see 17, 18) but received little attention until it was recommended for preparing derivatives of α-amino acids by Billman and Harting in 1948 (19) and was adapted to peptide synthesis by Sheehan and Frank (20), who utilized the procedure of Ing and Manske (21) for cleaving the phthaloyl protecting group with hydrazine under mild conditions. L-glutamic acid is racemized on melting with phthalic anhydride, giving the DL-phthalimido derivative, although a two step procedure gives the optically active compound (22). Experience in these and other (23) laboratories has indicated that racemization may be avoided in the case of other α-amino acids, showing that the use of a two step procedure by other workers (24) has been an unnecessary precaution. The α-phthalimido nitriles were particularly desirable in the present study because they can undergo neither the dissociation of the amino nitriles or the cyclization reactions of other acylamino acid derivatives.

Accordingly α-phthalimido-β-phenylpropionic acid (I) was prepared from phenylalanine, converted to the acid chloride (II) with thionyl chloride, and to the amide (III) by allowing the chloride to react with ammonium hydroxide. On refluxing the above amide with thionyl chloride for two hours, only unreacted starting material could be isolated, although dihydrocinnamamide was converted to the nitrile in a few minutes by this reagent. Furthermore the nitrile was not obtained from the amide on refluxing with phosphorus oxychloride, refluxing with a mixture of phosphorus oxychloride

and phosphorus pentachloride, melting with phosphorus pentoxide, melting with phthalic anhydride, warming with phosphorus pentachloride in dioxane, or refluxing in chloroform with phosphorus pentoxide for two hours. However, refluxing the amide in chloroform with phosphorus pentoxide for three days, gave the desired nitrile (IV). The properties of the phthalimido compounds involved in the preparation of the nitrile are given in Table I, page 67 which lists all of the phthalimido derivatives prepared in the present investigation. The reactions leading to the nitrile are given below:

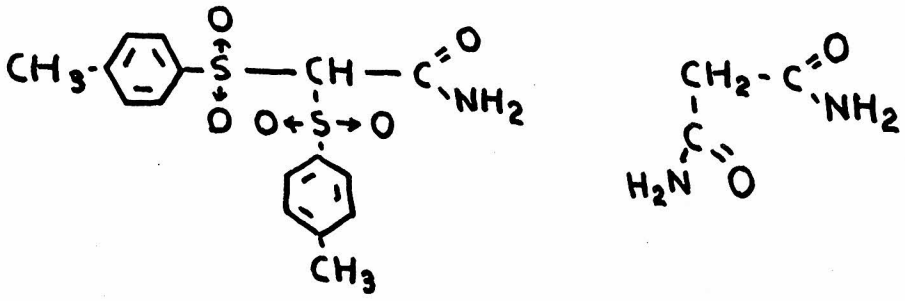






IV

Subsequent to the unsuccessful attempts to dehydrate the amide reported above, a literature search revealed that thionyl chloride is known to fail in the dehydration of other amides (25). These are similar to α-phthalimido-β-phenylpropionamide in having an electron attracting group on the α-carbon:



A series of accidental observations on the behavior of α-phthalimido-β-phenylpropionamide with phosphorus pentachloride eventually led to a second and somewhat unusual method for obtaining the nitrile which tended to confirm the structure of the latter compound. The reaction is discussed under a separate heading immediately following this one.

In later work a third method of preparing the nitrile was used. The method is a modification of a procedure, published in March, 1955 (26), in which benzenesulfonyl chloride in pyridine is used to dehydrate amides. When α-phthalimido-β-phenylpropionamide was allowed to react at 90° (the reported reactions were all run below 70°) with the above reagents a water insoluble product was obtained which was separated into nitrile (28%) and recovered amide (75%) by extracting the mixture with a benzene chloroform mixture, in which only the nitrile is soluble. However, boiling the reaction mixture for eight minutes resulted in a nearly quantitative yield of the desired nitrile. The most important advantage of the benzenesulfonyl chloride method was disclosed when it was found that the optically active L-α-phthalimido-β-phenylpropionamide was dehydrated to the nitrile. No racemization was observed using this method, while the dehydration with phosphorus pentoxide apparently gave a partially racemized product which however could be purified only by recrystallization. The utility of the benzenesulfonyl chloride method was again demonstrated when it was used in the preparation of phthalimidoacetonitrile from the amide. As has been mentioned Radde (6) first prepared this compound by distilling the amide from phosphorus pentoxide.

One desirable route to α-amino acid derivatives acylated by groups other than the phthaloyl group would involve cleavage of the phthaloyl group from the phthalimido nitrile, followed by acylation of the resulting α-amino nitrile. Such a cleavage (using hydrazine) was attempted, but no product was isolated, and the presence of a hydrogen cyanide odor indicated that the anticipated base

catalyzed decomposition of the α-amino nitrile had occurred to some extent. This made it likely that an optically active α-amino nitrile would have been at least partially racemized during the cleavage reaction, and therefore this general procedure was not investigated further.

Another approach to other acylated α-amino nitriles is exemplified by attempts to dehydrate acetylphenylalanine amide, using either phosphorus pentoxide in chloroform or benzenesulfonyl chloride in pyridine. Preliminary work gave only uncharacterized oils, but it is possible that these could have been converted to crystalline derivatives without purification. Although a cyclization reaction would be favored by phosphorus pentoxide, such a reaction would seem less likely to occur in pyridine. It may be concluded that the above route is still a possible alternative to the one actually employed, i.e., the one which involves cleavage of the phthalimido group from derivatives obtained from the α-phthalimido nitrile.

An attempt to prepare an acetamido nitrile via a phosphorus compound of the type mentioned in the next section failed.

D. PHOSPHORUS CONTAINING DERIVATIVES OF THE α-PHTHALIMIDO AMIDES

During the attempts to dehydrate α-phthalimido-β-phenylpropionamide reported above, it was observed that no amide could be precipitated from a hot dioxane solution in the presence of phosphorus pentachloride by adding hexane, in which the amide is insoluble. A series of experiments revealed that the amide rapidly reacted with phosphorus pentachloride in dioxane at 40° C., and that a derivative (VI) containing phosphorus and chlorine crystallized out of the resulting clear solution on adding hexane and allowing the mixture to stand open to the atmosphere. The compound decomposed with gas evolution at 180° C., giving a dark glass from which α-phthalimido-β-phenylpropionitrile identical with that reported above was isolated in good yield. Although the phosphorus compound gave excellent crystals from a benzene-chloroform mixture, good analyses could not be obtained.* However a phosphoramidic dichloride structure for the compound can be assigned with fair certainty from comparison with similar cases in the literature, and crystalline derivatives were prepared by reaction of the presumed phosphoramidic dichloride with methanol and with n-butanol both of which gave fair analyses for the expected compounds. The probable reactions are given below:

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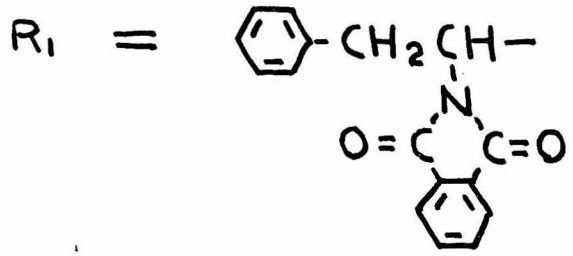
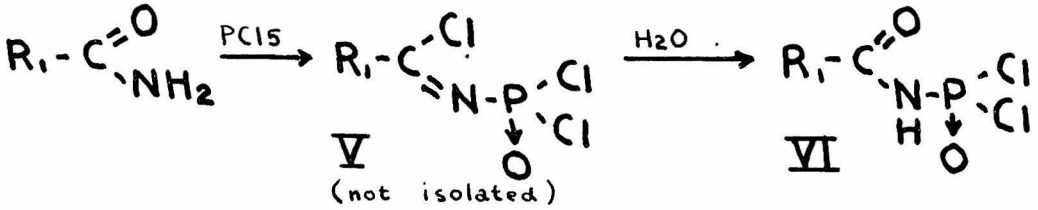
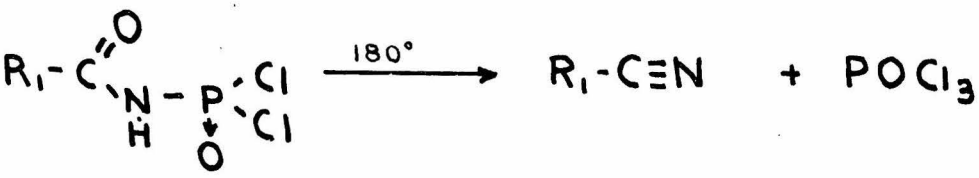
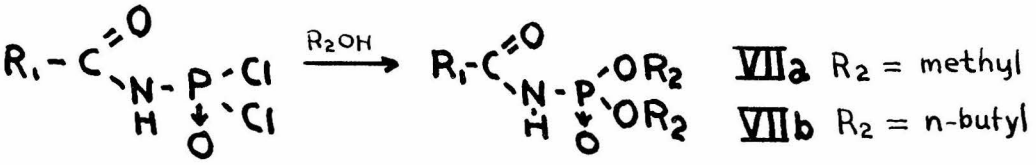
*All microanalyses reported in this thesis were done by Dr. A. Elek, 4763 W. Adams Blvd., Los Angeles 16, Calif.









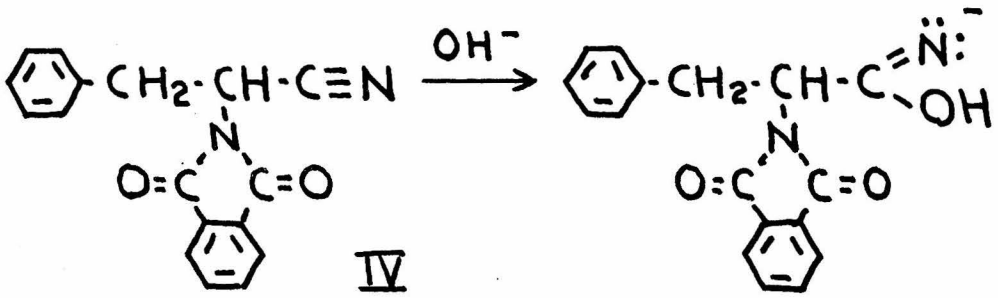
The reaction of amides with phosphorus pentachloride to give phosphorus containing compounds (analogues of V in the above equations) was first observed by Wallach in 1876 (27). It remained for later workers to show that this is probably the usual reaction observed (28, 29). None of these workers purified the intermediate imide chlorides, of type V, by crystallization, although some of them were crystalline solids. The phosphoramidic dichlorides formed by partial hydrolysis were purified by crystallization, however, and served to indicate the course of the reaction. The thermal decomposition to nitriles was observed in the case of N-benzoyl-

phosphoramidic dichloride, which gave benzonitrile on heating (29).

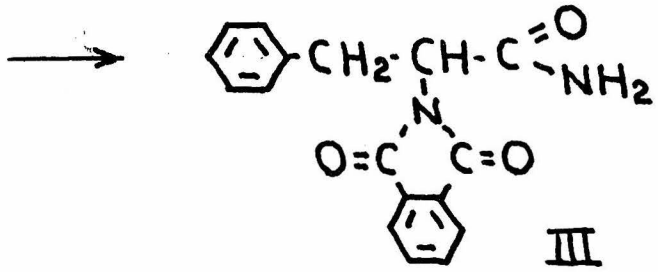
E. THE REACTION OF NUCLEOPHILIC REAGENTS WITH PHTHALIMIDO ACID DERIVATIVES

1. Rates of Nucleophilic Reactions

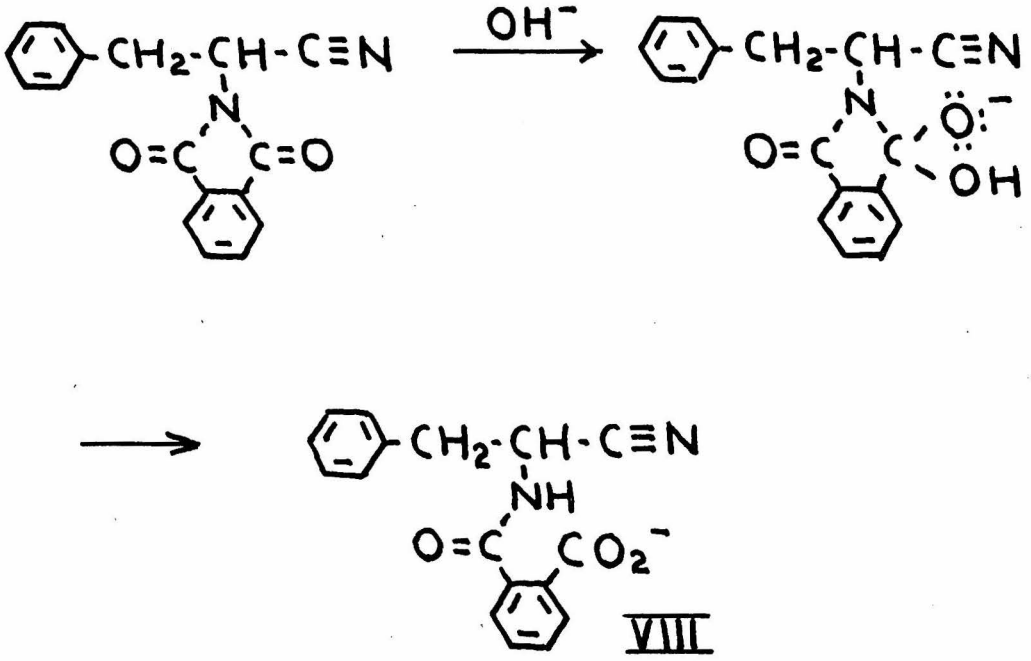
The reactions of many α-phthalimido acid derivatives with nucleophilic reagents may be regarded as competitions between sites in the molecule for the nucleophilic agent. As an example, α-phthalimido-β-phenylpropionitrile may react with hydroxide ion to give the amide III (addition to the nitrile group) or to give a phthalamic acid derivative VIII (addition to the phthalimido carbonyl group):



IV



III



VIII

Reactions of this type take on an added interest if considered in connection with recent attempts to formulate a quantitative theory of nucleophilic reaction rates. Such theories have been given by Swain and Scott (30) and by Edwards (31). In both theories a number, n, is assigned to each nucleophilic reagent which may be roughly designated as its nucleophilic power. Likewise the molecule which reacts with the nucleophilic reagent is assigned a characteristic number, s, which may be called the susceptibility of the substrate to nucleophilic power. These numbers are not used to calculate rates of a single reaction, but are used to determine the relative rates at which two nucleophilic reagents attack the same substrate. The Swain and Scott relationship is:

$$\text{Log}\frac{k}{k_o} = sn;$$

k = rate of reaction with nucleophilic reagent

k_o = rate of reaction with water

Although the scope of this equation has not been extensively explored, it is presumed to apply to substrates as widely varied as methyl bromide and benzoyl chloride (30). Wiberg has given an independent table of nucleophilic powers based on rates of addition to benzonitrile, noting that this order might not apply to esters (32, 33).

We may begin an examination of the application of the Swain-Scott equation by considering the case where both the nitrile group and the phthalimido group in the above example have the same susceptibility, s, to nucleophilic power, n. As a reference rate we may take the experimental finding that the reaction of hydroxide ion takes the second course given above, as shown by the isolation of α-(o-carboxybenzamido)-β-phenylpropionitrile* from the reaction of α-phthalimido-β-phenylpropionitrile with aqueous sodium hydroxide. On the basis of the above assumptions, every nucleophilic reagent will react to open the phthalimido ring faster than it will add to the nitrile group:

Let k_a^N be the rate constant for reaction of nucleophilic reagent a with the nitrile group.

Let k_a^P be the rate constant for reaction of nucleophilic reagent a with the phthalimido group.

Let k_o^N and k_o^P be the corresponding rate constants for the reactions where hydroxide ion is the nucleophilic reagent, this being the reference rate in this case.

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*The properties of this compound are recorded in Table II, p. 70 which lists all compounds prepared by use of phthaloylphenylalanine derivatives as intermediates.

$$\log \frac{K_a^P}{K_o^P} = sn_a \qquad \log \frac{K_a^N}{K_o^N} = sn_a$$

$$\log K_a^P - \log K_a^N = (sn_a + \log K_o^P) - (sn_a + \log K_o^N)$$

$$\log \frac{K_a^P}{K_a^N} = \log \frac{K_o^P}{K_o^N} \quad ;$$

the rate ratio is independent of the nucleophilic reagent.

When reaction of the phthalimido ring does not occur faster than reaction of the nitrile group it becomes of interest to determine the reason for the observed behavior. Possible reasons are:

(1) In acid solution one may observe reactions of the protonated nitrile group, which is to be considered as a different substrate than the unprotonated nitrile group.

(2) The nitrile group may have a higher susceptibility to nucleophilic power than the phthalimido group, contrary to the special case assumed above. In this case it might compete successfully for reagents of higher nucleophilic power than hydroxide ion, but not for reagents of lower nucleophilic power.

(3) The phthalimido ring may react abnormally fast with reagents having high base strength. The Edwards relationship, mentioned above, essentially allows for such an effect by adding a correction term to the Swain-Scott relationship. This term is the product of a susceptibility, β, times a base strength, H.

(4) The nature of the products may be controlled by considerations of equilibrium rather than rate. That is, with certain reagents the phthalimido ring may be unopened at equilibrium.

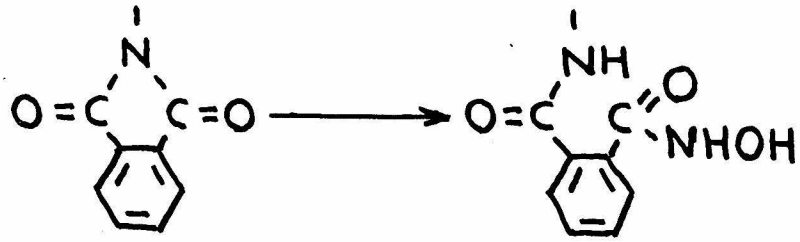
(5) The Edwards equation, and hence the Swain-Scott equation may fail for α-phthalimido acid derivatives.

2. The Reaction of α-phthalimido Acid Derivatives with Hydroxylamine; a New Reagent for Cleaving the Phthaloyl Group

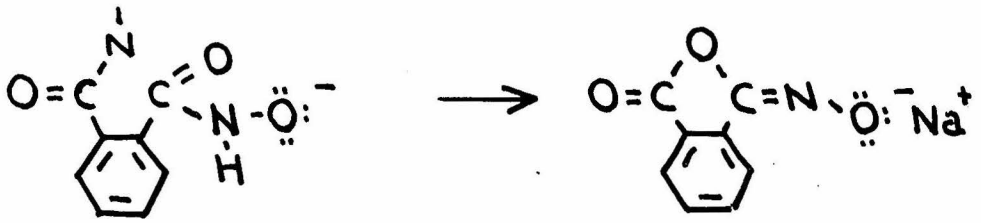
The reactivity of the phthalimido group of α-phthalimido acid derivatives toward nucleophilic reagents is not likely to vary much among all of the members of the phenylalanine series in which the carboxyl group has been replaced by an uncharged group such as the amide or nitrile group. Dilute sodium hydroxide appears to react rapidly with the phthalimido group in α-phthalimido-β-phenylpropionitrile. (Although the solid does not rapidly dissolve in aqueous sodium hydroxide, it is not precipitated from alcohol by aqueous sodium hydroxide although water gives rapid precipitation.) We may make another application of the Swain-Scott equation and predict that the many nucleophilic reagents having nucleophilic power equal to or greater than that of hydroxide ion will also react rapidly with the phthalimido group of this compound and with the phthalimido group of other electrically neutral members of the series.

