THE SYNTHESIS OF PEPTIDES

By

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INTRODUCTION

Investigators in many fields of scientific research have been probing the protein molecule as it occurs in toxins, enzymes, hormones, antibiotics and many types of virus. The study and synthesis of relatively simple peptides has contributed enormously to a better understanding of the protein nature of many of these naturally occurring, biologically active principles.

Interest in practical methods for the synthesis of peptides is increased by the discovery of the peptide nature of gramicidin (1), tyrocidin (2), and diplococcin (3), important substances with antibiotic properties. The structure of Gramicidin S has been thoroughly investigated by Synge and co-workers (4) who have shown it to be a cyclic polypeptide, possessing a sequence of amino acids as follows:

L-valyl-L-ornithyl-L-leucyl-D-phenylalanyl-L-prolyl-. Harris and Work (5) have attempted to prepare synthetic pentapeptides related to Gramicidin S. They found that L-leucyl-L-phenylalanyl-L proline inhibited the growth of S. aureus and S. hemolyticus at dilutions of 1:1000. They observed no significant difference in the antibacterial activity of the "natural" and "unnatural" isomers. In this connection, it may be of some interest in the study of the mechanism of action of the sulfonamides, that a peptide which contains p-aminobenzoic acid has been found in yeast (6).

Harrington (7) has shown that thyroxine can be isolated from the thyroid gland by hydrolysis with barium hydroxide, and the amino acid is obtained in racemic form. The protein thyroglobulin, to which thyroxine is attached through a peptide linkage, yields, L-thyroxine on hydrolysis with the aid of pepsin and trypsin (8). According to the present conception diiodotyrosine and thyroxine are linked together to form a peptide which in combination with other amino acids, forms thyroglobulin. When thyroglobulin undergoes hydrolysis, the intact peptide is released into the blood stream (9).

Studies have been made of the <u>in vivo</u> synthesis of peptides. Borsook and Huffman (10) have presented very interesting data based on thermodynamic considerations. They considered the formation of DL-leucylglycine from DL-leucine and glycine. At the pH of the tissues all the reactants are in the neutral form, and their ionization constants can be ignored. Using the relationship, $\Delta F^{\circ} = -RT\ln K$, where ΔF is the free energy of formation and K is the equilibrium constant they determined the equilibrium constant from the values of ΔF° to be 298.1. It is assumed that ΔF in solution is not likely to be significantly different from that of the solids. The reaction is given as:

glycine (solid) DL-leucine (solid) → DL-leucylglycine + H_2O (liq)

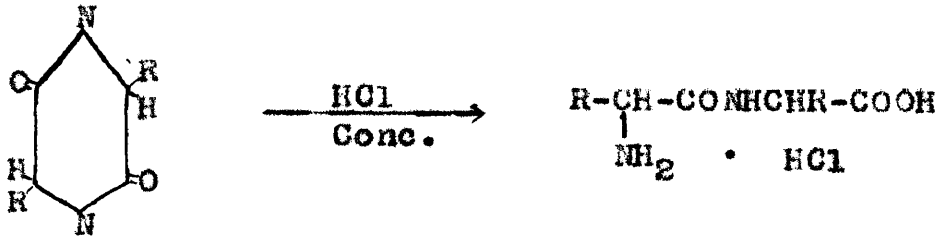
F_2 = (-114,420)-(-56,720) - (-89,020)-(99,340)=7520 cals.

From which K, the equilibrium constant, is $10^{-5.5}$. The equilibrium of the reaction lies so far on the side of hydrolysis, that it is practically certain that the synthesis of the peptide bond from amino acids of this type *in vivo* can take place only when it is associated with another energy-yielding reaction. The work of Cohen and McGilvery (11,12), seems to substantiate these thermodynamic considerations. These workers showed that rat liver slices or homogenates can synthesize p-aminohippuric acid from p-amino-benzoic acid and glycine. The reaction does not proceed anaerobically nor in the presence of oxidation inhibitors. The energy for the formation of the peptide bond is derived from oxidative processes and probably proceeds through a high energy phosphorylated intermediate. Friedberg (13), using labeled isotopes, emphasized the activity of the intestinal wall in peptide synthesis.

Excellent reviews of the early work on the chemistry of the peptides can be found in the collected papers of Fischer (14), and of Abderhalden (15). Bergmann has presented a review of the field up to the year 1923 (16) and Greenstein has summarized the literature up to 1945 (17).

The first claim for the synthesis of a peptide was made by Curtius (18), who prepared benzoylglycylglycine but was unable to debenzoylate his product to obtain the desired glycyglycine. The first successful synthesis of a peptide was accomplished by Fischer in 1901 (19). The synthesis involved the partial hydrolysis of 2,5-diketo-

piperazine with fuming HCl, yielding glycylglycine -HCl. The free racemic peptide was isolated by preparing the silver salt and decomposing it with hydrogen sulfide.



Greenstein (20) in like manner prepared L-cystinyl-L-cystine. This method of synthesis is limited to the preparation of symmetrical dipeptides. If an unsymmetrical anhydride is used, a mixture of two dipeptides is produced and great difficulty is encountered in the separation of the product. To overcome these disadvantages Fischer (21) resorted to the α-haloacyl derivative of an amino acid, e.g.

$$R\text{-}CHX\text{-}CONHCHR'CO_2H \xrightarrow{NH_3} \underset{\displaystyle NH_2}{\overset{\displaystyle |}{RCH}}\text{-}CONHCHR'CO_2H$$

He thus prepared diglycylglycine; and by using this method in conjunction with others he obtained the octadecapeptide (12).