From its rate of reaction with thiol esters (34), hydroxylamine may be judged to be nearly as nucleophilic as hydroxide ion. However, the reactivity of hydroxylamine toward esters is enormously increased in the presence of alkoxide ion, as is known from

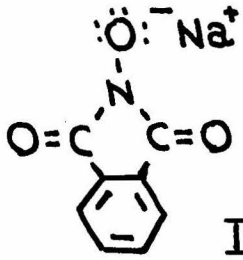
qualitative observations concerning the ferric hydroxamate test for esters. It seems likely that this effect is due to conversion of hydroxylamine to the NH_2O^- anion. As expected from these considerations, it was found that all derivatives of phthaloylphenylalanine tested reacted with basic hydroxylamine, giving a positive hydroxamate test. These derivatives included the nitrile, the amide, and methyl imidic ester, the amidoxime, and even the acid itself. The reaction is undoubtedly an opening of the phthalimido ring:



When α-phthalimido-β-phenylpropionic acid methyl ester was allowed to react with hydroxylamine and sodium methoxide in methanol in an attempt to convert the methyl ester to the hydroxamic acid, the above ring opening reaction occurred instead. This is shown by the fact that a slower second reaction occurred in which the phthalic acid residue was cleaved completely as a deep red sodium salt, IX, as shown by the following equation:

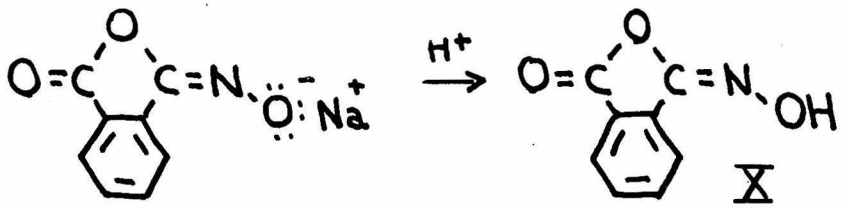


or

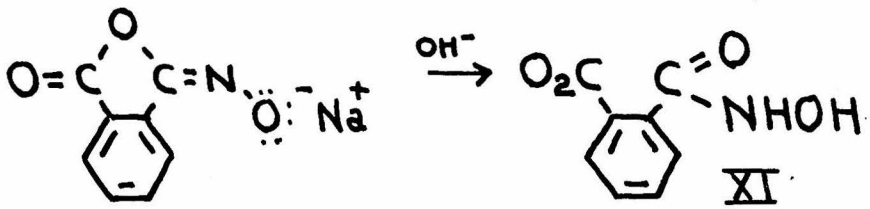


IX

The sodium salt was identified by titration with acid to give a colorless crystalline solid X which gave no ferric chloride test, and which was converted by sodium hydroxide solution first to the original red salt, then to a colorless hydroxamic acid XI:



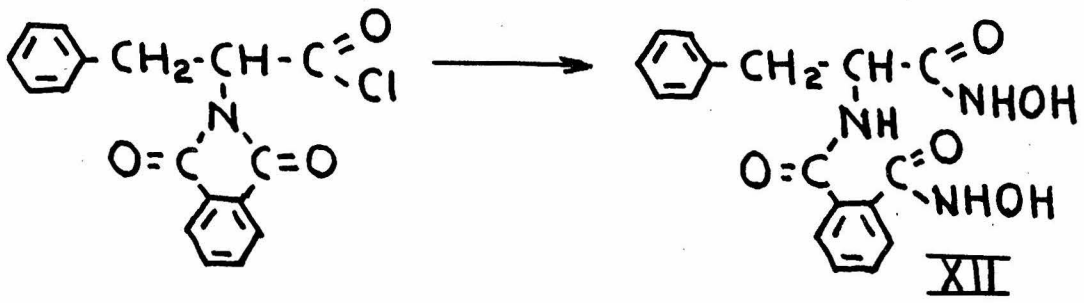
X



XI

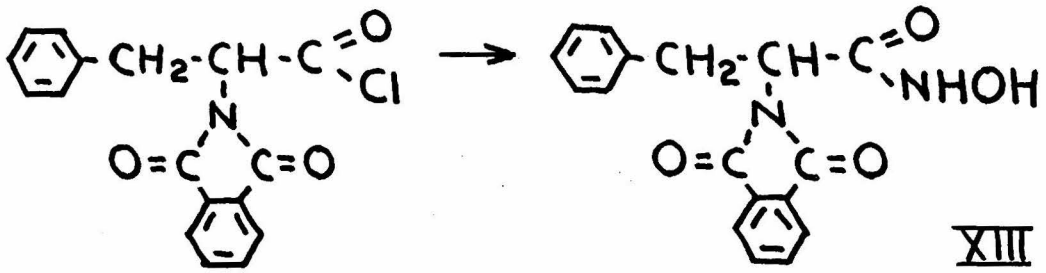
The colorless solid X was found to be described in the literature, where it is called phthaloxime (35). This cleavage of the phthaloyl group was eventually utilized in a later part of this investigation, as is described below.

Since the above experiment did not result in conversion of methyl α-phthalimido-β-phenylpropionate to the desired hydroxamic acid, preparation of the latter from the acid chloride and hydroxylamine was attempted, using benzene as solvent for the solid chloride (see 36). Unfortunately the susceptibility of the phthalimido group to nucleophilic attack was still not understood at the time, and a large excess of crystalline hydroxylamine (37) was used. A solid which could not be crystallized was obtained, which probably consisted partly of the dihydroxamic acid XII:



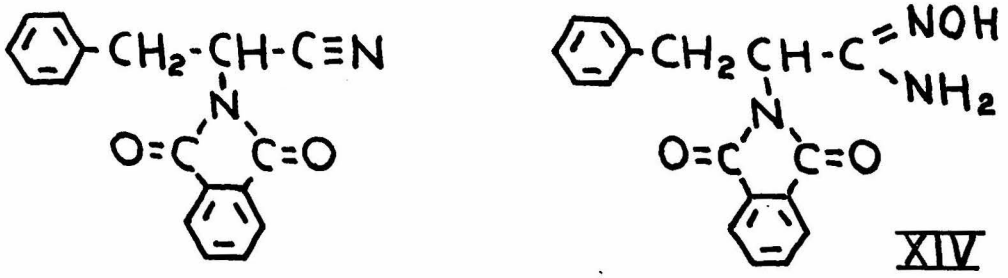
XII

Because hydroxylamine is not soluble in benzene, the reaction was then attempted using dimethylformamide to dissolve each reactant separately before mixing. Dilution of the homogeneous reaction mixture with water to the cloud point and cooling gave crystals of the hydroxamic acid XIII, which was desired for comparison with other derivatives of phthaloylphenylalanine of the type mentioned in the introduction.



XIII

As mentioned above, the phthalimido ring of α-phthalimido-β-phenylpropionitrile appears to be rapidly opened by hydroxylamine in the presence of base. An entirely different result was obtained, however, when hydroxylamine was used in the presence of acid, i.e., where hydroxylamine hydrochloride was the acid. In this case the hydroxylamine added to the nitrile group, giving a good yield of α-phthalimido-β-phenylpropionamidoxime, XIV:



XIV

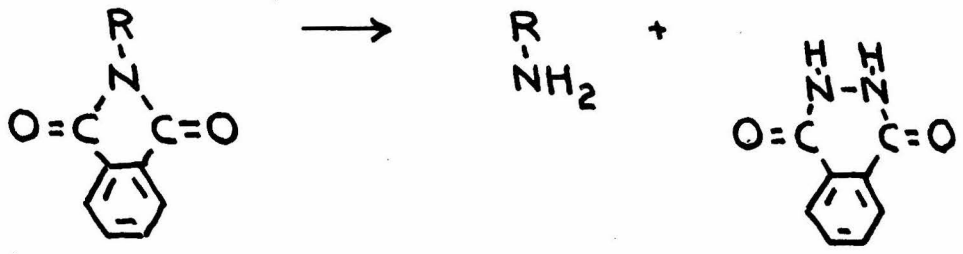
It seems possible that in this case the protonated nitrile group is the reacting species, and that this accounts for the change in the point of attack by hydroxylamine.

The chemical properties of the amidoxime obtained above are of interest, in view of the fact that it is an acylamino derivative similar to the ones envisioned as possible new substrates for enzymatic studies. The properties of the compound would be expected to resemble those of other aliphatic amidoximes reported in the

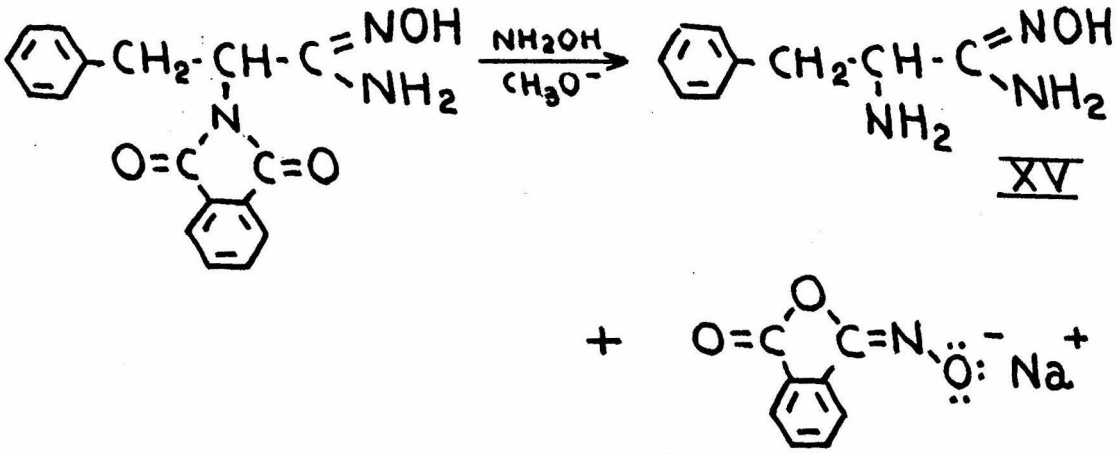
literature. The following statement concerning hydrolytic behavior is found in Houben-Weyl (38): "The aliphatic amidoximes, especially the first members of the series, are unstable. Even with water, and more easily with dilute acid or base, they decompose to acid, hydroxylamine, and ammonia. The aromatic ones are far stabler; they can even be boiled with hydrochloric acid without change" (underlining added). A literature search yielded no further information on hydrolysis of aliphatic amidoximes.

One of the first observations made on the behavior of α-phthalimido-β-phenylpropionamidoxime was that it momentarily seemed to go into solution in 6N hydrochloric acid and then give an insoluble precipitate. This behavior was thought to be due to the hydrolysis of the soluble hydrochloride of the basic amidoxime to the insoluble amide. Some time later a closer investigation revealed that the precipitate actually consisted of the amidoxime hydrochloride from which the amidoxime could be recovered by the action of base. This latter finding made it seem much more likely that the aliphatic amidoximes would have enough hydrolytic stability to serve as substrates, and the preparation of such substrates through replacement of the phthaloyl group by other acyl groups was undertaken.

The popularity of the phthaloyl group as a protecting group in amino acid chemistry is due to the finding that it may be removed by hydrazine under mild conditions (20, 21):



In the case under consideration there seemed to be a chance that hydrazine would react with the amidoxime group in competition with the desired cleavage reaction. On the other hand, hydroxylamine and sodium methoxide had been found to cleave the phthaloyl group, and seemed less likely to give undesirable side reactions. Because of this, and because it was desired to know more about the newly discovered cleavage by hydroxylamine, and because the latter reaction gave an impressive bright red solution, the cleavage was carried out with hydroxylamine. The sodium salt of phthaloxime is somewhat soluble in the methanol reaction mixture but is insoluble in ethyl acetate. Accordingly a procedure for separating the amino amidoxime from the sodium salt was worked out in which the reaction mixture was evaporated to dryness and the product was extracted with ethyl acetate. In this way crystalline α-amino-β-phenylpropionamidoxime XV was obtained in fair yield:

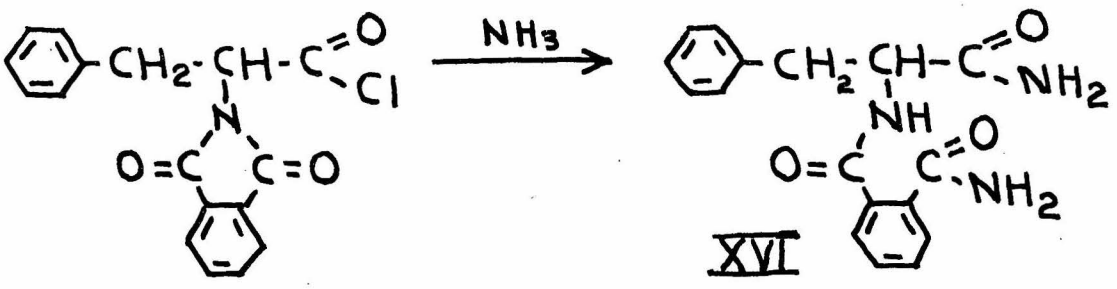


Furthermore, when this reaction was repeated using optically active α-phthalimido-β-phenylpropionamidoxime no evidence of racemization was encountered. Although the amino amidoxime could not be crystallized in this case, acylation of the ethyl acetate extract gave good yields of the crystalline, optically active, acylated product. The latter product will be discussed in another section.

The α-amino-β-phenylpropionamidoxime reported above appears to be the first example of an α-amino amidoxime. The only related compound revealed by a literature search is the amidoxime of hippuric acid, benzamidoacetamidoxime, reported in a Czechoslovakian journal in 1933 (39).

3. The Reaction of Phthalimido Acid Derivatives with Ammonia and Amines

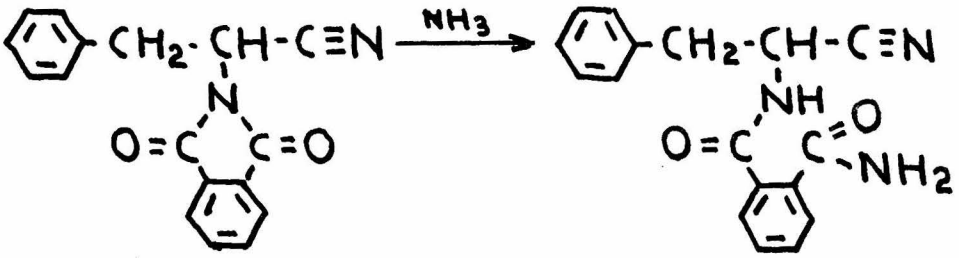
Ammonia is more nucleophilic than hydroxide ion according to Edward's table (31), and by the principles discussed above would be expected to react rapidly with phthalimido acid derivatives to give a ring opened compound. This was not realized at the time when amides were first prepared by reacting α-phthalimido-β-phenylpropionyl chloride with ammonia or amines. However, the variable yields obtained on attempting to purify early batches of amide are now attributed to a side reaction such as that postulated above. The ring opened compound was formed exclusively when a batch of amide was accidentally allowed to stand overnight in ammonium hydroxide, giving α-phthalamamido-β-phenylpropionamide XVI:



Reacting the acid chloride with ethyl amine gave a similar result. From one reaction with ethyl amine both the phthalimido N-ethyl amide and the ring opened product were isolated by fractional crystallization.

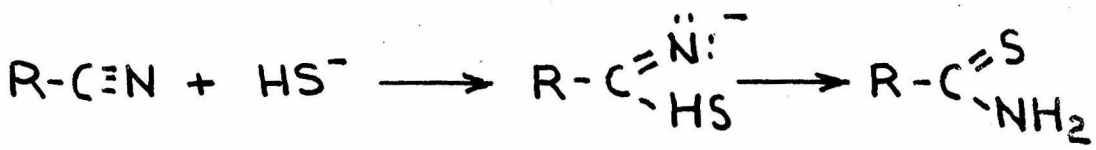
An interesting property of the ring opened amide is that on heating to the melting point it was converted to the phthalimidoamide with loss of ammonia. The same reaction was brought about by phosphorus pentoxide in boiling chloroform. A test tube experiment indicated the the ring opened ethylamide lost ethyl amine in an analogous reaction.

It is now apparent that the conditions used in an attempt to convert α-phthalimido-β-phenylpropionitrile to the corresponding thioamide (see below) were such that the phthalimido ring must have been opened by ammonia during the reaction. In this case, which will be discussed in detail later, the nitrile was dissolved in alcoholic ammonia and then saturated with hydrogen sulfide, a procedure which would be expected to lead to the following intermediate reaction:



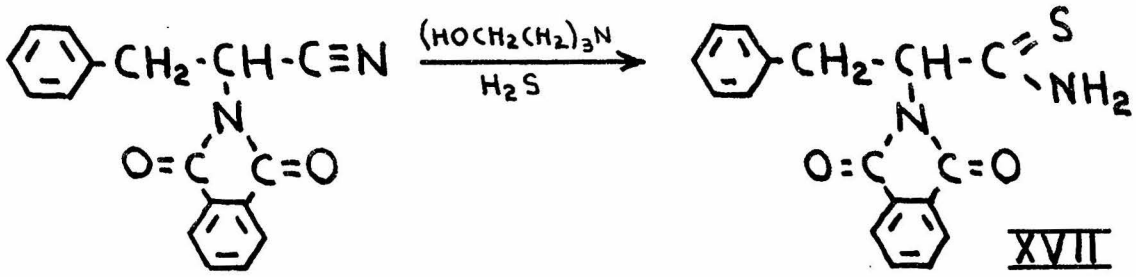
4. Reaction of α-phthalimido-β-phenylpropionitrile with Hydrogen Sulfide:

Thioamides are ordinarily prepared by allowing nitriles to react with hydrogen sulfide in the presence of a basic catalyst such as ammonia or an amine. It seems likely that such reactions represent a nucleophilic attack on the nitrile group by the hydrosulfide anion:

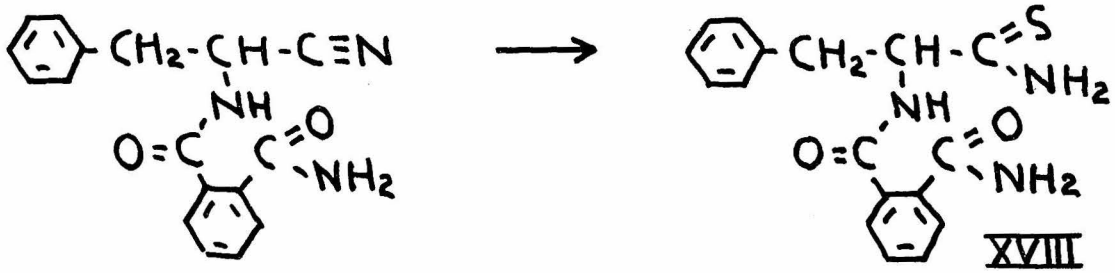


In the present study several attempts were made to convert α-phthalimido-β-phenylpropionitrile to the thioamide. In early attempts ammonia was used as the basic catalyst, resulting in a ring opened product, as described below. However, the desired α-phthalimido-β-phenylpropionthioamide XVII was obtained in a later preparation in which triethanolamine was employed as the catalyst. Although

it is reported that phthalimidoacetonitrile is converted to the thioamide in good yield by the latter procedure (5), α-phthalimido-β-phenylpropionitrile seems to have been only partially converted under similar conditions. The resulting thioamide crystallized from the reaction mixture only after an initial precipitate of unreacted nitrile had been separated by filtration. The equation for the reaction is the following:



In the early attempts to convert phthalimido-phenylpropionitrile to the thioamide the nitrile was first dissolved in alcohol saturated with ammonia. When hydrogen sulfide was passed into the ammoniacal solution of nitrile, a crystalline precipitate formed in about an hour. The product was recrystallized to give cotton-like needles which melted with decomposition, liberating both ammonia and hydrogen sulfide. (Thioamides decompose to the nitrile and hydrogen sulfide on heating.) It can now be seen that the expected compound is the one indicated in the following reaction:

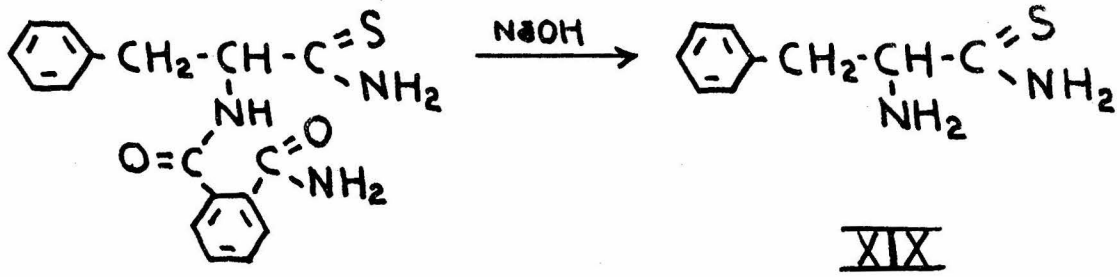


Other facts are not accounted for on the basis of the above formula. The yields of product in two separate preparations were 100 per cent and 103 per cent, based upon the above formula. Additional product was no doubt still in solution in the discarded filtrate. A series of observations established that the substance underwent a change at temperatures from 90 to 150 degrees, which was marked by partial melting and resolidification accompanied by a loss of weight of about seven per cent. The explanation now seems to be that the thioamide forms some kind of solvate with methanol, which loses methanol on heating. As evidence for this view, material heated until it had resolidified and then crystallized from acetonitrile decomposed about 173°, but the same material crystallized from methanol melted and resolidified at about 100° and then melted about 171°.