L-leucyl-triglycyl-L-leucyl-octaglycylglycine

By the same route Abderhalden and Fodor (23), prepared a nonadecapeptide.

$$RCHBr\,COCl + R'\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}CO_2H \longrightarrow RCHBr\,CONH\text{-}CHR'\text{-}CO_2H$$

$$\xrightarrow[\text{sealed tube}]{NH_3,\ 25\%} RCH(NH_2)\text{-}CONH\text{-}CHR\text{-}CO_2H$$

Bertho and Maier (24) devised an alternate method for replacing the α-halogen by the amino group. The halogenated peptide is treated with sodium azide, thus replacing the halogen by the azido group. Subsequent catalytic hydrogenation reduces the latter to the amino group.

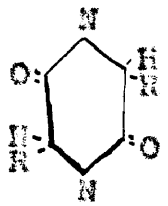
The α-haloacyl halide procedure involves many practical difficulties. It can be used only with mono-amino acids. In certain instances, treatment of the halogen-substituted amide with excess of ammonia results in hydrolysis, to the corresponding hydroxyl-amide (25).

Peptides such as phenylalanylglycine can be prepared only in poor yields by this method because of dehydrohalogenation by the excess of ammonia to form a cinnamide (26).

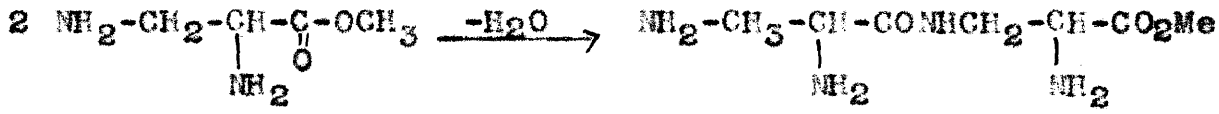
$$C_6H_5\text{-}CH_2\text{-}CHBr\text{-}\underset{\underset{O}{\|}}{C}\text{-}NHCH_2CO_2H \xrightarrow[-HBr]{NH_3} C_6H_5\text{-}CH{=}CH\text{-}\underset{\underset{O}{\|}}{C}\text{-}NHCO_2H$$

Esters of α-amino-mono-carboxylic acids and peptides are converted by heat to diketopiperazines.

$$2R\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}\underset{\underset{O}{\|}}{C}\text{-}OCH_3 \xrightarrow[-H_2O]{\Delta}$$

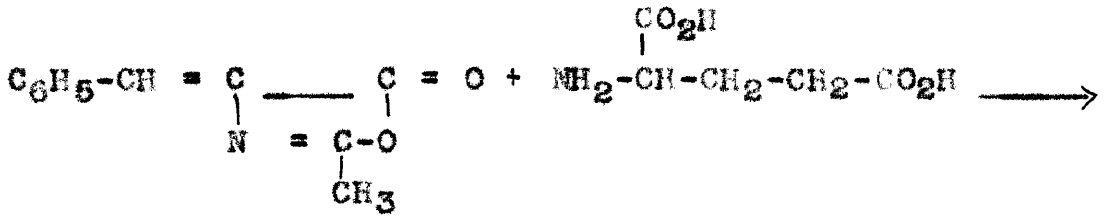


If, however, there is present a β-amino or ϵ-amino group, a dipeptide ester results (27).



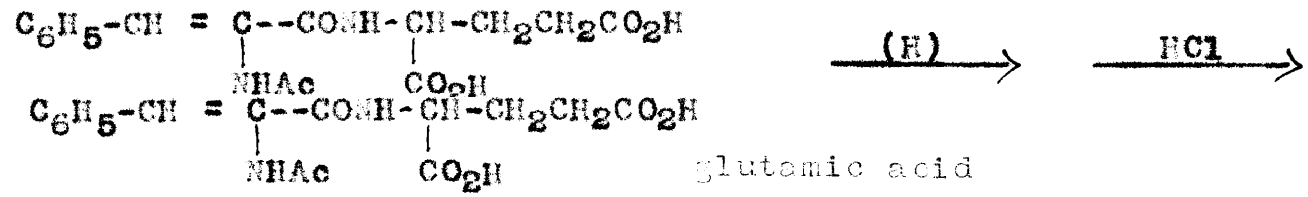
This method was successfully used by Fischer and Abderhalden in their synthesis of large, long-chained polypeptides.

The azlactone synthesis of peptides was introduced by Bergmann and co-workers (28). This method is based on the Erlenmeyer synthesis of α-amino acids (29). An aromatic aldehyde is condensed with an N-acylated glycine in the presence of a dehydrating agent to form an azlactone (oxazolone). It was found that the azlactones, although stable toward cold water, react vigorously with amines. Catalytic hydrogenation and removal of the N-acyl radical yields the desired dipeptide:

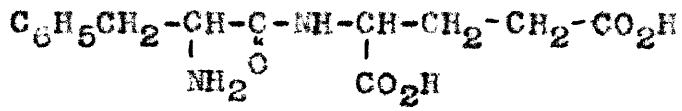


Azlactone of N-acetamino-cinnamic acid

L-Glutamic acid

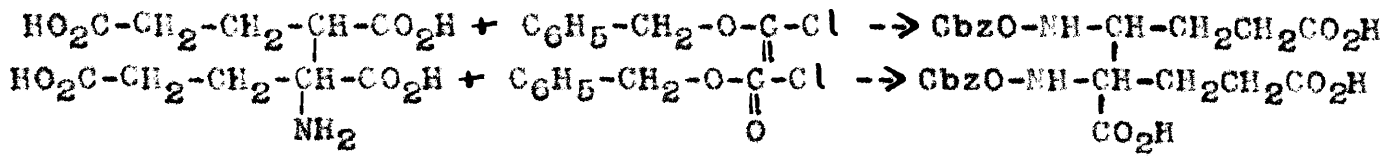


glutamic acid



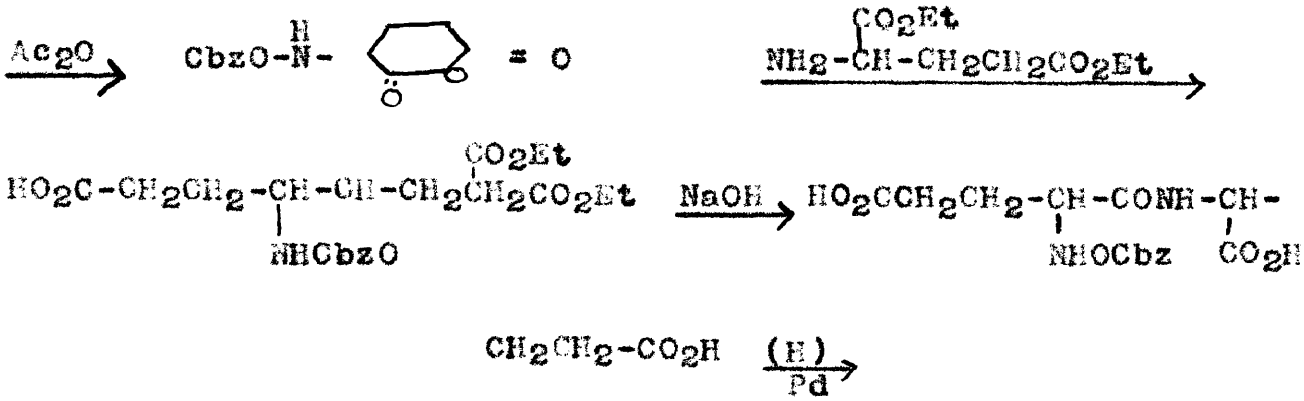
L-phenylalanyl-L-glutamic acid
D-phenylalanyl-L-glutamic acid

The azalactone procedure made possible the synthesis of peptides containing complex acids which could not be obtained by the earlier methods of Fischer. Unfortunately, during the hydrolysis of the acetyl group other parts of the peptide molecule are also attacked. A more serious objection is the formation of the DL-peptide mixture. A good method for the synthesis of pure optically active peptides was found in the "Carbobenzyloxy" Synthesis (30). By this method Bergmann was able to prepare any peptide containing simple or complex amino acids. The masking of the amino group is effected by a Schotten-Baumann technique with $C_6H_5CH_2-O-\underset{\underset{O}{\|}}{C}-Cl$, the benzyl ester of chloroformic acid. The removal of the masking group is accomplished by catalytic hydrogenolysis, producing toluene, carbon dioxide and the free amino group. This method affords access to pure optically active peptides that have been used as substrates for studies on the nature of enzyme action, and for investigations of physico-chemical properties of peptides containing more than one dissociated amino group and one dissociated carboxyl group (31).



CbzO-chloride

Glutamic Acid



Glutamylglutamic Acid

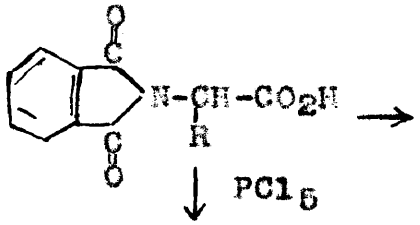
Among the workers who have successfully employed Bergmann's procedure are Dunn (102), Fruton (103), Greenstein (104), Harrington (32) du Vigneaud (33), and their co-workers. However, certain practical difficulties limit the applicability of Bergmann's method. For example, sulfur-containing peptides cannot be treated in the usual manner. Large excess of palladium catalyst must be employed in hydrogenolysis of the carbobenzyloxy group and the yields are poor.

The blocking group may alternatively be removed under more drastic conditions. In the preparation of gluthathione, glutamyl-cysteyl-glycine, Harrington and Mead (32) used phosphonium iodide and du Vigneaud and Miller (33) sodium and liquid ammonia to remove the masking group. Another disadvantage in the Bergmann method is the marked tendency for

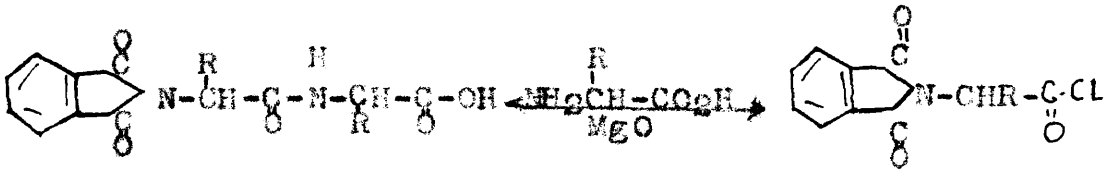
the carbobenzyloxyamino-acyl chlorides to decompose to the N-carboxy (Leuchs') anhydrides. Furthermore, the acyl chlorides are generally oils and the carbobenzyloxyamino acid derivatives cannot be obtained in crystalline form readily.