It should be mentioned that there has been difficulty in getting correct analyses for compounds in this series. Although carbon and hydrogen analyses in general support the structures proposed above, some nitrogen and sulfur analyses are in error by more than a per cent. Because the reported nitrogen and sulfur values are usually not consistent with any simple ratio of nitrogen atoms to sulfur atoms, there is no chance that structures can be found which <u>will</u> fit the analytical data.

Probably the most interesting result of the thioamide work came as a consequence of attempts to elucidate the structure of the products mentioned above by hydrolysis. Heating a sample of the methanol-thioamide adduct with dilute sodium hydroxide gave a solution from which crystals separated on cooling. They proved to be

α-amino-β-phenylpropionthioamide XIX as shown by solubility in dilute hydrochloric acid and analysis of the compound recrystallized from benzene:



XIX

This appears to be the first example of conversion of an amino acid to the amino thioamide. However, there is some work on amino thioamides reported in the literature, and this will be discussed below, in view of its relation to the present work.

As has been mentioned, the preparation of α-phthalimido-β-phenylpropionthioamide in this investigation utilized a procedure described in the literature for the preparation of phthalimidoacethioamide. Namely, it is reported that phthalimidoacetonitrile obtained from chloroacetonitrile and potassium phthalimide (but which might have been obtained from glycine) was allowed to react with hydrogen sulfide in the presence of triethanolamine giving phthalimidoacethioamide (5). This would seem to represent the first example which has been found in which the nitrile group reacts faster than the phthalimido group under basic conditions in which there is little chance that the protonated

nitrile group is the reactive species. Closer examination of the reaction conditions, however, reveals that such reasoning is false. The triethanolamine was used to the extent of only ten mole percent compared to the nitrile, while sixty percent of product was isolated. Ten percent of the nitrile may well have been used up in a reaction in which the phthalimido ring was opened to form an ammonium salt of the thio acid. Further attack on the phthalimido ring would have resulted in formation of the free thio acid, however, and there is some evidence that such a reaction is thermodynamically impossible in ethanol solution. In the presence of hydrogen chloride, the corresponding oxygen acids react in the reverse direction, giving phthalimido acids, although admittedly protonation of the water may supply driving force for this reaction. As for the nitrile group, it may have reacted in a protonated form, since an excess of hydrogen sulfide was used. It would be interesting to see if the yield of thioamide in the above experiment could be improved by using less amine, as predicted.

Other aminothioamides in the literature have been prepared by the addition of hydrogen sulfide to the amino nitriles obtained in the Strecker synthesis. The reaction is not very satisfactory. An early attempt to carry it out on aminoacetonitrile failed (40), and later work by Abe in Japan resulted in formation of products other than the aminothioamide when hydrogen sulfide was introduced directly into a Strecker reaction mixture (41). However, some of Abe's papers report using amino thioamides as reactants in other reactions, and,

although their preparation is not described in Chemical Abstracts, they were obviously obtained in a Strecker type reaction. One of his papers actually mentions the thioamide of phenylalanine (41a), but examination of the original paper revealed that no analysis or melting point for the compound is given. No other information could be obtained, since the paper is in Japanese.

Acylamino thioamides have been prepared from the acylamino nitriles, hydrogen sulfide, and ammonia (42, 43). They are described as stable crystalline materials.

5. Reaction of α-phthalimido-β-phenylpropionitrile with Alcohol; Chemical Properties of the Resulting Imino Ester.

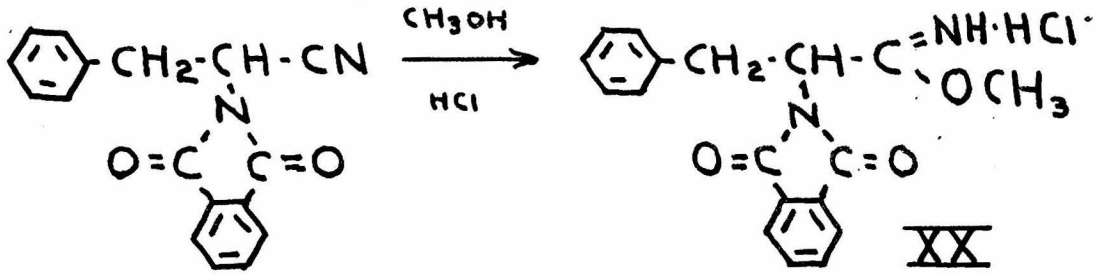
Nitriles are known to react with alcohols and hydrogen chloride to give imino ester hydrochlorides (imino ether hydrochlorides):

$$R_1\text{-}CN + R_2OH \xrightarrow{HCl} R_1\text{-}C\begin{matrix}=NH\cdot HCl\\ \diagdown OR_2\end{matrix}$$

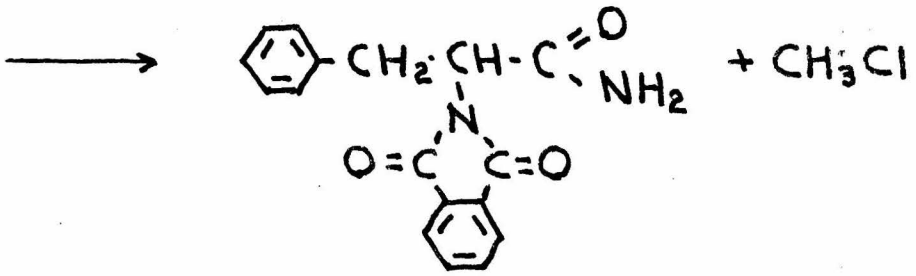
In the case of trichloroacetonitrile, reaction with alcohol occurs in the absence of hydrogen chloride, giving the imino ester (44). This latter type of reaction does not occur with α-phthalimido-β-phenylpropionitrile, as the nitrile was recovered unchanged after refluxing with ethanol containing a trace of hydrogen chloride.

When the above nitrile in benzene was treated with one mole of methanol and excess hydrogen chloride in benzene, a precipitate of the imino ester hydrochloride XX formed in good yield within a few hours. Its structure was confirmed when it was found to melt at the same temperature as the amide, indicating conversion to the

amide on heating. The pyrolysis product was identified as the amide by a mixed melting point determination. This conversion to an amide on pyrolysis is characteristic of imino ester hydrohalides. (See 45 for references). The equations for these reactions follow:



XX

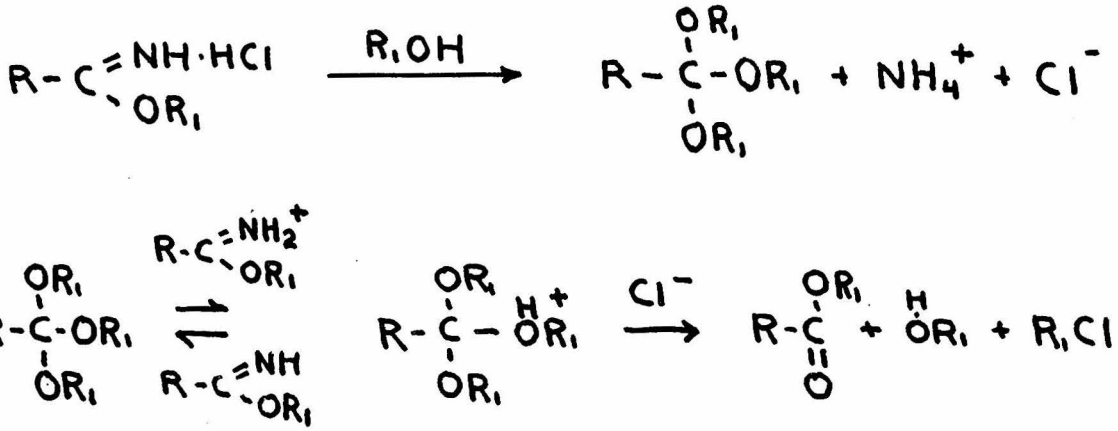


The first of the above reactions may be assumed to involve attack by the alcohol on the protonated nitrile group. The second has been shown to be an S_N2 displacement of oxygen by halide (45, 46).

The imino ester hydrochloride was converted to the free imino ester by sodium methoxide, but the yield of crystalline product was very low. The imino ester dissolved in water momentarily and then gave a white precipitate, presumably of the ordinary ester, which is formed in acid catalyzed hydrolysis of other imidic esters.

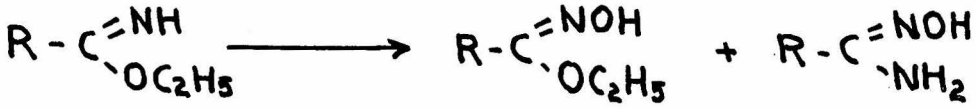
As has been mentioned in the section dealing with nitriles, phthalimidoacetonitrile was eventually prepared from glycine and converted to the methyl imino ester hydrochloride. The behavior of this imino ester hydrochloride on dissolving in water was identical with that of the phenylalanine derivative just described. In this case the white precipitate formed by hydrolysis was separated, recrystallized, and identified by mixed m.p. as the expected methyl phthalimidoacetate.

When an attempt was made to convert the imino ester hydrochloride to the ortho ester by reaction with methanol at room temperature, the major products of the reaction were the amide and the normal ester, as shown by isolation of the products and mixed melting point identification. A literature search revealed that similar difficulties are encountered in attempts to prepare the ortho esters of other acids containing substituents on the alpha-carbon. McElvain has conducted a study which shows the origin of the products in such cases (45). The amide is due to the reaction already mentioned in which methyl chloride is formed from the imino ester hydrochloride. The normal ester seems to be formed from a similar cleavage of the ortho ester:



The alcoholysis of imino ester to ortho ester is slowed greatly by the alpha-substituent, so that the ortho ester produced is in contact with acidic imino ester hydrochloride long enough for the above cleavage reaction to occur.

Addition of an imino ester to hydroxylamine hydrochloride solution has resulted in formation of both hydroximic ester and amidoxime in the same reaction (47):



In this work, methyl α-phthalimido-β-phenyliminopropionate hydrochloride was mixed with hydroxylamine hydrochloride in methanol and one mole of sodium methoxide was added. The hydroximic ester was isolated in twenty per cent yield.

The imino ester and the hydroximic ester described above were prepared, along with the amidoxime mentioned earlier, with the intention of attempting to cleave the phthaloyl group from the derivatives which seemed most promising. Since the amidoxime was obtainable in better yield, and since it appeared to have good hydrolytic stability, it was chosen for the cleavage study. Further study of the imino ester and hydroximic ester has not been undertaken.

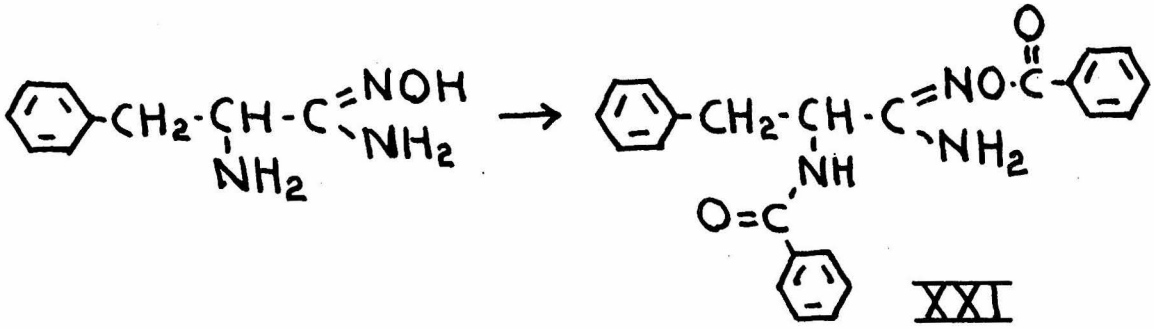
It may be mentioned that both α-amino imino esters (48) and α-acylamino imino esters (49) are known. Both types are derived from amino nitriles obtained by Strecker synthesis, which means that these methods of preparation are not suitable for obtaining

optically active compounds.

F. The Preparation of Acylaminoamidoximes

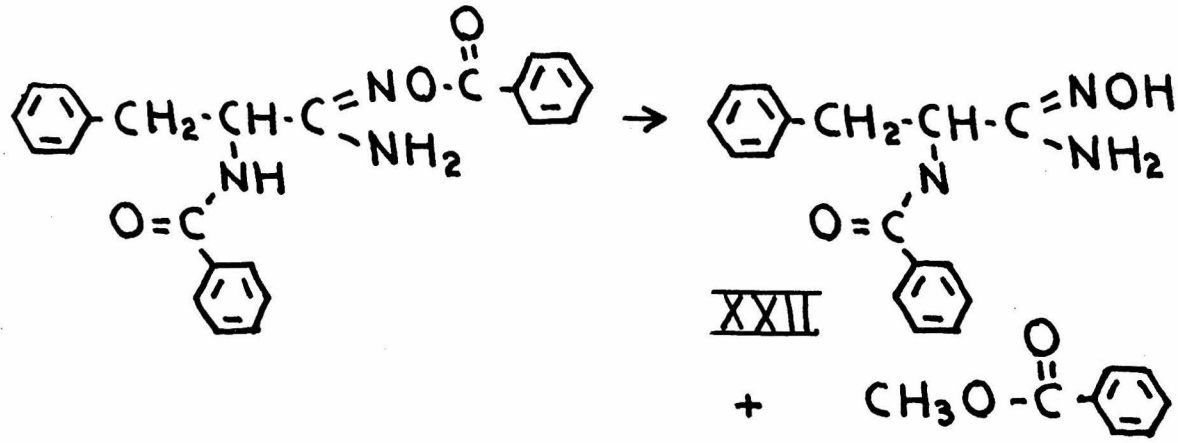
Most of the functional derivatives of α-amino acids which contain doubly bound nitrogen replacing the carbonyl oxygen of the carboxyl group are alike in that the free amino compounds (of the type obtained on cleaving off the phthaloyl group) can be acylated on either the amino nitrogen or the doubly bound nitrogen. It was anticipated that this situation would cause difficulty in preparing suitable acylamino derivatives by way of the amino derivatives obtained on cleavage of the phthaloyl group. It seemed that procedures would have to be developed for selective acylation of the amino nitrogen.

Accordingly, α-amino-β-phenylpropionamidoxime, described previously, was benzoylated in ethyl acetate in the presence of sodium bicarbonate. Exactly one mole of benzoyl chloride was used in an attempt to benzoylate only the amino group. A product was obtained, whose analysis indicated it to be a mixture of mono- and dibenzoyl derivatives. Amidoximes are known to be readily acylated on the oxygen atom, indicating that the following reaction had occurred.



XXI

It was decided to prepare a pure dibenzoyl derivative for comparison with the above product. On recrystallizing the resulting product from methanol it was observed that, although only just enough boiling solvent was used to slowly dissolve the compound, very little product crystallized out on cooling. The only reaction which could conceivably have occurred seemed to be one involving the benzoylated amidoxime function, which might result in formation of benzoic acid or methyl benzoate. The solution was found to have a strong odor of methyl benzoate, indicating that the following reaction had occurred:



The product is the acylated amidoxime which was desired from the beginning, and further study revealed that in the presence of a trace of sodium methoxide as catalyst the above reaction occurred quantitatively in less than ten minutes, enabling the monobenzoyl derivative XXII to be obtained in good yield as a crystalline solid.

As has been mentioned, L-α-amino-β-phenylpropionamidoxime was not isolated in the present work. The ethyl acetate extract of the product from phthaloyl cleavage was benzoylated directly giving a crystalline dibenzoyl derivative which was recrystallized from acetonitrile to give an analytical sample. It was converted to

a crystalline monobenzoyl derivative having a specific rotation of -85 degrees. The solubility of the monobenzoyl derivative in water was too low for it to be a useful substrate. Accordingly the monoacetyl derivative was prepared via the diacetyl compound, using acetic anhydride as the acylating agent. It had a low specific rotation, but unlike the benzoyl compound, it could be distinguished from DL-monoacetyl derivative by its melting behavior. The L-derivative melted ten degrees higher than the DL-derivative, while a mixture melted ten degrees lower than the DL-derivative, despite the fact that decomposition occurred at the melting point. The L-α-acetamido-β-phenylpropionamidoxime possessed the desired higher solubility in water and could be crystallized as long needles from warm water.

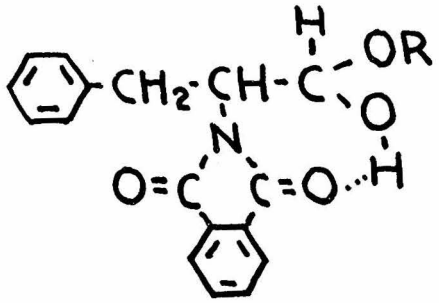
C. THE PREPARATION AND REACTIONS OF α-PHTHALIMIDO ALDEHYDES.

The availability of an easy method for making the α-phthalimido nitrile from phenylalanine made it desirable to utilize the nitrile as an intermediate to synthesize amino acid derivatives not easily accessible by other means. For this reason the reduction of the nitrile to the corresponding aldehyde was investigated.