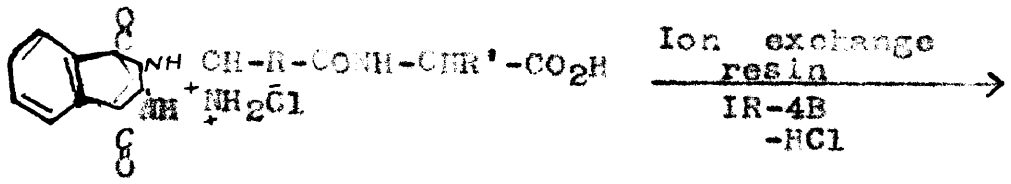
In place of the unstable chlorides, the corresponding acid azides have been employed. These are not isolated after preparation by the interaction of hydrazine and the ester followed by treatment of the hydrazide with nitrous acid. Immediate coupling with an amino acid is necessary to avoid losses through rearrangements.

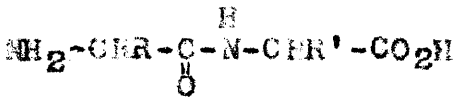
Another scheme for protecting the amino group has been recently described by two independent groups of workers (34, 35), in which the phthalyl group has been used as a masking group in the preparation of amines and amino acids. Ing and Manske (36) reported the cleavage of N-alkyl phthalimides with alcoholic hydrazine hydrate, a reaction first observed by Gabriel (37). The following equation illustrates the method (35):

$N_2HCHR\text{-}CO_2H$ + phthalic anhydride ⟶  → PCl_5

1) $N_2H_4 \cdot H_2O$

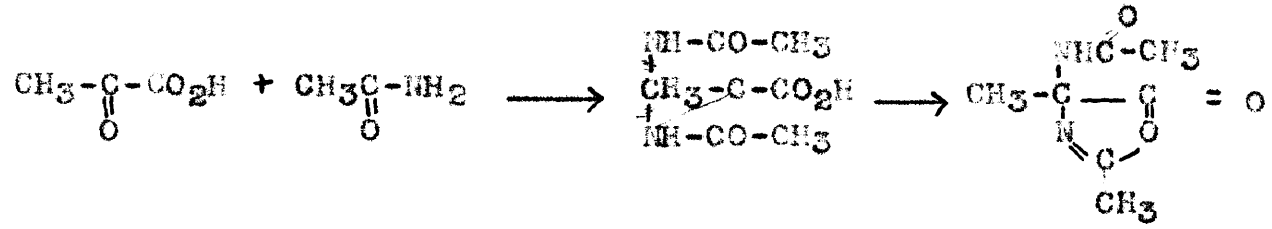
2) $\xrightarrow{HCl}$ 

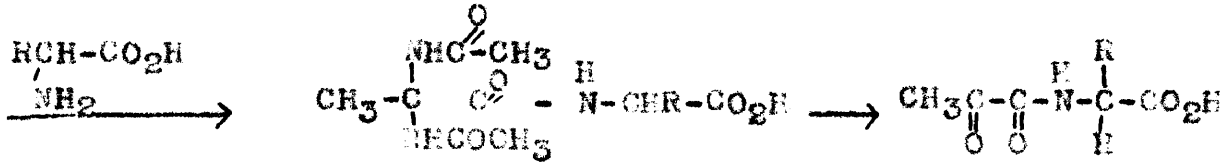


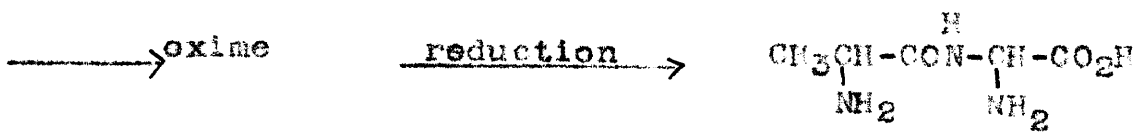


The advantages of the phthalyl method lie in the availability of phthalic anhydride and the ease of crystallization of the phthalyl derivatives. Although the carbobenzoxy amino acids are unstable, the phthalylamino acyl chlorides are stable, crystallizable solids obtainable in 80 per cent yield. An interesting use of ion exchange resins is reported by Sheehan and Frank (35) for conversion of the peptide hydrochloride to the free peptide. Extensive use of this method is not reported, and it remains to be seen how applicable it will become especially in preparing the higher peptides.

Shemin and Herbst (38) have devised a peptide synthesis involving α-keto acids:







By this method a number of peptides containing alanine have been prepared. The limitation of this method lies in the preparation of the pyruvyl intermediates. This synthesis may have biochemical significance since Herbst and Shemin (39) reported a biochemical synthesis of DL-alanyl-alanine from pyruvylalanine by transamination.

Polymerization reactions have also been reported in the preparation of polypeptides. Katchalski et. al. (40) reported the isolation of a polylysine obtained from α-carbobenzyloxy-L-lysine anhydride. This latter substance, if dried <u>in vacuo</u> at 105° melts and undergoes rapid polymerization, yielding a polycarbobenzyloxylysine, which is water soluble and hydrolyzed by trypsin. It may serve as a model in protein research.