It was found possible to carry out a Stephen reduction using stannous chloride and hydrogen chloride in ether if certain modifications were introduced. Some benzene was used as solvent along with ether in order to dissolve the nitrile. No solid complex was formed during the reduction, as with some other nitriles. However, it was found that when the ether layer was poured off and the remaining viscous liquid was hydrolyzed with water and extracted with benzene, a

solid product could be obtained on stripping off the benzene which gave a precipitate with 2, 4-dinitrophenylhydrazine. The DNP derivative could not be recrystallized, but a semicarbazone was prepared which analyzed correctly for the semicarbazone of α-phthalimido-β-phenyl-propionaldehyde.

In a later preparation the aldehyde XXIII itself was crystallized from ethanol water. The analysis, however, indicated that one mole of ethanol was contained in the product. A series of experiments showed that crystallization from different alcohols gave different products, as shown by the different melting points. The products could be crystallized from non-alcoholic solvents such as benzene hexane without change. They contained an oxygen-hydrogen bond, as shown by the infra red spectrum of the ethanol adduct. The compounds are thought to be hemiacetals which possess unusual stability in the present case because of the highly positive character of the carbonyl group and probably because of formation of an unusual type of intramolecular hydrogen bond:



Finally, the free aldehyde itself was eventually crystallized from petroleum ether.

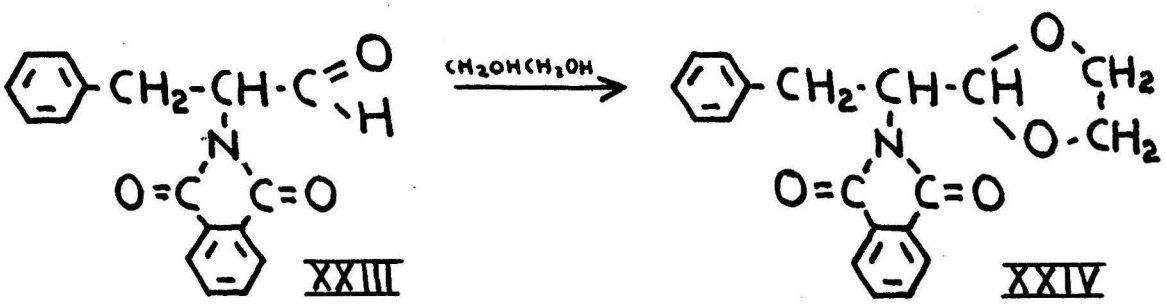
In March, 1955, Chemical Abstracts published a report of the preparation of α-phthalimido-β-phenylpropionaldehyde by another method. The reported melting point (50) was twenty degrees lower than for the compound obtained in this study by reduction of the nitrile, and the reported melting point of the semicarbazone was thirteen degrees lower. The aldehyde had been prepared by Rosenmund reduction of the acid chloride. This method was first employed in the case of phthalimido acid chlorides by Radde in 1922 (6), and since discovery of the hydrazine cleavage of the phthalimido group the method has been used as a step in the preparation of amino acetals (51). It apparently has been the only general method available for converting an amino acid to an aldehyde derivative up to now. The reduction of the phthalimido nitrile would now seem to be an alternative route of comparable ease. Although the later method involves additional steps, the preparation of a special catalyst and catalyst poison is not necessary. In the present work the Rosenmund reduction of α-phthalimido-β-phenylpropionyl chloride was repeated in order to clear up the melting point disparity noted above. Addition of hexane to the toluene reaction medium gave a mixture of oil and crystals, from which the crystals were separated and recrystallized twice from hexane to give material melting only three degrees lower than the product from the Stephen reduction. A mixed melting point showed no depression. The semicarbazone of the product from Rosenmund reduction also melted at the same temperature as that obtained via Stephen reduction. In the latter case the disparity in melting points may be due to different rates of heating, since the semicarbazone decomposes at the melting point. The disparity in in the aldehyde melting points is unexplained, although there is indication

that failure to purify the Rosenmund reduction product extensively gives a very impure product with low melting point.

The Stephen reduction, like the Rosenmund reduction, appears to proceed without racemization. L-α-phthalimido-β-phenylpropionitrile was successfully reduced to the crystalline, optically active aldehyde by the Stephen method.

The aldehyde reported above was of interest because of the possibility of replacing the phthaloyl group by an acetyl group or other acyl group. Such an acylamino aldehyde would have a structure analogous to typical substrates of α-chymotrypsin. Studying the compound as a competitive inhibitor might reveal something about the function of the carbonyl group in binding molecules to the active site of the enzyme.

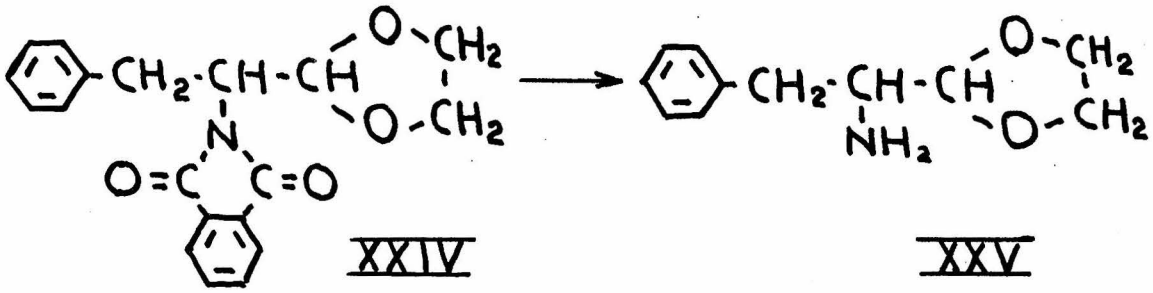
Following the method of Balenovic and coworkers (51), the aldehyde was protected by converting it to the ethylene glycol acetal before attempting to cleave the phthaloyl group:



XXIII XXIV

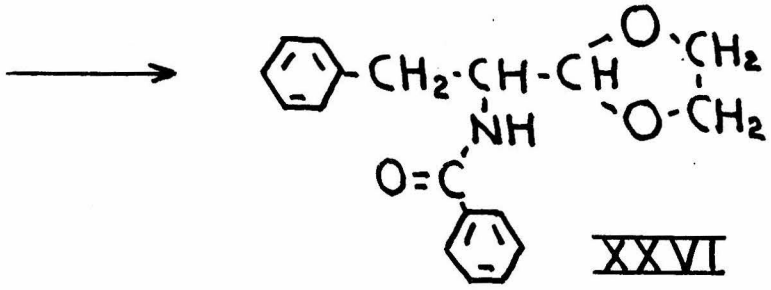
The action of hydrazine on the acetal resulted in an oil from which some crystals of uncleaved starting material slowly separated, indicating incomplete reaction. It was decided to repeat the cleavage and benzoylate the crude reaction mixture in an attempt to obtain a crystalline product. The presence of unreacted hydrazine in the crude reaction mixture was

undesirable, as it would result in contamination of the product with insoluble benzhydrazide or dibenzoylhydrazine. Accordingly, the cleavage was carried out using hydroxylamine, which would give as a side product the more soluble benzohydroxamic acid after benzoylation. The cleavage, followed by benzoylation of the ethyl acetate extract, gave the desired α-benzamide-β-phenylpropionaldehyde ethylene glycol acetal, which crystallized from the ethyl acetate on concentrating the solution:



XXIV

XXV



XXVI

Further work, which would presumably involve preparing an optically active acetamido derivative and attempting to convert the acetal back to an aldehyde, has not been undertaken.

H. EXPERIMENTAL

α-Phthalimido-β-phenylpropionic acid.--DL-Phenylalanine (190.8 g., 1.15 mole) was ground in a mortar and mixed with 170.8 g. (1.15 mole) of phthalic anhydride. The mixture was placed in a 2-l round bottomed flask with ground joint (which is fitted with a reflux condenser when the acid is converted to the acid chloride), and the flask was slowly introduced into a pan of butyl phthalate heated to 185-200° C. Steam bubbled out of the melting mixture as reaction occurred, and in about thirty minutes the flask containing the clear brown molten product was removed and allowed to cool. The resulting solid α-phthalimido-β-phenylpropionic acid was used directly for the preparation of the acid chloride. A separate preparation using 0.0725 mole of each reactant was crystallized from alcohol water to give 20.53 g. of acid (96%), m.p. 176-177.5° (lit. (19) 174-175°).

α-Phthalimido-β-phenylpropionyl chloride.--To the crude acid described above was added 380 ml. of redistilled thionyl chloride (the technical product was fractionated to remove colored lower boiling impurities). The flask was fitted with a reflux condenser, and the mixture was heated on a water bath until the gas evolution had ceased. The hot solution was poured into a 2-l beaker, using fifty additional ml. of thionyl chloride to wash the flask. Five hundred ml. of hexane was added to the mixture, giving an immediate crystalline precipitate of the acid chloride. The mixture was covered and cooled several hours, and the acid chloride was separated by suction filtration, using a sintered-glass Buchner funnel protected with a drying tube. The solid was washed on the funnel with 200 ml. of hexane and dried by drawing dry air through the funnel.

The crude α-phthalimido-β-phenylpropionyl chloride, m.p. 135-137°, was used directly for the preparation of the amide. It may be recrystallized from benzene-hexane if a purer product is needed.

α-phthalimido-β-phenylpropionamide.--The crude acid chloride described above was added to 1 liter of cold concentrated ammonium hydroxide and allowed to stand for several hours with occasional stirring*. The resulting solid was separated by filtration and dried in air to give 288 g. (85% based on phenylalanine) of crude α-phthalimido-β-phenylpropionamide. The crude amide may be purified in about 80% yield by recrystallization from boiling 95% ethanol, but it is soluble only to the extent of 1 g. in 65 ml. Other low boiling solvents (benzene, acetone) are no better, and for large scale work boiling methyl cellusolve was used. The solution tended to darken, but on washing the amide free of solvent with ethanol a white product was obtained in yields which varied considerably, but which averaged about 70% if the solvent was reused several times. An analytical sample was obtained by recrystallizing the amide several times from ethanol, m.p. 236.8-237.2°.

Anal. calc. for $C_{17}H_{14}O_3N_2$: C, 69.37; H, 4.80; N, 9.52.

Found: C, 69.37; H, 4.75; N, 9.46.

L-α-Phthalimido-β-phenylpropionamide.--L-Phenylalanine (5.045 g., 0.0305 mole) was mixed with 4.51 g. (0.0304 mole) of ground phthalic anhydride and heated in an oil bath at 185° for eight minutes or at 150° for thirty minutes. It is reported that racemization occurs on heating at 180° for thirty minutes (20). The crude L-α-phthalimido-β-phenylpropionic acid was converted directly to the acid chloride.

*It was shown later that opening of the phthalimido ring occurred on long standing with ammonia. Twenty minutes reaction time was found to be satisfactory. The ring opening reaction probably accounts for the variable yields obtained on recrystallization.

This was accomplished by refluxing the crude acid with 15 ml. of thionyl chloride for about twenty minutes. One hundred ml. of hexane was then added, and the mixture was cooled overnight. The resulting solid was separated by filtration and converted to the amide. A second crop of acid chloride was obtained as an oil on stripping the hexane from the filtrate, and was separately converted to the amide. The acid chloride has also been obtained by the use of phosphorus pentachloride (20). The above crystals of acid chloride were converted to amide by treatment with 25 ml. of concentrated ammonia for twenty minutes. The above second crop of acid chloride was treated with ten ml. of concentrated ammonia. Recrystallization of the combined products from 400 ml. of 95% ethanol gave a total of 7.4 g., (82%) after concentrating the solution to give additional crops. The melting point of various batches was within four degrees of that recorded for a pure sample, 226-227.5°. The specific rotation was not measured because no good solvent for the amide could be found.

Anal. calc. for $C_{17}H_{14}O_3N_2$: C, 69.37; H, 4.80; N, 9.52

Found: C, 69.75; H, 4.96; N, 9.85.

α-Phthalimido-β-phenylpropionitrile (phosphorus pentoxide method.--α-Phthalimido-β-phenylpropionamide (75 g., 0.255 mole) was suspended in 2200 ml. of hot reagent grade chloroform in a 3-liter round-bottomed flask fitted with a reflux condenser. The mixture was cooled below the boiling point and 35 g. of phosphorus pentoxide was added. The mixture was refluxed 24 hours, and an additional 33 g. of phosphorus pentoxide was added. The mixture was again refluxed 24 hours, and 13 g. of phosphorus pentoxide was added. The mixture was

refluxed another 24 hours, and the chloroform solution was filtered to give a water-clear filtrate containing the nitrile. The chloroform was removed by distillation, using the steam cone, and the liquid nitrile was poured into a beaker, using chloroform to rinse the flask. On cooling, the nitrile crystallized. It was washed with hexane and filtered, to give 54.6 g. (77.5%) which was pure enough for use in subsequent reactions. If desired, the above quantity of nitrile could be recrystallized from 300 ml. of benzene and 300 ml. of hexane to give product which melted partially at about 125°, resolidified, and finally melted at 134-136°.

Anal. calc. for $C_{17}H_{12}O_2N_2$: C, 73.90; H, 4.38; N, 10.14.
Found: C, 73.91; H, 4.45; N, 10.09.

D-α-Phthalimido-β-phenylpropionitrile (phosphorus pentoxide method). --D-α-Phthalimido-β-phenylpropionamide (3.9 g.) was converted to the nitrile by the method given above for the preparation of the DL-nitrile. The crude product, 2.87 g., m.p. 135-143°, was recrystallized from 15 ml. of benzene and 40 ml. of hexane to give 2.3 g., m.p. 140-146°. Another recrystallization gave 2.03 g., m.p. 143-147°; $(\alpha)_D^{25°}$ + 89.6° ± 1° (C, 2.2% in $CHCl_3$). Two recrystallizations from methanol (1 g. from 40 ml.) gave 0.8 g., m.p. 151.5-153°; $(\alpha)_D^{25°}$ + 96.0° ± 1° (c, 2.1% in $CHCl_3$).

α-Phthalimido-β-phenylpropionitrile (benzene sulfonylchloride method). --α-Phthalimido-β-phenylpropionamide (2.76 g., 0.0094 mole) was dissolved in 10 ml. of boiling pyridine in a beaker. Benzenesulfonyl chloride (6 ml., 0.0325 mole) was added, and the mixture was boiled for ten minutes. The mixture was cooled in an ice bath, and 25 ml. of

water was added slowly. The resulting solid nitrile was filtered, washed with water, and dried to give 2.56 g. (99%) crude product, m.p. 126-131°, with some resolidification at about 126°.

L-α-Phthalimido-β-phenylpropionitrile (benzene sulfonylchloride method.--L-α-Phthalimido-β-phenylpropionamide (5 g.) was converted to the nitrile by the procedure described for the DL-amide. The crude nitrile (4.57 g., 97%) was recrystallized from 175 ml. of methanol to give 4.21 g. (89.6%, based on amide), m.p. 150-153.2°, $(\alpha)_D^{25°}$ - 103° ± 1°.

Anal. calc. for $C_{17}H_{12}O_2N_2$: C, 73.90; H, 4.38; N, 10.14

Found: C, 73.97; H, 4.47; N, 10.09

N-(α-Phthalimido-β-phenylpropionyl) phosphoramidic dichloride.--α-Phthalimido-β-phenylpropionamide (12 g., 0.0408 mole) was suspended in 168 ml. of dioxane in a beaker, and phosphorus pentachloride (16.8 g., 0.0808 mole) was added. Stirring and warming to about 40° gave a clear solution which was decanted from some excess phosphorus pentachloride. Five hundred and twenty ml. of hexane was added to the solution, which was then poured into five crystallizing dishes in the hood and allowed to stand for about two hours. Crystals formed during this exposure to moist air. They were separated by filtration and recrystallized from 160 ml. of chloroform and 160 ml. of benzene, giving 14.56 g. (86.5%), m.p. 170-171° dec.

Anal. calc. for $C_{17}H_{13}O_4N_2PCl_2$: C, 49.65; H, 3.19; N, 6.81; P, 7.53; Cl, 17.25.

Found in analyses of three separate samples:

C, ---, ---, 56.34; H, ---, ---, 4.16; N, 5.08, 5.21, 5.03;

P, ---, 6.37, 6.90; Cl, 32.55, 25.13, 16.22.

The only consistent analyses for those for nitrogen, and these lead to an unreasonable molecular weight, assuming two nitrogens per molecule. The crystals were well formed, transparent prisms. The first two samples were crystallized from benzene chloroform, while the last was crystallized from benzene only.

Dimethyl N-(α-phthalimido-β-phenylpropionyl) phosphoramidate.--N-(α-phthalimido-β-phenylpropionyl) phosphoramidic dichloride (1 g.) was boiled for a few minutes with 5 ml. of methanol. The solution was filtered and allowed to cool, giving well formed crystals which were recrystallized from five ml. of methanol to give an analytical sample, m.p. 207°-208.5°. It was later found that after melting about 210°, the compound resolidifies and melts above 220°. The higher melting compound was not investigated.

Anal. calc. for $C_{19}H_{19}O_6N_2P$: C, 56.71; H, 4.76; N, 6.96; P, 7.7.

Found: C, 56.14; H, 4.73; N, 6.32; P, 7.08.

Di-n-butyl N-(α-phthalimido-β-phenylpropionyl) phosphoramidate.--The preparation was similar to that of the methyl derivative reported above. The 0.3 g. obtained on cooling the buytl alcohol solution of product was recrystallized from four ml. of carbon tetrachloride to give an analytical sample, m.p. 136-139°.

Anal. calc. for $C_{25}H_{31}O_6N_2P$: C, 61.72; H, 6.42; N, 5.76; P, 6.36.

Found: C, 60.30; H, 6.30.

α-(o-Carboxybenzamido)-β-phenylpropionitrile.--α-Phthalimido-β-phenylpropionitrile (1.68 g., 0.0061 mole) was dissolved in 40 ml. of methanol, and 20 ml. of water was added. The resulting suspension of

oil was stirred for ten minutes with 15 ml. 1M sodium hydroxide, and then acidified with 4 ml. 6M hydrochloric acid. The resulting solid was difficult to recrystallize, but this was finally accomplished by allowing an alcohol solution of it to evaporate slowly. This recrystallization was repeated twice from alcohol-water, giving an analytical sample, m.p. 165° dec.

Anal. calc. for $C_{17}H_{14}O_3N_2$: C, 69.37; H, 4.80; N, 9.52.

Found: C, 69.49; H, 4.97; N, 9.63.

The structure of the compound was confirmed by heating a sample in an oil bath at 165°. After bubbling ceased the residue was cooled and recrystallized from a benzene hexane mixture, giving the original α-phthalimido-β-phenylpropionitrile, which was identified by melting point and mixed melting point.

α-Phthalimido-β-phenylpropionhydroxamic acid. --α-Phthalimido-β-phenylpropionyl chloride (7 g., 0.0224 mole) was dissolved in 25 ml. of dimethyl formamide and mixed rapidly with a solution of 1.47 g. (0.0448 mole) free hydroxylamine in 12 ml. of dimethyl formamide. The resulting warm solution was cooled and diluted with an equal volume of water. Cooling in the refrigerator gave crystals of product which were separated by filtration. A second crop was obtained from the filtrate on adding more water, giving a total of 3.88 g., (55.7%). It may be mentioned that on repeating this preparation on a larger scale, much greater difficulty in crystallizing the product was encountered. The analytical sample was recrystallized from aqueous alcohol.