A macromolecular polypeptide of the structure:

$$HO_2C\text{-}\underset{R}{\underset{|}{CH}}\text{-}\overset{R}{\overset{|}{N}}\left[\underset{O}{\overset{\|}{C}}\text{-}\underset{R}{\underset{|}{CH}}\text{-}\overset{H}{\overset{|}{N}}\right]_n\text{-}\underset{O}{\overset{\|}{C}}\text{-}CH(R_{n+2})NH_2$$

with large values of n has been synthesized by Woodward and Schramm (41). As yet, this synthesis has had no practical utility and probably will be of extremely limited use in preparing natural peptides.

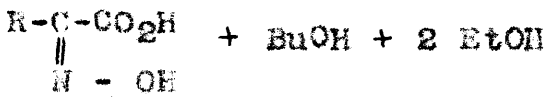
In all of the methods that have been described, except Fischer's and Herbst's, the free amino acid is required at the outset. A more desirable synthesis would involve the simultaneous formation of the peptide and the amino acid. The following is a very brief resume of the background of a

synthesis of the type which is the basis of this thesis.

The preparation of oximino acids was accomplished by the Bouvenault and Locquin (44) nitrosation of substituted acetocetic esters by Haulin (42):

This reaction is limited to those substituted aceto-acetic esters where R is an alkyl or an unsubstituted benzyl group.

A more generally applicable method for synthesizing oximino acids, useful in the preparation of compounds where R is a highly substituted aromatic nucleus, was reported by Barry (46). In this modified procedure, nitrosation of a substituted malonic or acetoacetic ester is accomplished by means of an alkyl nitrite and sodium ethoxide. The yields obtained are between 60 per cent to 75 per cent.



Barry was able to obtain higher yields when the alkyl nitrite and the substituted malonic acid interacted in the presence of hydrogen chloride. By this method Mattocks (47)

successfully prepared such complex oximino acids as β- (3,4-diethoxyphenyl) -α-oximinopropionic acid and its methylenedioxy analog.

The ease with which oximino acids are synthesized and converted into the corresponding amino acids by hydrogenation suggested the possibility that they could be used in the synthesis of peptides. Hamlin using Hartung's conditions for hydrogenation (79) (palladium chloride on charcoal in acidified ethyl alcohol) could obtain a number of amino acids in fairly good yields.

$$R\text{-}\underset{\underset{N\text{-}OH}{\|}}{C}\text{-}CO_2H \xrightarrow[Pd,\ HCl]{H_2} R\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}CO_2H$$

Mattocks used the same procedure in the preparation of amino acids with substituted aromatic nuclei.

It was observed by Bouveault and Locquin (44) that loss of CO_2 and H_2O and formation of nitriles result when α-oximino acids are heated with dilute mineral acids. Waters and Hartung (50) found that the oximino acids were resistant to alkaline hydrolysis. Bouveault and Locquin (49) succeeded in hydrolyzing these acids by a cumbersome procedure employing 85 per cent formic acid and lead chamber crystals. It was desirable at the time to obtain the keto acids which could be converted to acyl chlorides and coupled with amino acids. Subsequent oximination and reduction would yield a dipeptide. A procedure of this type was developed by She-

min and Herbst (38). All attempts to prepare the ideal intermediate R-C(=N-OH)-COCL were unsuccessful (50).

In 1938 Adkins and Reeve (51) developed a synthesis for threonine depending on the catalytic reduction of the O-ethyl ether of oximinoacetoacetic ester. This preparation indicated that it is possible to prepare O-ethers of the oximino acids from which the acid chlorides could be obtained as shown by Waters (50).

$$\text{R-C(=N-OH)-CO}_2\text{H} \longrightarrow \text{R-C(=N-OR')-CO}_2\text{H} \longrightarrow \text{R-C(=N-O-R')-COCl} \xrightarrow{\text{R''-CH(NH}_2\text{)-CO}_2\text{H}}$$

$$\longrightarrow \text{R-C(=N-OR')-CONH-CHR''-CO}_2\text{H} \longrightarrow \text{R-CH(NH}_2\text{)-CONH CHR''-CO}_2\text{H}$$

The lower alkyl oximino ethers have been synthesized by the treatment of the oxime with an alkyl iodide in the presence of sodium ethoxide. The first O-alkyl ether of a ketoxime was prepared by Trapesonzjanz (52). Meyer and Ceresole (53) in 1882 and Janny 1883 (54) prepared the O-benzyl other of acetone oxime. Previously Conrad and Bischoff (55) had prepared the benzyl ether of oximinomalonic ester. The evidence favoring the present day position regarding the structure of these O ethers is presented by Taylor and Baker (56).

Hantzsch (57) prepared a group of oximino ethers of oximinoacetic and propionic acids by heating the oximino acid with chloracetic acid with aqueous potassium hydroxide

for six to eight hours.

No thorough investigation of the catalytic dealkylation of oximino ethers has been reported. Jones and Major (58) using platinum oxide in aqueous ethanol containing hydrochloric acid, studied the reduction of the hydrochlorides of O-methylacetoxime, O-ethylacetoxime and O-methyldiethylketoxime. The products obtained corresponded to O,N-dialkyl hydroxyamine, according to the equation:

$$R'\text{-}\underset{\displaystyle R''}{\overset{|}{C}} = N\text{-}OR''' \cdot HCl \longrightarrow R\text{-}\underset{\displaystyle R''}{\overset{|}{CH}}\text{-}\overset{\displaystyle H}{\overset{|}{N}}\text{-}OR'''$$

A tendency to cleavage with formation of NH_4Cl was also noted. The catalytic reduction of aryloximino groups by Jones and Major under the same conditions yielded primary and secondary amines along with unrecovered oximino ether. Adkins and Reeve (59) reduced catalytically an ethoximino group in their synthesis of threonine. The conditions employed were drastic; they used a pressure of three hundred atmospheres and a temperature of 90°. Raney nickel was the catalyst.

The use of the benzyl group to mask the labile oximino group was suggested by Waters and Hartung (50) because of the ease of catalytic debenzylations noted in other compounds. Ipatiev (60) found that benzyl alcohol could be converted to toluene and dibenzyl using an iron catalyst at 350° and 96 atmospheres. Rosenmund (61) cleaved benzyl benzoate into toluene and benzoic acid with palladium on barium sulfate

in xylene solution. A few years later (62) Rosenmund extended the reduction to mandeloacetates. Palladium on barium sulfate was employed by two Japanese workers (63) in the reduction of acetals of benzadehyde. A German patent (64) reports the catalytic debenzylation of ethers. Benzyl and benzal derivatives of sugars have been split by means of platinum black (65). Freudenberg and co-workers found that benzyldiacetoneglucose, if hydrogenated in ethanol, did not split as readily as in acetic acid. Sodium and alcohol were effective in the reduction of these benzylated sugars.

Benzylidene residues have been catalytically removed from sugars by means of palladium (66). Fischer employed palladium in acetic acid according to the procedure of Freudenberg in the debenzylation of benzylated sugars. Based on the experiences of Fischer and Freudenberg, Bergmann and Zervas (30) developed their classical reduction of the carbobenzyloxy group in their peptide synthesis. The procedure also involved the use of methanol together with acetic acid.

As mentioned previously du Vigneaud (33) used sodium and liquid ammonia, while Harrington (32) employed phosphonium iodide to remove the carbobenzyloxy group.

Hartung and Crossley (69) considered pallidium to have a greater debenzylating action than platinum. Their experiments utilized both metals supported on charcoal. It was found that palladized-charcoal catalyzed debenzylation more rapidly than platinum-oxide or platinum black. In some

cases the use of platinum interferes with the debenzylation by reducing the aromatic ring.

In their studies of catalytic debenzylations of O- and N- benzyl groups in the presence of palladium, Baltzly and Buck (70) found that alkyl groups in the α-position of benzyl alcohol have a stabilizing effect, which is still more pronounced with carboxy and amino groups. Substitution on the aromatic rings with -OH, -OMe, $-NH_2$, -Cl, $-NR_3$ and CH_3 facilitates debenzylation.

On the other hand, more extended aromatic systems as 4-phenylbenzyl, are more labile than the benzyl group. This observation suggests that the 4-phenylbenzyloximino - group may be employed in the peptide synthesis to be described below in cases where the debenzylation is difficult.

Simonoff (71) effected debenzylation using palladium catalysts and presented a thorough review of the catalysts that have been used for this process. Mattocks (72) obtained quantitative yields in debenzylating N-benzyl-β-alanine by using a platinum-palladium mixture.

The use of Raney nickel as a debenzylating catalyst was studied by Van Duzee and Adkins (73) in their general survey of the hydrogenolysis of ethers. These authors showed that benzyl ethers are more easily hydrogenolysed than other types of ethers. They used reaction temperature over 100°C.

While the debenzylation of O-benzyl ethers is readily accomplished, the reduction of the oxime involves special

conditions. In neutral solvents the oximes like the nitrites are converted to secondary amines when hydrogenated. Hartung and co-workers (74) recommended the addition of three or more equivalents of hydrogen chloride to the alcoholic solution of the oxime. Under these conditions high yields of the primary amines are obtained. Glacial acetic acid has been used as the solvent and its beneficial effects are presumably due to the formation of oxime acetates which, according to Rosenmund and Pfankuch (75), are reduced in higher yields to the primary amines than the free oximes.

Winans and Adkins (76) found that oximes reacted with hydrogen over nickel under mild conditions. The reaction began at room temperature and was quite exothermic. The final temperature of the bomb rose to 40° or 50° without external heating.

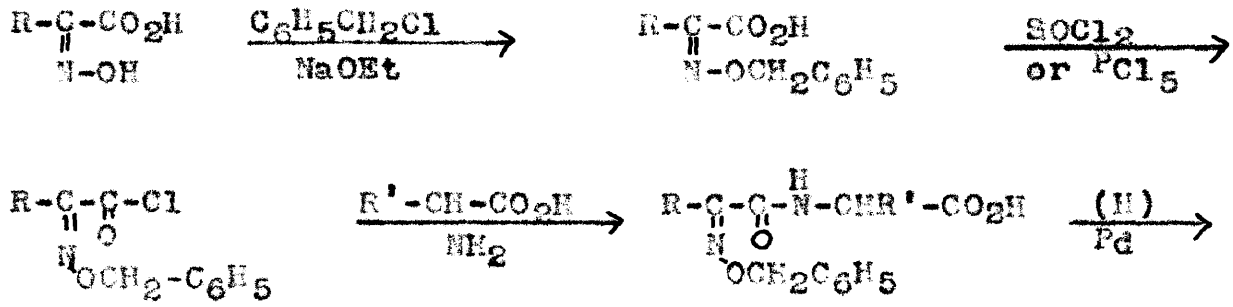
Paul (77) found that aldoximes under normal conditions could be reduced with Raney nickel. A mixture of primary and secondary amines resulted and the proportion of secondary amine formed was considerable. If ammonia was added to the reaction mixture secondary amine formation was suppressed. This observation was used, as will be shown later, in the reduction of the benzyloximino group in alkaline media. However, under higher pressures, ketoximes were reduced to primary amines, predominantly, with yields 80-95 per cent at 70°C and 50 atmospheres.