Anal. calc. for $C_{17}H_{14}O_4N_2$: C, 65.80; H, 4.55; N, 9.03.

Found: C, 65.67; H, 4.52; N, 6.37, 8.37.

α-Phthalimido-β-phenylpropionamidoxime. -- α-Phthalimido-β-phenylpropionitrile (8.28 g., 0.03 mole) was dissolved in 120 ml. of boiling methanol. A solution of hydroxylamine containing some hydroxylamine hydrochloride was prepared by adding 30 ml. of 1M sodium methoxide in methanol to 69 ml. of 0.5M hydroxylamine hydrochloride in methanol and cooling the mixture in the refrigerator. After half an hour the hydroxylamine solution was decanted from precipitated sodium chloride and added to the methanol solution of nitrile. The reaction mixture was heated under reflux for three hours and then cooler overnight. The white crystals of product were separated by filtration (5.74 g., 62.1%), m.p. 198-204° dec. Distilling off most of the methanol under reduced pressure gave a second crop of 0.83 g., bringing the total yield to 71%. Recrystallization from methanol gave an analytical sample.

Anal. calc. for $C_{17}H_{15}O_3N_3$: C, 66.01; H, 4.89; N, 13.59.

Found: C, 66.13; H, 4.97; N, 13.57.

L-α-Phthalimido-β-phenylpropionamidoxime. -- L-α-Phthalimido-β-phenylpropionitrile was converted to the amidoxime by the procedure described above, using 10.20 g. nitrile, 290 ml. of methanol, 37 ml. of 1M sodium methoxide, and 42.4 ml. of 1M hydroxylamine hydrochloride. The L-amidoxime was too soluble in methanol to crystallize directly from the reaction mixture. Accordingly the solution was evaporated under reduced pressure to 100-125 ml., and 60 ml. of water was added in portions, giving a mass of fine needles which were separated by filtration after the mixture had been cooled in the refrigerator. This gave 8.35 g. (73%), m.p. 164-171° dec., $(\alpha)_D^{25°}$ - 108° ± 1° (c, 2.7%

in methanol)

Anal. calc. for $C_{17}H_{15}O_3N_3$: C, 66.01; H, 4.89; N, 13.59.

Found: C, 66.23; H, 4.87; N, 12.81, 14.19.

α-Amino-β-phenylpropionamidoxime. -- α-Phthalimido-β-phenylpropionamidoxime (3.3 g., 0.0107 mole) was dissolved in 150 ml. of hot methanol. To the solution was added 32 ml. of $\frac{1}{3}$ M hydroxylamine in methanol, prepared by mixing solutions of hydroxylamine hydrochloride and sodium methoxide, and 10.7 ml. of 1M sodium methoxide. The solution immediately turned yellow due to formation of the sodium salt of phthaloxime. In two hours the color had become deep red, and the solution was evaporated to dryness under reduced pressure. This operation was accomplished best by use of a rotating flask evaporator, since the sodium salt of phthaloxime precipitates and causes bumping in a conventional apparatus. The resulting solid was extracted with about 25 ml. of hot ethyl acetate, and the mixture was filtered. Crude α-amino-β-phenylpropionamidoxime crystallized from the solution on cooling (0.93 g., 52%), m.p. 115-117.5°. If desired, this product may be crystallized from about 10 ml. of water to give thin white plates, m.p. 117-118, in about 70% yield.

Anal. calc. for $C_9H_{13}ON_3$: C, 60.31; H, 7.31; N, 23.45.

Found: C, 60.27; H, 7.26; N, 23.48.

O-Benzoyl-α-benzamido-β-phenylpropionamidoxime. -- α-Amino-β-phenylpropionamidoxime (0.43 g., 0.0024 mole) was dissolved in 200 ml. of ethyl acetate. Benzoyl chloride (0.995 ml., 0.00862 mole) was dissolved in 20 ml. of ethyl acetate; this amount was used because of a mistake in calculation, it being desired to use 0.0048 mole of benzoyl chloride. Half the benzoyl chloride solution

was added to the amidoxime in ethyl acetate and the mixture was allowed to stand twenty minutes in order to let half of the acylation take place under anhydrous conditions. Then 4.8 ml. of 1M potassium carbonate and the rest of the benzoyl chloride was added. In thirty minutes the ethyl acetate layer was separated and dried with magnesium sulfate. Evaporation under reduced pressure to about 50 ml. gave 0.7 g. (75%) of the desired dibenzoyl derivative. This was recrystallized from about 140 ml. of acetonitrile to give 0.4 g., m.p. 206-7° dec. on rapid heating, although later experience with the optically active dibenzoyl derivative indicates that recrystallization was unnecessary.

L-O-Benzoyl-α-benzamido-β-phenylpropionamidoxime.--L-α-Phthalamido-β-phenylpropionamidoxime (5 g., 0.0162 mole) was treated with hydroxylamine and sodium methoxide in order to cleave off the phthaloyl group, using the procedure described above for the DL-compound. Since preliminary experiments had given the amino amidoxime only as a water soluble syrup, the ethyl acetate extract of the amino amidoxime was diluted to 200 ml. with reagent grade ethyl acetate and benzoylated directly with 5 ml. of benzoyl chloride, using 5 ml. of 1M potassium carbonate. The desired dibenzoyl derivative crystallized directly from the ethyl acetate phase after a few minutes. It was separated by filtration, washed with ethyl acetate, and dried, giving 2.91 g. (46.5%). An analytical sample was obtained by recrystallization from acetonitrile, m.p. 204-211° dec, $(\alpha)_D^{25°}$ - 28.0° ± 1° (c, 2.4% in dimethyl formamide).

Anal. calc. for $C_{23}H_{21}O_3N_3$: C, 71.30; H, 5.46; N, 10.85.
Found: C, 71.38; H, 5.51; N, 10.94.

α-Benzamido-β-phenylpropionamidoxime. --O-Benzoyl-α-benzamido-β-phenylpropionamidoxime (0.4 g.) was boiled with 50 ml. of methanol containing a few drops of 1M sodium methoxide. A clear solution was formed rapidly, indicating formation of the more soluble product. The solution of product was divided into two parts. Water was added to one part, giving fine white needles which were separated by filtration and dried, 0.090 g. (61.5%). The other half of the methanol solution was evaporated to dryness, dissolved in hot acetonitrile, filtered, and cooled, giving cottony crystals (0.074 g., 50.5%). An analytical sample was recrystallized from acetonitrile, m.p. 200-202° dec.

Anal. calc. for $C_{16}H_{17}O_2N_3$: C, 67.82; H, 6.05; N, 14.83.
Found: C, 67.90; H, 6.09; N, 14.83.

L-α-Benzamido-β-phenylpropionamidoxime. -- L-O-Benzoyl-α-benzamido-β-phenylpropionamidoxime (1 g.) was boiled for nine minutes in 75 ml. of methanol containing nine drops of 1M sodium methoxide in methanol. The solution was evaporated to dryness in vacuo, and the residue was dissolved in about 70 ml. of acetonitrile and centrifuged hot. The clear solutions were poured out and allowed to cool, giving 0.32 g. (43.7%), $(\alpha)_D^{25^\circ}$ - 85.2° ± 1° (c, 2.3% in dimethyl formamide).

O-Acetyl-α-acetamido-β-phenylpropionamidoxime. -- α-Amino-β-phenylpropionamidoxime (0.5 g., 0.00279 mole) was dissolved in 200 ml. of reagent grade ethyl acetate. Acetic anhydride (0.552 ml., 0.00585 mole) was dissolved in 10 ml. of ethyl acetate. Half the acetic anhydride solution was added to the amidoxime solution, and the mixture

was allowed to stand ten minutes. Then 5.85 ml. (0.00585 mole) 1M potassium carbonate and the remainder of the acetic anhydride was added. After ten minutes the solution was evaporated under reduced pressure to about 30 ml., giving 0.56 g., (76.3%) of the desired diacetyl derivative, m.p. 160-162° dec.

Anal. calc. for $C_{13}H_{17}O_3N_3$: C, 59.30; H, 6.51; N, 15.96.

Found: C, 59.32; H, 6.61; N, 15.97.

α-Acetamido-β-phenylpropionamidoxime. --O-Acetyl-α-acetamido-β-phenylpropionamidoxime (0.56 g.) was boiled for nine minutes with 15 ml. of methanol containing four drops of 1M sodium methoxide in methanol. The resulting solution was evaporated to dryness in vacuo and recrystallized from hot water, giving 0.31 g. (65.8%) of white needles, m.p. 156-162° dec.

Anal. calc. for $C_{15}H_{15}O_2N_3$: C, 59.71; H, 6.83.

Found: C, 59.82; H, 6.56

L-O-Acetyl-α-acetamido-β-phenylpropionamidoxime. -- The phthaloyl group was cleaved from 4.15 g. (0.0134 mole) of L-α-phthalimido-β-phenylpropionamidoxime, using the procedure already described. The resulting ethyl acetate extract of the amino amidoxime was diluted to 100 ml. and acetylated, using 2.52 ml. (0.0268 mole) of acetic anhydride and 26.8 ml. of 1M potassium carbonate, employing the procedure described above for the DL-compound. Concentrating the dried ethyls acetate layer in vacuo gave 1.44 g., (48.5%) of product.

L-α-Acetamido-β-phenylpropionamidoxime. -- L-O-Acetyl-α-acetamido-β-phenylpropionamidoxime (1.44 g.) was treated with methanol containing sodium methoxide, using the procedure described

above for the DL-compound. The product was crystallized from 30 ml. of water, giving 0.89 g. (73.5%), m.p. 167-169° dec. Mixed with the DL-compound of m.p. 156-158° dec., the m.p. was 140-150° dec., giving evidence that racemization did not occur during any step of the preparation. This evidence was desired because of the low specific rotation, $(\alpha)_D^{25°}$ - 11.1° ± 4° (c, 2.1% in absolute ethanol).

Anal. calc. for $C_{11}H_{15}O_2N_3$; C, 59.71; H, 6.83; N, 18.99.

Found: C, 59.80; H, 6.98; N, 17.69, 19.75.

α-Phthalamamido-β-phenylpropionamide. -- During the preparation of α-phthalimido-β-phenylpropionamide by the method described earlier, the crude amide was accidentally allowed to stand 36 hours in concentrated ammonium hydroxide. The resulting α-phthalamamido-β-phenylpropionamide did not crystallize from the volume of alcohol used to recrystallize the amide, but evaporation to smaller volume resulted in very slow precipitation of very fine cottony needles, m.p. 205-210° dec. The observed decomposition on melting was found to consist of loss of ammonia, with formation of α-phthalimido-β-phenylpropionamide. The latter compound solidified in the melting point tube above 210° and then melted at 227-229° (Found for pure amide, m.p. 236.8-237.2). The phthalamamido compound was recrystallized with difficulty from ethanol. At times a cold supersaturated solution in ethanol very slowly formed large prisms which appeared to be a solvate, as the prisms disintegrated to a white powder on filtering and drying.

Anal. calc. for $C_{17}H_{17}O_3N_3$: C, 65.58; H, 5.50; N, 13.50.

Found: C, 65.52; H, 5.57; N, 13.41.

α-Phthalimido-β-phenylpropionamide (from α-phthalamamido-β-phenylpropionamide). -- The phthalamamido derivative described above was heated in a beaker by means of an oil bath at 240°. The powder melted with loss of ammonia. The melt was cooled and converted directly to α-phthalimido-β-phenylpropionitrile.

α-Ethylphthalamamido-β-phenyl-N-ethylpropionamide. -- The compound was prepared by the method described above for the preparation of α-phthalamamido-β-phenylpropionamide, using aqueous ethyl amine instead of aqueous ammonia. It was recrystallized from ethanol-water, m.p. 180-182° with loss of ethyl amine, as shown by holding pH paper above the melting compound.

Anal. calc. for $C_{21}H_{25}O_3N_3$: C, 68.64; H, 6.86.

Found: C, 68.71; H, 6.81.

α-Phthalimido-β-phenyl-N-ethylpropionamide. -- An unknown amount of ethyl amine gas was passed into a solution of α-phthalimido-β-phenylpropionyl chloride in benzene. The mixture was evaporated to dryness to give a solid. Four g. of this material was recrystallized from about 200 ml. of ethyl acetate to give white prisms, m.p. 183.5-184°, which were recrystallized to give an analytical sample.

Anal. calc.: for $C_{19}H_{18}O_3N_2$: C, 70.79; H, 5.63.

Found: C, 70.87; H, 5.58.

Evaporation of the ethyl acetate filtrate from the first crystallization gave a second compound which proved to be α-ethylphthalamido-β-phenyl-N-ethylpropionamide, identical with that obtained in the preparation described above, as shown by mixed melting point.

The α-phthalimido-β-phenyl-N-ethylpropionamide was prepared with the idea of converting it to the imide chloride, which would be useful in making N-ethyl amidines or imino esters. However, this project has not been carried out.

α-Phthalimido-β-phenylpropionthioamide. -- α-Phthalimido-β-phenylpropionitrile (13.8 g., 0.05 mole) was dissolved in 300 ml. of ethanol and 0.745 g. (0.005 mole) of triethanolamine was added. The mixture was heated to near the boiling point, and hydrogen sulfide was slowly bubbled through the hot solution for two days. The reaction mixture was then placed in the refrigerator, giving some solid which was separated by filtration, and which was judged from the melting point to be mostly unreacted nitrile. A portion of the filtrate was allowed to evaporate slowly, giving large prisms of a higher melting substance which proved to be the desired thioamide. It was recrystallized from alcohol water, m.p. 160.5-162°.

Anal. calc. for $C_{17}H_{14}O_2N_2S$: C, 65.78; H, 4.55; N, 9.03; S, 10.33

Found: C, 65.98; H, 4.55; N, 9.04, S, 10.34.

Preparation of the compound thought to be α-phthalamamido-β-phenylpropionthioamide and its alcohol solvate. -- α-Phthalimido-β-phenylpropionitrile (8 g.) was dissolved in 120 ml. of ethanol saturated with ammonia by warming the mixture slightly. The mixture was saturated with hydrogen sulfide and allowed to stand for a few hours. White crystals formed which were separated by filtration and washed with ether, and allowed to dry in air, giving 9.72 g. This represents a yield of 90%, assuming that the compound is α-phthala-

mamido-β-phenylpropionthioamide containing one mole of ethanol, or a yield of 103% for the same compound containing no ethanol. The evidence for the existence of an alcohol adduct was obtained in a later preparation in which methanolic ammonia was used instead of ethanolic ammonia. The product from this preparation was recrystallized from methanol (3.73 g. from 150 ml. gave 2.49 g.), and the product was heated under vacuum in an Abderhalden drying pistol, using boiling cyclohexanol, b.p. 161°, as the heating fluid. Partial melting and resolidification occurred, accompanied by a 6.18% loss of weight. The resulting solid could be recrystallized from acetonitrile (0.3 g. from 6 ml.) to give a product having m.p. 173-177° dec. However, when the solid was recrystallized from methanol, partial melting and resolidification at 100-160° was again observed, accompanied by a 7.35% loss of weight. Calc. for loss of CH_3OH: 8.50%.

Anal. calc. for $C_{17}H_{17}O_2N_3S$: C, 62.36; H, 5.23; N, 12.83.

Found: C, 62.98; H, 5.48; N, 11.31.

Anal. calc. for $C_{17}H_{17}O_2N_3S:CH_3OH$: C, 60.14; H, 5.89; N, 11.69; S, 8.92.

Found: C, 59.90; H, 5.64; N, 10.75; S, 8.26.

Phenylalanine thioamide.--The compound thought to be a methanol adduct of α-phthalamamido-β-phenylpropionthioamide (4.10 g., 0.0114 mole) was heated to 85° with 20.6 ml. of 0.612M sodium hydroxide (0.0126 mole), giving a clear yellow solution. A solid crystallized out on cooling which was purified and identified as phenylalanine thioamide (0.5 g., 24.3%), on the basis of its solubility and elementary analysis. The above crude product was recrystallized

from 4 ml. of ethanol and 8 drops of water, giving 0.285 g., recrystallized again to give an analytical sample, yellowish plates, m.p. 135-136.3° dec. The compound was insoluble in water and dilute sodium hydroxide, soluble in dilute hydrochloric acid.

Anal. calc. for $C_9H_{12}N_2S$: C, 59.96; H, 6.71; N, 15.54; S, 17.79.

Found: C, 60.05; H, 6.72; N, 15.63; S, 17.72.

Preparation of the compound which may be α-phthalamamido-β-phenylthiopropionic acid. -- Acidification of the filtrate from the above preparation of phenylalanine thioamide gave an oily solid which was recrystallized with some difficulty from ethanol-water, giving an analytical sample after three recrystallizations, m.p. 190-195° dec. with loss of ammonia. The structure is indicated by the solubility in base, the loss of ammonia on heating, and the analysis, despite the incorrect values obtained for nitrogen and sulfur.

Anal. calc. for $C_{17}H_{16}O_3N_2S$: C, 62.18; H, 4.91; N, 8.53; S, 9.76.

Found: C, 62.51; H, 5.01; N, 6.84; S, 10.94.

Methyl α-phthalimido-β-phenyliminopropionate hydrochloride. -- α-Phthalimido-β-phenylpripionitrile (7.72 g., 0.0280 mole) was dissolved in 60 ml. of benzene, and 9.3 ml. of 3M methanol in benzene was added. In two days a large amount of precipitate had formed. It was separated by filtration and dried by passing dry air through the funnel, giving 8.04 g. (83.3%), m.p. 234-236, indicating that conversion to the amide occurred on heating. The product was used directly in further experiments.

Methyl α-phthalimido-β-phenyliminopropionate. --The hydrochloride described above (8.04 g., 0.0233 mole) was dissolved in 100 ml. of dry methanol, and 6.3 ml. of 3.7M sodium methoxide (0.0233 mole) was added. The mixture was evaporated to dryness under reduced pressure and extracted with 50 ml. of ether. The solution was evaporated to about 15 ml., and 5-10 ml. of hexane was added, giving oily crystals. These were separated by filtration and recrystallized from methanol-water, giving only 0.43 g., (6.0%), m.p. 124.8-126.8.

Anal. calc. for $C_{18}H_{16}O_3N_2$: C, 70.11; H, 5.23; N, 9.09.
Found: C, 70.07; H, 5.29; N, 9.06.

Methyl α-phthalimido-β-phenylpropionhydroximate. -- Methyl α-phthalimido-β-phenyliminopropionate hydrochloride (7.90 g., 0.0229 mole) was dissolved in 160 ml. of methanol, and 23 ml. of 1M hydroxylamine (prepared from the hydrochloride and sodium methoxide) was added. The mixture was evaporated to dryness in vacuo and extracted with 50 ml. of benzene. Addition of hexane gave 1.44 g. (20%) of product m.p. 162-168° dec. The material was recrystallized from methanol, giving large prisms, m.p. 172-180° dec.

Anal. calc. for $C_{18}H_{16}O_4N_2$: C, 66.66; H, 4.97; N, 8.64.
Found: C, 66.58; H, 4.90; N, 8.56.

The major product of the above reaction (4.17 g.) was not soluble in benzene. The crude product melted at 170-205° dec., and was apparently not α-phthalimido-β-phenylpropionamide of α-phthalimido-β-phenylpropionamidixome, as might be expected.