In the reduction of α-oximino acids according to Hartung's procedure, Hamlin (79) observed that during the

hydrogenation, one-half of the theoretical amount was taken up rapidly, the remainder required four to five times longer periods of shaking. Hamlin postulated the intermediate formation of the imine. Waters (50), however, found that the imino is not formed, but rather an equal mixture of the completely reduced α-oximino acid and the unreduced product. Waters considered that the free amino acid formed acted as an anticatalyst and thus slowed down the reduction. Weaver (78) used a variety of procedures and catalysts for reduction of the benzyloximino group to the free amine. However, he could not obtain pure products possessing the properties of the desired peptides. Weaver suggested the formation of diketopiperazines in explanation of his results. Apparently debenzylation took place readily but the reduction to the peptide stage was difficult.

RESEARCH AIM

Previous reports from these laboratories indicate the possibility of synthesizing peptides by a new route. Weaver, using the α-benzyloximino acids described by Waters, set out to prepare peptides by the following methods:



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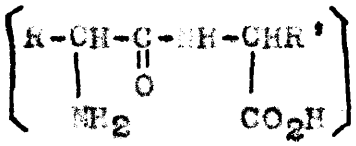


The procedure was shown to be feasible up to the stage indicated I. The reduction of I, although taking up the calculated amount of hydrogen did not form the expected pure dipeptide, but rather yielded largely the corresponding diketopiperazines. These results, though unexpected, were encouraging in that they suggested that the peptide undoubtedly was formed during the reduction and before cyclization. Cyclization probably will not occur with intermediates for higher peptides.

This thesis describes the following:

1. The coupling of α-benzyloximino acid chlorides

with amino acids under various conditions.

2. The purification and separation of N-α-benzyloxiamino acid derivatives of amino acids, dipeptides, and tripeptides.

3. Studies in the reduction of the benzyloxiamino group.

4. The isolation and preparation of dipeptides and tripeptides.

5. Attempts to prepare higher peptides.

EXPERIMENTAL SECTION

INTERMEDIATES

In the preparations to be described later, the following commercially obtainable chemicals were used: butyl alcohol, phosphorous pentachloride, benzyl chloride, ethyl malonate, benzaldehyde, ethyl acetate, petroleum ether, glycine (Eastman Kodack Co.), DL-alanine (Eastman Kodak Co.), DL-leucine (Eastman Kodak Co.). Ethyl malonate obtained from Eastman Kodak Co., was re-distilled prior to use.

A. n-Butyl nitrite.

n-Butyl nitrite, prepared according to the direction of Hamlin (79) was stored in the refrigerator and re-distilled before use.

B. Malonic Esters

Ethyl benzylmalonate.

The preparation of this compound is described in Organic Syntheses (80). For the present study it was synthesized by two methods.

1. A slight modification was employed to simplify the isolation of the substance.

In a 2-liter, 3-neck round bottom flask, fitted with an efficient stirrer, dropping funnel, and a condenser, and provided with a drying tube, was placed a liter of absolute alcohol (distilled from magnesium ethoxide). Forty-six grams (2 moles) of sodium ribbon was added gradually (one hour). To the hot refluxing solution was added all at once,

480 grams (3 moles) of redistilled ethyl malonate. A white curdy precipitate was formed. The mixture was heated and 253 grams (2 moles) of benzyl chloride, as suggested by Weaver (78), was added slowly over a period of one hours. The reaction mixture was refluxed for 24 hours and allowed to cool after which ethanol saturated with hydrogen chloride was added until the reaction mixture was neutral to moist litmus. The salt was removed on a large Buchner funnel and washed with three 50 ml. portions of commercial ethanol. From the filtrate and washings, the excess ethanol was removed under reduced pressure (water pump), using a three-liter modified Claisen flask. The residue was transferred to a one-liter modified Claisen flask and distilled. The first fraction, the excess ethyl malonate (b_3 up to 174°) was recovered and approximately 345 grams (b_3 174-6) of ethyl benzylmalonate was obtained (69% yield). A dark reddish viscous liquid, presumably ethyl dibenzylmalonate, remained in the flask.

2. Higher yields of ethyl benzylmalonate were obtained by the following method (81):

$$C_6H_5CHC + CH_2(CO_2Et)_2 \xrightarrow{NaOEt} C_6H_5CH{=}CH(CO_2Et)_2$$

$$\xrightarrow[(H)]{Pd(Pt)} C_6H_5\overset{CH_2}{\overset{|}{-}}CH(CO_2Et)_2$$

One hundred grams (.63 mol.) of ethyl malonate, dried

by distilling under reduced pressure from anhydrous sodium carbonate, and 70 grams (.66 mol.) of freshly distilled benzaldehyde were mixed and 2 grams of piperidine added. The mixture was kept in a stoppered flask for two days and heated on a water bath for 12 hours. The reaction mixture was taken up in ether, washed first with water, then with dilute hydrochloric acid, dried over anhydrous sodium sulfate, and distilled under reduced pressure. The benzalmalonic ester was collected at 185-186/11 mm. The yield was about 70-80 per cent. It solidified upon cooling. One hundred grams (.2 mol.) of ethyl benzalmalonate was dissolved in an equal volume of ethanol (95%) and hydrogenated on the Parr apparatus at 50 pounds and room temperature. Five grams of 10 per cent Pd-Charcoal containing .15 grams of chloroplatinic acid (cf. page 56 for the preparation) was used to effect the hydrogenation. The product was worked up in the usual manner and yields ranged between 80-85 per cent.