α-Phthalimido-β-phenylpropionaldehyde. -- α-Phthalimido-β-phenylpropionitrile (11.04 g., 0.04 mole) was dissolved in 80 ml. of benzene, and 80 ml. of ether was added. Anhydrous stannous chloride

(15.12 g., 0.080 mole) was suspended in 160 ml. of dry ether in a 500 ml. Erlenmeyer flask and hydrogen chloride gas was passed in until the stannous chloride phase turned to a clear, heavy liquid. The solution of nitrile was added, causing the stannous chloride phase to turn opaque white. Hydrogen chloride was again run in until a clear liquid was obtained. This process is speeded considerably if the flask is stoppered and shaken at frequent intervals. The mixture was allowed to stand five hours at room temperature and then placed in the refrigerator for 36 hours. The ether layer was poured off and discarded, since an earlier experiment showed that it contains little nonvolatile material. The excess hydrogen chloride was blown off with nitrogen, and the product was shaken for ten minutes on a mechanical shaker with 40 ml. of water and 240 ml. of benzene. The benzene layer was separated, and the water layer, along with any solid present, was diluted with 40 additional ml. of water and shaken for twenty minutes with 240 additional ml. of benzene. The second benzene extract was separated and combined with the first. The benzene extracts were combined, dried briefly in two stages with magnesium sulfate, filtered, and evaporated in vacuo, using a warm water bath as heater to avoid local overheating of the product. During the evaporation some solid tended to separate. This was at first thought to be a polymer of the aldehyde, but later observations on the stability of the aldehyde make it seem more likely that it is some tin compound. When the benzene had been removed, the resulting oil solidified (8.3 g., 74.4%). That the product was not pure aldehyde is indicated by the low yield on recrystallization. Extracting 6.56 g. with 175 ml. of boiling petrolium

ether (85-100°) and pouring off the solution left an insoluble residue which apparently was not aldehyde. The petroleum ether solution first turned cloudy on cooling in the refrigerator, and then gave crystals of aldehyde (3.04 g., 34.3% overall yield), m.p. 94.5-96°. Recrystallization from hexane (0.28 g., from 20 ml.) gave an analytical sample, m.p. 96.3-96.8°.

Anal. calc. for $C_{17}H_{13}O_3N$: C, 73.11; H, 4.69; N, 5.02.
Found: C, 73.49; H, 4.78; N, 5.09.

The crude aldehyde obtained on evaporation of the benzene extract in a separate preparation gave a precipitate with 2,4-dinitrophenylhydrazine, but the derivative could not be recrystallized. However, a crystalline semicarbazone was prepared by the method described in Shriner and Fuson's text. The recrystallization from acetonitrile (0.2 g. from 25 ml.) gave an analytical sample, m.p. 238-240° dec. on rapid heating.

Anal. calc. for $C_{18}H_{16}O_2N_4$: C, 64.27; H, 4.79; N, 16.66.
Found: C, 64.24; H, 4.82; N, 16.63.

α-Phthalimido-β-phenylpropionaldehyde from Rosenmund reduction. -- The preparation was carried out according to the directions of Foye (50), giving a toluene solution from which the aldehyde was obtained as a mixture of oil and crystals by addition of a large amount of hexane. The crude crystalline product was melted at about 61-64° with resolidification occurring before melting was complete, the final melting point being 80-85°. It is possible that Foye observed the earlier transition, accounting for his reported m.p. of 75-78°, although the earlier transition was no longer observed in the present

work after the product was recrystallized from hexane. This recrystallization (0.4 g. from 40 ml. of hexane) gave product having m.p. 90.5-92°. Recrystallization raised this to 92-93°. Mixed with product from Stephen reduction of m.p. 94-96°, the m.p. was 95-96°.

α-Phthalimido-β-phenylpropionaldehyde ethyl hemiacetal.--When crude α-phthalimido-β-phenylpropionaldehyde from Stephen reduction was dissolved in ethanol by warming, and water was added to the cloud point, crystals of the ethyl hemiacetal formed on cooling. The substance could be recrystallized from benzene-hexane (0.5 g. from 10 ml. benzene and 40 ml. of hexane). The hemiacetal melted with evolution of a gas, probably ethanol, m.p. 122-124° on rapid heating.

Anal. calc. for $C_{19}H_{17}O_3N$: C, 70.14; H, 5.89; N, 4.30.

Found: C, 69.51, 70.65; H, 5.89, 5.84; N, 4.11.

α-Phthalimido-β-phenylpropionaldehyde n-butyl hemiacetal.--α-Phthalamido-β-phenylpripionaldehyde (0.2 g.) was boiled with 2 ml. of n-butyl alcohol, cooled, and the hemiacetal crystallized by addition of 4 ml. of hexane, giving 0.09 g. This was recrystallized from 2 ml. of benzene and 4 ml. of hexane, giving an analytical sample, m.p. 102-104°.

Anal. calc. for $C_{21}H_{23}O_4N$: C, 71.27; H, 6.56; N, 3.96.

Found: C, 71.80; H, 6.57; N, 4.02.

α-Phthalimido-β-phenylpropionaldehyde methyl hemiacetal.--This substance, prepared by the procedure described for the ethyl derivative, had m.p. 119-121° dec.

L-α-Phthalimido-β-phenylpropionaldehyde.--L-α-Phthalimido-

β-phenylpropionitrile (1.69 g.) was converted to the aldehyde by the Stephen method, using the procedure described for the DL-compound. The product was recrystallized from 25 ml. of 85-100° petroleum ether, giving 0.73 g. (42.8%), m.p. 115-117°, $(\alpha)_D^{25°}$ - 157° ± 2° (c, 2.03% in chloroform).

Anal. calc. for $C_{17}H_{13}O_3N$: C, 73.11; H, 4.69.

Found: C, 73.38; H, 5.15.

α-Phthalimido-β-phenylpropionaldehyde ethylene glycol acetal. -- Following the procedure of Balenovic et al (51), α-phthalimido-β-phenylpropionaldehyde (4 g.) in 200 ml. of benzene was mixed with 4 ml. of ethylene glycol and 0.2 g. of -toluenesulfoned acid. The water formed in the reaction was removed as the azeotrope by slow distillation over the course of eight hours, adding benzene as needed. The benzene solution was washed with water, dried, and evaporated in vacuo, giving a solid which was recrystallized from 8 ml. of benzene and 30 ml. of hexane. Yield: 3.74 g. (80.8%), m.p. 108-109.5°.

Anal. calc. for $C_{19}H_{17}O_4N$: C, 70.57; H, 5.30; N, 4.33

Found: C, 70.44; H, 5.38; N, 4.31.

α-Benzamido-β-phenylpropionaldehyde ethylene glycol acetal. -- α-Phthalimido-β-phenylpropionaldehyde ethylene glycol acetal (1.08 g., 0.00333 mole) in 40 ml. of methanol was refluxed 3 hr. with 3.33 ml. of 1M hydroxylamine in methanol and 3.33 ml. of 1M sodium methoxide in methanol. The dark red solution was evaporated to dryness on a rotating flask evaporator and extracted with hot reagent grade ethyl acetate. The extract was benzoylated, using 0.38 ml. (0.00333 mole)

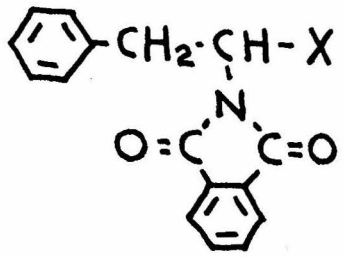
of benzoyl chloride and 3.33 ml. of 1M potassium carbonate. The resulting ethyl acetate solution was dried and concentrated giving the product, white crystals (0.36 g., 36.2%), m.p. 118.5°.

Anal. calc. for $C_{18}H_{19}O_3N$: C, 72.70; H, 6.44; N, 4.71.

Found: C, 72.49; H, 6.68; N, 4.65.

TABLE I

Physical Constants of α-Phthalimido-β-phenylpropionic Acid Derivatives of General Type:

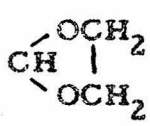


Compound		X	Melting point of DL-compound[a]	Melting point of optically active compound[b]	$(\alpha)_D^{25^\circ}$ Specific rotation of optically active compound[c]
I	Acid	CO_2H	176-177.5°[d]	Not isolated[e]	
II	Acid chloride	COCl	135-137°	Not isolated[f]	
III	Amide	$CONH_2$	226-227.5°	236.8-237.2	Not taken[g]
	N-ethyl-amide	$CONHC_2H_5$	167.3-168°		
IV	Nitrile	CN	125°, then 134-136°	151.5-153°	$-103^\circ \pm 1^\circ$ (c, 2.1% in chloroform)

TABLE I (Cont'd)

XIII	Hydroxamic acid	CONHOH	165° dec.		
XIV	Amidoxime	$C(=NOH)NH_2$	198-204° dec.	164-171° dec.	-108° ± 1° (c, 2.7% in methanol)
XVII	Thioamide	$CSNH_2$	160.5-162°		
XX	Methyl imino ester hydrochloride	$C(=NH)OCH_3 . HCl$	234-236°		
	Methyl imino ester	$C(=NH)OCH_3$	124.8-126.8°		
	Methyl hydroximic ester	$C(=NOH)OCH_3$	172-180° dec.		
XXIII	Aldehyde	CHO	94.5-96° [h]	115-117°	-157° ± 2° (c, 2.03% in chloroform)
	Ethyl hemiacetal of aldehyde	$CH(OC_2H_5)OH$	122-124° dec.		
	n-Butyl hemiacetal of aldehyde	$CH(OC_4H_9)OH$	102-104° dec.		

TABLE I (Cont'd)

	Methyl hemiacetal of aldehyde	$CH(OCH_3)OH$	119-120° dec.
XXIV	Ethylene glycol acetal of aldehyde		108-109.5°

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[a] Uncorrected melting points are reported, in view of the fact that two different methods for calibrating the thermometer gave correction curves which disagreed by as much as two degrees. However both calibrations indicated that the uncorrected thermometer readings are within two degrees of the correct value.

[b] Melting points are for the L-compounds, although in some cases the same melting point has been observed for the D-compound, as expected. Compounds prepared from L-phenylalanine are called L-compounds.

[c] All specific rotations are for the L-compound.

[d] Reported (13) 174-175°; (20) 177.5-179°.

[e] Reported (23) 183-185°.

[f] Reported (23) 82-83°.

[g] No good solvent for the amide was found.

[h] Reported (50) 75-78°.

TABLE II

Physical Constants of Compounds Prepared by the Use of α-Phthalimido-β-phenylpropionic Acid Derivatives as Intermediates

Compound		Melting point of DL-compound	Melting point of optically active compound	$(\alpha)_D^{25^\circ}$ Specific rotation of optically active compound [a]
VIII	α-(o-Carboxybenzamido)-β-phenylpropionitrile	165° dec.		
IX	Phthaloxime	235-238° [b]		
XV	α-Amino-β-phenylpropion-amidoxime	117-118.5°	Not isolated	
XVI	α-Phthalamamido-β-phenylpropionamide	205-210° dec., then 227-229°		
	α-Ethylphthalalamido-β-phenyl-N-ethylpropionamide	180-182° dec.		
XXI	O-Benzoyl-α-benzamido β-phenylpropionamidoxime	206-207° dec.	204-211° dec.	-28° ± 1° (c, 2.4% in dimethyl formamide)

TABLE II (Cont'd)

XXII	α-Benzamido-β-phenyl-propionamidoxime	200-202° dec.		-85.2° ± 1° (c, 2.3% in dimethyl formamide)
	O-Acetyl-α-acetamido-β-phenylpropionamidoxime	160-162° dec.		
	α-Acetamido-β-phenyl-propionamidoxime	156-162°	167-169° dec.	-11.4° ± 4° (c, 2.1% in ethanol)
XVIII	α-Phthalamamido-β-phenylpropionthioamide	173-177° dec.		
XIX	Phenylalanine thioamide	135-136.3° dec.		
XXVI	α-Benzamido-β-phenylpropion-aldehyde ethylene glycol acetal	118.5°		

[a] Specific rotations of the L-compound.

[b] Reported (52) 220-226°.

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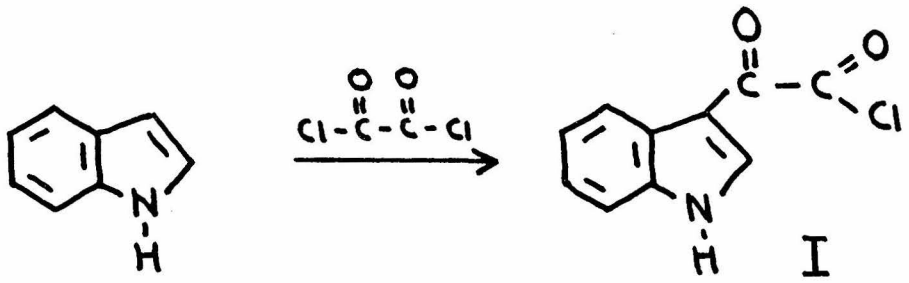
PART II: DERIVATIVES OF 3-INDOLECARBOXYLIC ACID

A. INTRODUCTION

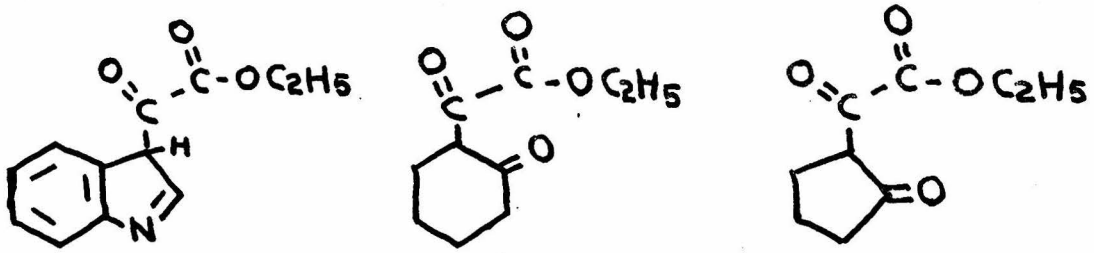
One of the ways of studying the α-chymotrypsin catalyzed hydrolysis of acylamino acid derivatives involves using substrates in which the acyl group is varied. For some time there has been an interest in these laboratories in studying the enzymatic hydrolysis of acylamino acid derivatives in which the acyl group is derived from 3-indolecarboxylic acid. The interest derives from the relation of such a substrate to tryptophane derivatives, which possess the same indole group in a different position in the molecule.

In December 1954 a communication appeared describing the product from reaction of indole with oxalyl chloride as 3-indoleglyoxalyl chloride I (1):



I

The work reported here arose from the idea that the corresponding ester might eliminate carbon monoxide on heating, providing an easy route to ethyl 3-indolecarboxylate. The idea is based on the resemblance of one of the tautomeric structures of ethyl 3-indoleglyoxalate to esters such as III which are known to eliminate carbon monoxide (2):



II III IV

Closer inspection of the literature revealed that the more closely related structure, IV, does not eliminate carbon monoxide. However II was prepared and tested for carbon monoxide elimination. Carbon monoxide was not eliminated.

The project was abandoned, but it was decided to recrystallize some of the bright yellow acid chloride (I) for keeping in a chemical collection. When (I) was dissolved in hot tetrachloroethane there seemed to be a great deal of foaming, and no crystals came out of the resulting brown solution. It was decided to test for carbon monoxide evolution during this thermal decomposition. The test was accomplished using basic, ammoniacal silver nitrate (3) which was kindly loaned by Howard Mower*. The test for carbon monoxide was positive, making it seem possible that the following reaction had occurred:

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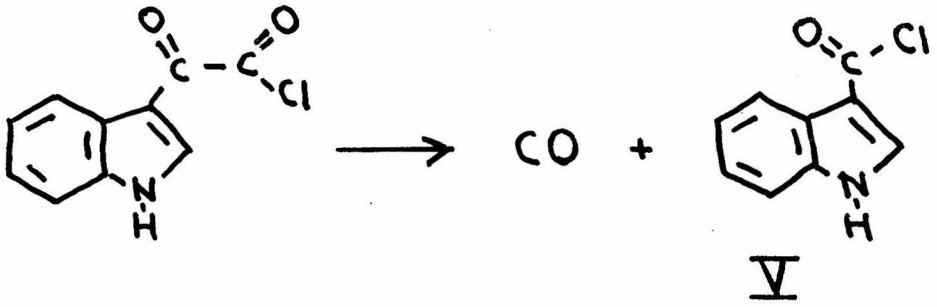
*The author is indebted to Dr. Mower for his lively interest and interesting comments during all phases of this work. Part of Dr. Mower's interest may stem from the fact that he was just beginning the preparation of 3-indolecarboxylic acid derivatives by another route when this investigation was undertaken.



V

The experiments described below show that this is actually the case, and that the indole-3-carbonyl chloride V which is formed can be used to acylate alcohols and amines, including amino acid esters. The products in the latter case are amino acid derivatives of the type originally desired.

One example of the above method for making acid chlorides has been found in the literature. In 1909 p-dimethylaminobenzoyl chloride was prepared by the reaction of oxalyl chloride with dimethylaniline (4). This reaction obviously involved a decarbonylation similar to that which is described above.

B. THE ISOLATION AND REACTIONS OF 3-INDOLECARBONYL CHLORIDE (V).

The preparation of crude 3-indolecarbonyl chloride (V) was accomplished by heating 3-indoleglyoxalyl chloride in tetrachloroethane at 115° for a few minutes and precipitating the brownish yellow product by the addition of a large excess of hexane. It appears to be a new compound, although the 2-indolecarbonyl chloride is known. When the crude product was added to methanol or ethanol, red solutions resulted, from which the corresponding esters of 3-indolecarboxylic acid could be crystallized by addition of water. Likewise, adding

the acid chloride to sodium hydroxide solution and acidifying the solution gave 3-indolecarboxylic acid. The melting points of all these products were in agreement with the values reported in the literature, although only the melting point of the acid serves to distinguish the starting material from 2-indolecarbonyl chloride. (It was originally reported that oxalyl chloride reacted with indole in the two position, although the recent communication (1) gives conclusive evidence that reaction is at the three position.)

Following this structure proof, there followed a series of attempts to acylate amino acid derivatives, all of which resulted in formation of uncrystallizable oils. The amino acid derivatives used were tyrosine ethyl ester, tyrosine amide, tyrosine hydrazide, phenylalanine, and phenylalanine amide. During this period it was found that the indolecarbonyl chloride could be purified by precipitation of the brown impurities, followed by slow crystallization of the yellow acid chloride.

It was next decided to study the acylation of simple amines in order to develop a good acylation method. The previously unreported anilide and p-toluide of 3-indolecarboxylic acid were easily obtained as white crystals by an ordinary Schotten-Baumann procedure. By the same procedure glycine ethyl ester was then successfully acylated, although the product was more difficult to crystallize. Finally L-phenylalanine methyl ester was acylated, giving a viscous oil which crystallized after standing 24 hours in the refrigerator. The resulting 3-indolecarbonyl-L-phenylalanine methyl ester was too insoluble in water to serve as a substrate, but might be convertible to

a more soluble substrate.

A preliminary experiment indicates that the ester may not be convertible to the hydrazide in the usual way, however. A practice experiment in which 3-indolecarbonylglycine ethyl ester was reacted with hydrazine gave a crystalline product which contained more nitrogen than the desired hydrazide. The structure of the product is not known, but it may be a compound resulting from opening of the indole ring by hydrazine. Such a reaction has been observed with another indole derivative (5).

C. EXPERIMENTAL

3-Indolecarbonyl chloride. -- 3-Indoleglyoxalyl chloride was prepared by the method described in the literature (1). When 10 g. of indole in 100 ml. of dry ether was cooled in an ice bath and added to 10 ml. of oxalyl chloride in 100 ml. of dry ether, yellow crystals of the product formed within a few minutes. They were separated by filtration, washed with hexane, and dried on a sintered glass suction funnel protected from moisture with stopper containing a drying tube, giving 16.07 g., 90.6%.

This 3-indoleglyoxalyl chloride was added to 150 ml. of tetrachloroethane which had been heated to 115-120° in an 800 ml. beaker. Carbon monoxide was rapidly evolved. Within a minute or two the gas evolution had slowed greatly, and the deep brown solution was cooled rapidly by placing the beaker in a pan of water. Hexane (450 ml.) was added to precipitate the 3-indolecarbonyl chloride as a brownish-yellow powder. This was separated by filtration on a sintered glass funnel protected with a drying tube and washed on the

funnel several times with hexane. Dry air was drawn through the funnel to dry the product, giving 7 to 10 g. (45-65% based on indole).

Recrystallization of the product was carried out as follows: the crude material was dissolved in 75 ml. boiling benzene and filtered from a large amount of insoluble material. To the brown filtrate was added 25 ml. of hexane. On cooling some precipitate formed, which was separated by filtration and discarded. The resulting solution was much lighter, the dark-colored impurities having been discarded in the precipitate. A few more ml. of hexane was added to the filtrate, again giving some solid which was separated by filtration and discarded. The light yellow-brown filtrate was diluted with about 75 ml. of hexane, added in portions over the course of half an hour. The resulting mixture was cooled, giving yellow crystals of 3-indolecarbonyl chloride. They were dried, using suction filtration with the funnel protected by a drying tube, giving 2.5-3.5 g. (16-23%, based on indole).

Ethyl 3-indolecarboxylate. -- Crude 3-indolecarbonyl chloride (3.06 g.) was added to 25 ml. of dry ethanol, and the product was caused to crystallize by addition of 30 ml. of water. The product was separated by filtration and recrystallized from 30 ml. of ethanol and 20 ml. of water, giving 1.81 g. (37%), m.p. 113-117°. After two recrystallizations the product had m.p. 119-123° (lit. (6) 118-119°).

Methyl 3-indolecarboxylate. -- The procedure described for the ethyl derivative was employed, except that the methanolic solution of ester was filtered from dark-colored impurities before water was added. The final product had m.p. 141-146.5°, but the wide

melting range may have been due to a change of crystal form near the melting point, since when the ester was allowed to resolidify in the melting point tube and was remelted, a m.p. of 144-146.5° was observed. Lit. (7) 147-148.

3-indolecarboxylic acid. -- Crude 3-indole carbonyl chloride (10.61 g.) 0.0592 mole) was added to 59 ml. of 1M sodium bicarbonate. On stirring only a small part of the solid dissolved. Filtering and acidifying the filtrate gave a white precipitate of 3-indolecarboxylic acid, which was separated by filtration and dried, 1.17 g., m.p. 217-219° dec. The insoluble residue from the first filtration dissolved in 60 ml. of 1M sodium hydroxide. Addition of 10 ml. of 6M hydrochloric acid gave a heavy precipitate of pink solid. This was separated and dried, 6.17 g., m.p. 214-217° dec., making the combined yield of product 7.34 g. (53.2%). Reported for 3-indolecarboxylic acid (8), m.p. 218°, 210-218°.

3-indolecarboxanilide. -- Recrystallized 3-indolecarbonylchloride in reagent grade ethyl acetate was added to an excess of aniline in reagent grade ethyl acetate. The solution was washed with water, hydrochloric acid, sodium hydroxide, and water. The ethyl acetate phase was dried with magnesium sulfate, and hexane was added giving crystals of the anilide. Recrystallizations from ethanol-water and from ethyl acetate-hexane gave product with m.p. 175.5-176.2°.

Anal. calc. for $C_{18}H_{12}ON_2$: C, 76.25; H, 5.12; N, 11.86.

Found: C, 76.39; H, 5.19; N, 11.79.

3-Indolecarbox-p-toluide. -- The preparation of this compound was similar to that of the anilide. The analytical sample had m.p.

200.9-201.1°.

Anal. calc. for $C_{16}H_{14}ON_2$: C, 76.78; H, 5.64; N, 11.19.

Found: C, 76.92; H, 5.65; N, 10.79.

3-Indolecarbonylglycine ethyl ester. -- Potassium carbonate (3.18 g., 0.0222 mole) was dissolved in about 5 ml. of water, and the solution was cooled in an ice bath. Glycine ethyl ester hydrochloride (1.55 g., 0.0111 mole) was added to the potassium carbonate solution and the mixture was stirred, liberating free glycine ester. The mixture was placed in a separatory funnel and shaken with 60 ml. of cold reagent grade ethyl acetate. Recrystallized 3-indolecarbonyl chloride (2 g., 0.0111 mole) was dissolved in 30 ml. of cold ethyl acetate, and the solution was added to the separatory funnel containing the glycine ester solution. The mixture was shaken, allowed to stand for 10 minutes, and the ethyl acetate phase was washed with water, hydrochloric acid, and water. The ethyl acetate solution was dried and evaporated to dryness, giving a residue which was crystallized from ethanol-water, yielding 0.84 g., (30.6%), m.p. 159-160°.

Anal. calc. for $C_{13}H_{14}O_3N_2$: C, 63.40; H, 5.73: N, 11.38.

Found: C, 63.51; H, 5.65; N, 11.58.

3-Indolecarbonyl-L-phenylalanine methyl ester. -- The procedure described above for the glycine ester derivative was followed, using 2 g. (0.0111 mole) of 3-indolecarbonyl chloride, 3.2 g. (0.0138 mole) of L-phenylalanine methyl ester hydrochloride, 4.18 g. (0.0253 mole) of potassium carbonate, 7 ml. of water, and a total of 90 ml. of reagent grade ethyl acetate. The product was an oil, which was dissolved in methanol. The solution was adjusted to the cloud point with

water, and the mixture was placed in the refrigerator. After 20 hours crystallization occurred, giving 1.08 g. (30%), m.p. 128-133°. Two additional recrystallizations were easily accomplished to give an analytical sample, m.p. 133-134°.

Anal. calc. for $C_{19}H_{18}O_3N_2$: C, 70.79; H, 5.63; N, 8.69.
Found: C, 70.81; H, 5.69; N, 8.73.

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PART III: METAL ION COMPLEXES OF AMIDOXIMES; THE INHIBITION OF THE ALPHA-CHYMOTRYPSIN CATALYZED HYDROLYSIS OF NICOTINYL-L-TYROSINE HYDRAZIDE BY L-α-ACETAMIDO-β-PHENYLPROPIONAMIDOXIME

A. METAL ION COMPLEXES OF AMIDOXIMES

1. Qualitative Observations. --The well known ferric hydroxamate test for esters is based on the fact that hydroxamic acids give a reddish purple color with ferric chloride in the presence of acid. In these laboratories this property has been utilized to measure hydroxamic acid concentrations quantitatively by a colorimetric method.

It has long been known that the amidoximes also give colored complexes with ferric chloride under certain conditions (1). In 1952 this property was made the basis of a qualitative test for nitriles. The nitrile is boiled for one minute with a hydroxylamine-hydrochloride mixture in propylene glycol, and ferric chloride is added to the solution, giving the reddish-purple amidoxime complex (2). Accordingly it was hoped that the ferric complexes of α-acylamino amidoximes could be used for their quantitative determination in the present investigation.

The first amidoxime obtained in the present investigation was α-phthalimido-β-phenylpropionamidoxime, described in Part I of this thesis. When an aqueous alcohol solution of this amidoxime was tested with ferric chloride, no color was produced. Since it was thought at this time that the amidoximes might be rapidly hydrolyzed by water the test was repeated in anhydrous alcohol solution, using ferric chloride in anhydrous alcohol for the test. A deep blue color was immediately produced under these conditions. Addition of water caused the color to fade within a few seconds. Later experiments showed that this behavior is not due to hydrolysis of the amidoxime as originally suspected, since the aqueous solutions of the amidoxime

could be evaporated to dryness to give a residue which gave the deep blue ferric chloride test when dissolved in absolute alcohol. It is now thought that the ferric complex is a very weak one, and that water, but not alcohol, can compete successfully with the amidoxime in coordinating with ferric ions. It was eventually found that α-benzamido- and α-acetamido-β-phenylpropionamidoxime gave ferric complexes in absolute alcohol which were destroyed by water, although the sensitivity to water did not appear to be so great.

From the experiments described above it can be seen that there was little promise of developing an analytical method for the amidoximes at hand based on ferric complexes. It may also be concluded that the above mentioned qualitative test for nitriles would fail for α-acetamido-β-phenylpropionitrile and for the α-benzamido derivative. Strangely enough the sole example of an acylamino amidoxime reported in the literature, benzamidoacetamidoxime, is reported to give a ferric chloride test (3). The test may have been carried out in alcohol solution, however.

It may be mentioned that ferric complexes of amidoximes are decolorized by strong acids, unlike the complexes of hydroxamic acids. This may be due to protonation of the amidoximes, which are weak bases. In the present study, α-phthalimido-β-phenylpropionamidoxime in fifty per cent ethanol was titrated with hydrochloric acid, giving an apparent pK_A of about 3.2. As would be expected, titration of α-amino-β-phenylpropionamidoxime gave a pH curve corresponding to protonation of only the α-amino group, whose apparent pK_A was 7.5.

Amidoximes are known to form complexes with basic cupric solutions, including Fehlings solution (1). These complexes sometimes separate as precipitates of so-called basic copper salts. It appears to be this behavior which has led the amidoximes to be considered acidic compounds. In the present case α-acetamido-β-phenylpropionamidoxime was found to form a deep yellow or yellow-green color with Fehling's solution. This behavior was made the basis of an analytical method, as is described below.

2. Quantitative Observations. -- In this investigation early observations of the spectra of the copper complex under various conditions were made on the complex of α-benzamido-β-phenylpropionamidoxime in aqueous alcohol solution. It is believed that these observations apply in full to the α-acetamido complex in aqueous solution.

The visible spectrum of a solution of the complex in the presence of both excess Fehling's solution and α-chymotrypsin showed a shoulder at about 375 millimicrons which was due to the complex. A blank containing Fehling's solution and enzyme showed absorption in this region which increased at shorter wavelength, and which accounted for the spectrum of the complex appearing as a shoulder rather than as a peak. Accordingly the spectrum of the complex was run using the enzyme-Fehling's solution blank instead of the water blank used previously. This time a maximum was observed at 375-380 millimicrons, the color due to cupric ion and enzyme having been subtracted from the spectrum.

Analyses were run by adding 2 ml. of Fehling's solution to a

mixture and diluting to 10 ml. The Fehling's solution was prepared by mixing equal parts of Fehling's solution A and Fehling's solution B. In the present case Fehling's A contained 14.66 g. of $CuSO_4 \cdot 5H_2O$ per liter; Fehling's B contained 69 g. of Rochelle salt and 15 g. of sodium hydroxide per liter. This is much less than the usual amount of sodium hydroxide employed in Fehling's solution.

At this point the more soluble α-acetamido-β-phenylpropionamidoxime was prepared in order to study the cupric complex. The Fehling's complex gave a peak at 375 millimicrons similar to the one obtained with the benzamido derivative, when a colored blank was used as described above, and the optical density changed only slowly with time (2 to 4% in 40 minutes). The optical density was approximately linear with concentration except at low concentration. The optical density reading was 0.06 at 10^{-4} mole/liter and 1.26 at 10^{-3} liter, showing the usable range of amidoxime concentration.

The experiments which have been described were done in the absence of any buffer in the solutions containing the cupric complex of the amidoxime. The buffering agent now commonly employed in kinetic studies in these laboratories is tris-hydroxymethylaminomethane in conjunction with its hydrochloride. However, the presence of this compound was found to prevent formation of the cupric complex of α-acetamido-β-phenylpropionamidoxime, undoubtedly because the tris-hydroxymethylaminomethane is itself an effective complexing agent which combines preferentially with the copper. This would be a serious difficulty in using the amidoxime complex to follow the rate of enzymatic hydrolysis, but it was still possible to carry out a

qualitative test for enzymatic hydrolysis of the amidoxime.

This was accomplished by adding two ml. of 0.005 molar aqueous amidoxime solution and one ml. of enzyme solution (3 mg. per ml.) to a five ml. beaker and adjusting to pH8. The resulting solution was quantitatively transferred to a 10 ml. volumetric flask and allowed to stand for more than an hour. Fehling's reagent was then added, the solution was made up to 10 ml., and the concentration of amidoxime was measured as described above. The optical density was found to be identical to that of two control solutions, both of which had been made basic with Fehling's solution before adding the enzyme.* It was estimated that less than one per cent hydrolysis could have occurred. The lack of hydrolysis is also indicated by the fact that the solution of complex remained clear and gave no precipitate. If the solution had contained an appreciable amount of hydroxylamine from hydrolysis of the amidoxime, a positive Fehling's test consisting of a yellow or orange precipitate of cuprous oxide would have been formed. The fact that hydroxylamine gives a Fehling's test was accidentally discovered during the development of the analytical method for the amidoxime, and it would undoubtedly complicate the study of amidoxime hydrolysis. A literature search has subsequently revealed that in 1884 Fehling's reagent was used to test for hydroxylamine formed by hydrolysis of acetamidoxime (1).

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*The same result was obtained in later experiments conducted at pH values of 5, 6, 7, and 9.

B. DETERMINATION OF THE ENZYME-INHIBITOR DISSOCIATION CONSTANT FOR ALPHA-CHYMOTRYPSIN AND L-α-ACETAMIDO-β-PHENYLPROPIONAMIDOXIME

1. Introduction

The studies reported above showed that L-α-acetamido-β-phenylpropionamidoxime was not hydrolyzed to any measurable extent by α-chymotrypsin at pH values ranging from 5 to 9. Accordingly it was decided to determine the enzyme-inhibitor dissociation constant L-α-acetamido-β-phenylprioionamidoxime and α-chymotrypsin by employing the amidoxime as an inhibitor in the α-chymotrypsin catalyzed hydrolysis of some convenient substrate. Comparison of the inhibitor constant with the inhibitor constants of other acetyl-L-phenylalanine derivatives might reveal whether the carbonyl oxygen atom corresponding to the carboxyl group in phenylalanine plays a part in binding those molecules having such a carbonyl group to the active site of α-chymotrypsin.

In the present study nicotinyl-L-tyrosine hydrazide was chosen as the substrate*. The α-chymotrypsin catalyzed hydrolysis of this compound has been shown (4) to follow the usual Michaelis-Menten equation. In the presence of added competitive inhibitor, and at low per cent hydrolysis where inhibition by the hydrolysis products is negligible, the equation is:

$$\frac{d\ S}{dt} = \frac{-k_3\ E\ S}{S + K_s\ 1 + \frac{I}{K_i}} \quad ; \quad \text{where (1)}$$

S = substrate concentration

---

*Donated by Richard Kerr.

$[E]$ = enzyme concentration

$K_s = \frac{k_2 + k_3}{k_1}$ for the reaction

$$E + S \underset{k_2}{\overset{k_i}{\rightleftharpoons}} ES \overset{k_3}{\longrightarrow} E + P_1 + P_2$$

$K_i = \frac{k_2}{k_1}$ for the reaction

$$E + I \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EI$$

The differential equation (1) gives the steady state reaction velocity, i.e., the velocity which is asymptotically approached if $[E]$ and $[S]$ are maintained at a constant value. For the present enzyme-substrate system such a steady state velocity is attained rapidly compared with the time required to hydrolyze an appreciable amount of substrate, so that instantaneous values of substrate concentration can be used in equation (1).

It is common practice to use the reciprocal of the above equation, and to consider the values of $d[S]/dt$ and of $[S]$ at the beginning of the hydrolysis, designated as v_o and $[S]_o$. Then:

$$\frac{1}{v_o} = \frac{1 + \frac{I}{k_i} K_s}{k_3 [E]} \frac{1}{[S]_o} + \frac{1}{k_3 [E]} \qquad (2)$$

$$\frac{[S]_o}{v_o} = \frac{1 + \frac{I}{k_i} K_s}{k_3 [E]} + \frac{1}{k_3 [E]} [S]_o \qquad (3)$$

The constants are readily evaluated for the equations in this form. From equation (3) it can be seen that a plot of $[S]_o / v_o$ versus $[S]_o$ gives a line with a slope of $1/k_3 [E]$ and an intercept of $(1+I/k_i)K_s/k_3[E]$.

The data obtained in the present investigation is shown plotted in this form in Figure I.

2. Experimental

In this study the rate was followed by measuring the appearance of hydrazine formed by hydrolysis of nicotinyl-L-tyrosine hydrazide. This was accomplished by use of an analytical method developed in these laboratories (4), employing a recent improvement of the method (5). Specifically, a reagent was prepared by mixing a solution of p-dimethylaminobenzaldehyde in ethanol (4 g. per 100 ml.) with an equal volume of hydrochloric acid (140 ml. of 36.7% hydrochloric acid, s. g. 1.186, made up to 1 liter). For carrying out an analysis a measured volume of the reagent was pipetted into a volumetric flask of five times the volume (e.g., 2 ml. in a 10 ml. flask), and water was added to dilute the solution, leaving room to add a one ml. sample of the solution to be analyzed. The solution to be analyzed was then introduced, the flask water was added to bring the mixture to the desired volume, the flask was placed in a constant temperature bath at 25° for 20-25 minutes, and the optical density of a sample was measured at 455 millimicrons by means of a Beckman Model B spectrophotometer, using one centimeter cells. The cell holder of the spectrophotometer was maintained at 25° by means of water circulated from a constant temperature bath.

Enzymatic hydrolyses were run at 25° in solutions which contained 0.02 moles per liter of buffer (tris-hydroxymethylaminomethane in conjunction with its hydrochloride, adjusted to pH 7.93) and one milligram per ml. of α-chymotrypsin (Armour lot 00592).

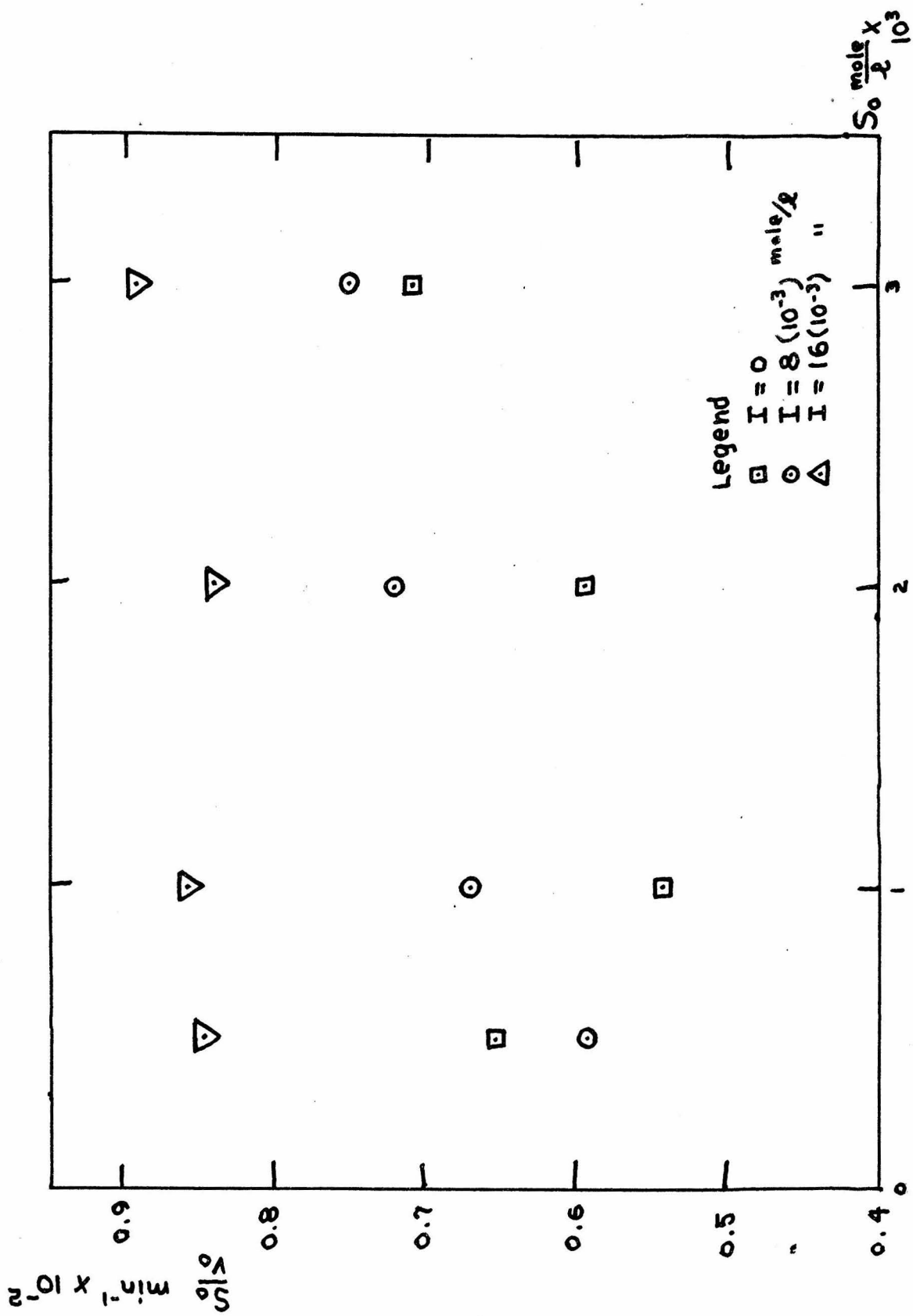

Figure 1. Inhibition of the enzymatic hydrolysis of nicotinyl-L-tyrosine hydrazide by L-α-acetamido-β-phenylpropionamidoxime plotted according to equation (3).

In this study the one ml. samples which were withdrawn from the hydrolysis mixture for analysis were diluted either to 10 ml. or to 25 ml. depending on the expected final concentration of hydrazine in the last sample. In no case was the optical density of the final solution allowed to exceed 1.5, since the optical density is not a linear function of hydrazine concentration above this optical density.

In the analytical method employed, there is a certain amount of color in a blank containing no hydrazine which must be subtracted from the observed optical density readings. Failure to subtract a suitable blank causes the first observed optical density to be abnormally large. It was found that in most cases the necessary blank correction can be achieved in the spectrophotometer by using a reference solution containing both enzyme and substrate added separately to the acidic p-dimethylaminobenzaldehyde solution. In earlier runs this was not done, and the alternate procedure of estimating the optical density reading at zero time by extrapolating a graph of optical density differences to zero time was employed.

The initial velocity in each run was estimated by a method, whose application to enzyme kinetics has been recently developed in these laboratories (6), in which a polynomial is found which approximates the optical density readings as a function of time. The initial velocity is taken to be the slope of the polynomial at zero time.

3. Discussion

The most obvious feature of the $[S]_o / v_o$ versus $[S]_o$ plot is that the points at an initial substrate concentration of 0.5 (10^{-3}) are seriously out of line, in that the initial velocity in the presence of

$8(10^{-3})$ moles / liter of inhibitor appears to be greater than the initial velocity in the absence of inhibitor. However examination of the original data shows that the per cent hydrolysis at the end of 16 minutes was 12.2% in the absence of inhibitor and 9.92% in the presence of $8(10^{-3})$ mole/l of inhibitor. Apparently the initial velocities obtained from the experimental optical densities do not reliably indicate the true initial velocities in this case, because if the inhibitor decreased the overall per cent hydrolysis, it must have slowed the velocity of hydrolysis at all stages. It may be noted that this argument is essentially one which introduces considerations of mechanism, although no particular mechanism of inhibition is postulated. Therefore, because the calculated initial velocities for $[S]_o = 0.5\ (10^{-3})$ mole/liter indicate acceleration of hydrolysis by added amidoxime when actually examination of the per cent hydrolysis leaves no doubt that the expected inhibition occurred, the points for $[S]_o = 0.5\ (10^{-3})$ mole/liter were rejected in calculating the value of K_2.

A straight line was then fitted to the remaining three points at each inhibitor concentration, and values of the constant term in equation (3), designated as b, were calculated. The desired inhibitor constant is calculated from the ratio of any two values of b. Thus:

$$\frac{b_{16}}{b_8} = \frac{1 + \frac{16(10^{-3})}{K_2}}{1 + \frac{8(10^{-3})}{K_2}} = \frac{8410}{6572} ;$$

From $b_{/6}/b_8$, $K_2 = 20.61\ (10^{-3})$.

Using the other possible ratios:

$$\text{From } \frac{b_8}{b_0}, \; K_i = 23.32\,(10^{-3})$$

$$\text{From } \frac{b_{16}}{b_0}, \; K_i = 22.28\,(10^{-3})$$

$$\text{Average } K_i = 22.1\,(10^{-3})$$

This value may be compared with the value of 31 ± 3 for K_s of acetyl-L-phenylalaninamide (4), which probably is an equilibrium constant comparable to K_i. No definite conclusion concerning the effect of the amidoxime group on K_i can be reached until the inhibitor constants of other acetyl-L-phenylalanine derivatives (such as the N-methylamide) are available. The above data indicates that the amidoxime is more strongly bound than the amide to the enzyme, suggesting that the amidoxime group does contribute to the binding. There is no indication that the failure of the amidoxime to undergo enzymatic hydrolysis is due to lack of binding to the enzyme.

It may be mentioned that a value of K_s may be obtained from the straight line fitted to the points in Figure 1 corresponding to zero inhibitor concentration:

$$= \frac{48.95}{6.34(10^{-3})} = 7.7\,(10^{-3})$$

$$K_s = \frac{b}{a} = \frac{48.95}{6.34(10^{-3})} = 7.7\,(10^{-3})$$

This is in agreement with the previously determined value, $9\,(10^{-3})$, a fact which tends to justify the procedure used in calculating the K_i values.

4. Tables of data.--The following tables give optical density readings observed in each run, plus other pertinent data. In cases where the first optical density is not zero, the first value is the value obtained by extrapolation of the optical density differences to zero time. Time intervals between each optical density reading are given in minutes. Dilution of 1/10 indicates that 1 ml. of the hydrolysis mixture was diluted to 10 ml. during the analysis. $[S]_o$ and $[I]$ are given in moles/liter times 10^3. v_o is given in moles/liter minute times 10^5. P is the power of the polynomial used to obtain v_o.

Run number 3
Time interval: 2
Dilution: 1/10
$S_o = 0.5$; $I = 0$
$v_o = 0.764$; P = 1

0.042
0.135
0.224
0.325
0.414
0.503
0.581
0.695
0.772

Run number 2
Time interval: 2
Dilution: 1/10
$S_o = 1$; $I = 0$
$v_o = 0.845$; P = 2

0.016
0.235
0.431
0.655
0.831
1.015
1.180
1.335
1.515

Run number 6
Time interval: 2
Dilution: 1/10
$S_o = 2$; $I = 0$
$v_o = 3.354$; P = 2

0.014
0.144
0.300
0.450
0.587
0.725
0.851
0.990
1.107

Run number 4
Time interval: 1.5
Dilution: 1/25
$S_o = 3$; $I = 0$
$v_o = 4.223$; P = 1

-0.044
0.119
0.280
0.438
0.585
0.748
0.895
1.022
1.169

Run number 10
Time interval: 2
Dilution: 1/10
S_o = 0.5
I^o = 8
v_o = 0.896
P = 2

0.000
0.041
0.186
0.300
0.361
0.392
0.480
0.520
0.594

Run number 9
Time interval: 1.5
Dilution: 1/10
S_o = 1
I^o = 8
v_o = 1.490
P = 2

-0.013
0.121
0.245
0.380
0.495
0.585
0.718
0.819
0.920

Run number 7
Time interval: 2
Dilution: 1/25
S_o = 2
I^o = 8
v_o = 2.771
P = 2

-0.012
0.115
0.248
0.372
0.488
0.610
0.722
0.822
0.942

Run number 8
Time interval: 1.5
Dilution: 1/25
S_o = 3
I^o = 8
v_o = 3.984
P = 2

-0.012
0.133
0.274
0.401
0.534
0.664
0.779
0.912
1.022

Run number 16
Time interval: 3
Dilution: 1/10
S_o = 0.5
I^o = 16
v_o = 0.626
P = 2

0.000
0.108
0.210
0.313
0.402
0.500
0.592
0.658
0.736

Run number 11
Time interval: 2
Dilution: 1/10
S_o = 1
I^o = 16
v_o = 1.159
P = 2

0.007
0.145
0.278
0.414
0.538
0.670
0.780
0.896
1.027

Run number 12
Time interval: 2
Dilution: 1/25
S_o = 2
I^o = 16
v_o = 2.376
P = 2

0.000
0.108
0.222
0.325
0.428
0.534
0.623
0.730
0.810

Run number 15
Time interval: 2
Dilution: 1/25
S_o = 3
I^o = 16
v_o = 3.358
P = 2

0.000
0.158
0.313
0.465
0.609
0.749
0.888
1.030
1.149

Optical density is proportional to hydrazine concentration in the concentration range employed, with the proportionality constant equal to 1.6707 (10^{-5}) mole/liter per optical density unit.

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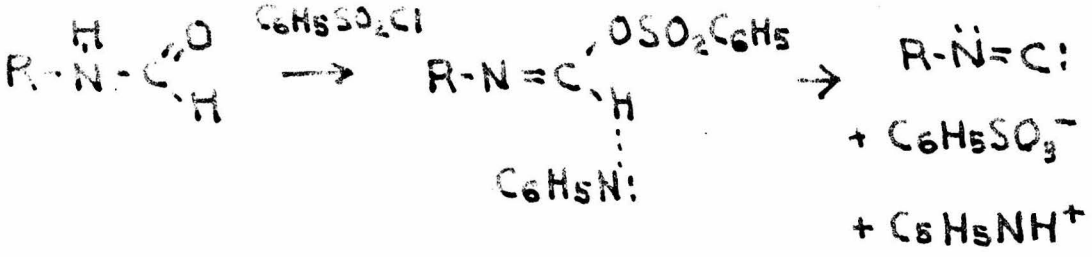
PART IV. PROPOSITIONS

1. Recent attempts to establish a quantitative measure of nucleophilic power have revealed that some molecules are very highly nucleophilic compared to many of the most common nucleophiles (1, 2). It is proposed that the property common to the very nucleophilic molecules is that they are negatively charged and contain several unshared pairs of electrons which occupy a large volume in space. This factor has heretofore been associated with polarizability of loosely held outer orbitals in strong nucleophiles like R-S:$^-$ and I$^-$. However, the present generalization allows inclusion of O-O-H$^-$ and :C N:$^-$ which contain only 2p electrons in the outer shell. It is further proposed that H-N-O:$^-$ will be found to be the strongest of all nucleophiles composed of first and second row elements in the periodic table.

2. Because of their unique electronic structure involving unfilled orbitals the isonitriles should be of unusual interest to organic chemists. The study of these compounds is hindered by the fact that the only common method of preparation gives low yields. It is suggested that the following reaction might provide a new method of preparation for isonitriles:



The idea is based on a recently proposed mechanism for the reaction of benzenesulfonyl chloride with unsubstituted amides (3).

3. It is proposed that Alexander's explanation of an optimum pH for formation of semicarbazones is wrong, and that no optimum pH is predicted from the mechanism of the reaction given by Alexander (4). That this is true can be seen from the fact that at low pH, assuming Alexander's mechanism, the reaction rate should be proportional to

$$\frac{(H_3O^+)\,(\text{aldehyde})}{K_A \text{ of aldehyde}} \qquad \frac{K_A \text{ of semicarbazide}\ (\text{semicarbazide})}{(H_3O^+)}$$

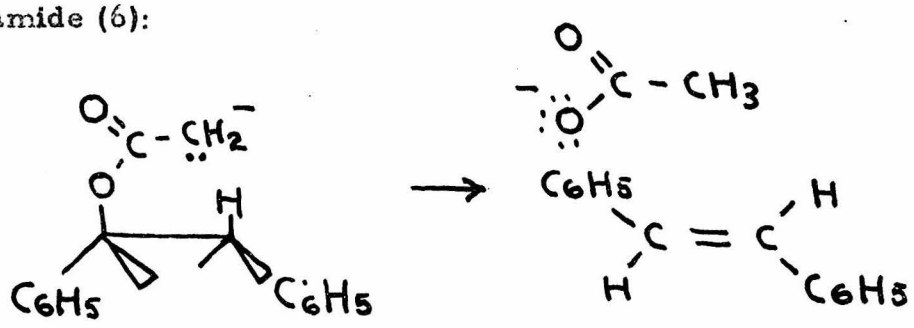
and hence would be independent of hydrogen ion concentration. It is proposed that Conant and Bartlett's suggestion that the reaction involves general acid catalysis (5) does lead to a kinetic expression which predicts a decrease in rate at low pH in the presence of buffer which is proportional to the buffer concentration.

4. It is proposed that the kinetics of hydroxamic acid formation and of amidoxime formation be studied at various pH values. Because of the availability of colorimetric analytical methods, such a study could be made with unusual ease, and the results would be of broad interest in view of the need to test and define the scope of the quantitative theories of nucleophilic reactions discussed in this thesis (1, 2). Specific reactions can be suggested which it would be of interest to study.

5. Apparently no example of an optically active secondary alkyl halide of known optical purity has been prepared, in view of the fact that all preparations have proceeded from the active alcohol and may have involved partial racemization. It is proposed that pure active 2-butyl chloride can be prepared by crystallization of a solid ester of the following active acid to constant rotation, followed by hydrolysis and decarboxylation:

$$CH_3CHClCH_2CH_2CO_2H$$

6. Curtin and Kellom have given the following mechanism for the cis-elimination of acetate in the presence of potassium amide (6):

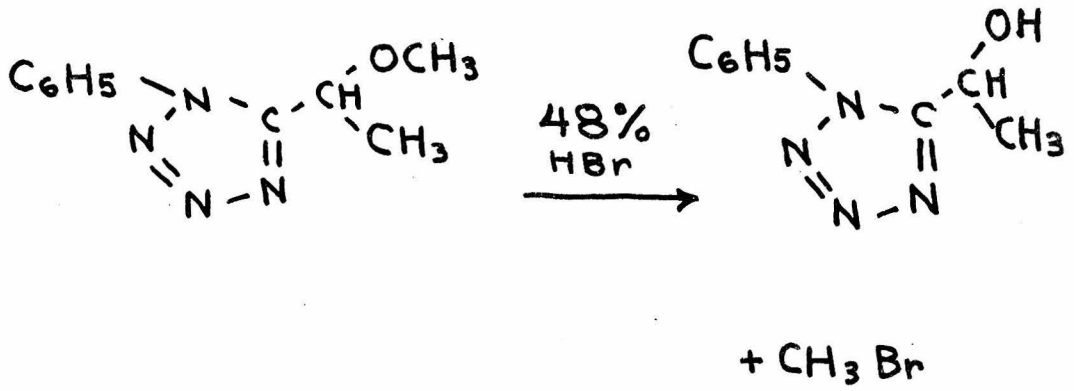


It is proposed the corresponding α-haloacetate would undergo an analogous elimination reaction when allowed to react with a metal such as magnesium or zinc. This would be a non-thermal cis-elimination occurring under "mild" conditions.

7. Fuson's generalization concerning the structure necessary for carbon monoxide elimination from keto acids (7) is helpful but fails to account for the carbon monoxide elimination from $CH_3COCO_2C_2H_5$. Since enolization has often been postulated as

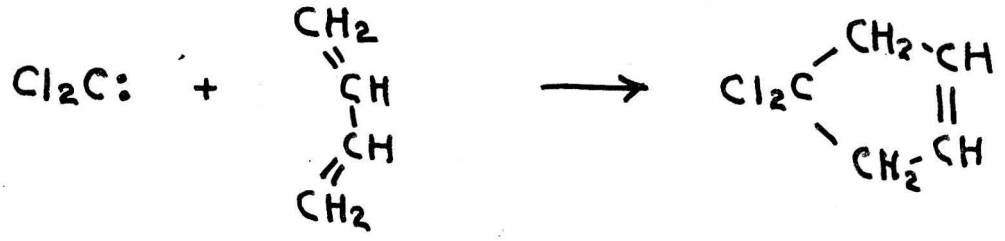
a step in carbon monoxide elimination reactions, it is suggested that the following keto esters be tested for carbon monoxide elimination in order to see whether enolization plays a part: $CF_3COCO_2C_2H_5$, $CF_3COCF_2COCO_2C_2H_5$, $(CH_3)_3CCOCO_2C_2H_5$. Methods of synthesis for these compounds can be suggested.

8. The following reaction was reported in the Journal of Organic Chemistry in 1954 (8):



The authors make the comment, "That this ether is split in this way is both interesting and significant, in that it shows that the methyl carbonium ion is more readily formed than the tetrazole cation". Actually the mechanism of this reaction no doubt involves S_N2 attack of the protonated ether by bromide ion or hydrogen bromide, which is expected to occur at the primary carbon, as observed.

9. Divalent carbon radicals have been proposed as intermediates in the formation of cyclopropanes from chloroform, hydroxide , and alkenes (9). It is proposed that if a diene is used instead of an alkene, an unusual type of Diels-Alder reaction could occur:



It is further proposed that an attempt be made to add isonitriles to alkenes, in view of the fact that these compounds represent stable divalent carbon radicals.

10. The Beckman rearrangement of aryl ketones may be regarded as a cyclic electrophilic substitution reaction of the aromatic ring, and is usually a concerted reaction, as shown by its stereospecificity. It is proposed that by deactivating the normal migrating aromatic group toward electrophilic substitution and activating the other aromatic group, an abnormal migration may be obtained. This would indicate that formation of $R_2C{=}N^+$ occurs as fast as the participation reaction.

11. One of the objections to the use of turbine engines in automobiles is that the motor does not serve as an effective brake on downgrades, as does a reciprocating engine. It is instructive to consider the cause of the braking effect in the latter case. Since each piston in an automobile is constrained to move back and forth in a straight line, it is obvious that the kinetic energy of a rapidly moving piston has been dissipated by the time it reaches the end of its stroke. Additional braking effect comes when energy must then be supplied to get the piston moving again. Since all this energy must

go somewhere, it is proposed that it passes off in the form of neutrinos, which have of course not been noticed because of the difficulty of detecting them.

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