C. Oximino acids

The oximino acids which were used as starting materials were prepared by the alkaline nitrosation procedure of substituted malonic esters described by Barry (46). It was observed that a very pure product was obtained by carrying out the reaction at -10°. A small excess of butyl nitrite was employed to insure complete nitrosation.

β-Phenyl-α-Oximinopropionic acid

To a two-liter, three-neck, round bottom flask equipped

with an efficient stirrer, dropping funnel, and a condenser bearing a drying tube, was added one liter of absolute alcohol. The flask was chilled and 11.5 grams (.5 equiv.) of sodium was added all at once. After the evolution of hydrogen was complete, 125 grams (.5 mol.) of ethyl benzylmalonate was added to the hot mixture, which was cooled with a Dry Ice-acetone bath to -10°, and 111 grams (.55 mol.) of freshly distilled butyl nitrite was added slowly so that the temperature did not rise over -10°. The mixture was transferred to a two-liter Claisen flask and the butanol and ethanol were removed under reduced pressure. Six-hundred ml. of water was added, the mixture cooled to 0° and carefully neutralized with concentrated hydrochloric acid, and then extracted with 10 per cent sodium hydroxide. The alkaline solution was heated on a hot plate for an hour to hydrolyze the ester. The alkaline solution was cooled and added to an ice-HCl slush. The tan precipitate was collected on a Buchner funnel, dried in air and recrystallized from petroleum ether. Yield, 90 per cent.

The original material melted as reported by Weaver (78) 168-169°. However, after two recrystallizations from ethyl acetate-petroleum ether (1:10) a faintly tan product was obtained that melted at 176°.

<u>α-Oximonobutyric acid</u>

$$Et\text{-}CH(CO_2Et)_2 \xrightarrow[BuONO]{NaOEt} Et\text{-}\underset{\underset{N\text{-}OH}{\|}}{C}\text{-}CO_2Et \xrightarrow{NaOH} Et\text{-}\underset{\underset{N\text{-}OH}{\|}}{C}\text{-}CO_2H$$

The procedure described under β-phenyl-α-oximinopropionic acid was followed. Yields obtained were essentially those reported by Weaver (78). The compound after recrystallization from ethyl acetate-petroleum ether melted at 153-154°.

α-Oximinopropionic acid

$$CH_3-\underset{\underset{N-OH}{\|}}{C}-CO_2H$$

This compound was prepared by the method of Inglis and Knight (82) in yields of 71 per cent of the theoretical. After recrystallization from ethyl acetate-petroleum ether, the compound melted at 179-180°.

β-(O-nitrophenyl)-α-oximinopropionic acid

$$(o\text{-}NO_2\text{-}C_6H_4)-CH_2-\underset{\underset{N-OH}{\|}}{C}-CO_2H$$

This compound was obtained through the courtesy of Dr. Oscar Kliore (83). It was impure and was recrystallized from ethyl acetate-petroleum ether to give a pale tan powder melting 159-160°.

II. THE BENZYLATION OF α-OXIMINO ACIDS

D. Benzyloximino Acids

The benzylation of the α-oximino acids was carried out according to the procedure described by Weaver (78). A

slight modification was found to yield a cleaner product. The ether extraction was eliminated by the isolation of the sodium salt of the α-benzyloximino acid, and could be washed free of the benzyl alcohol and the unreacted benzyl chloride.

β-Phenyl-α-benzyloximinopropionic acid

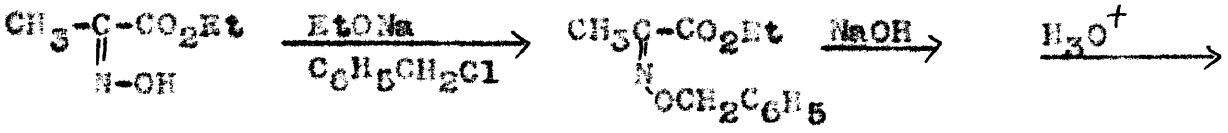
In a one liter, 3-neck round bottom flask equipped with a stirrer and drying tube were placed 500 ml. of commercial absolute ethanol and 11.5 grams (.5 equivalent) of freshly prepared sodium ribbon. After the sodium had dissolved, 45 grams (.35 mol.) of finely pulverized β-phenyl-α-oximinopropionic acid was added. Then the mixture was heated to refluxing and over a period of five minutes, 64 grams (.5 mol.) of benzyl chloride was added and the refluxing continued for three hours. One-hundred ml. of 20 per cent potassium hydroxide in 95 per cent ethanol was added and the mixture concentrated under reduced pressure to dryness. The product was suspended in ether and transferred to a Buchner funnel and washed five times with 50-ml. portions of ether. The filter cake was taken up in water and the free acid precipitated by addition to crushed ice and hydrochloric acid. The product was practically white and weighed 48.5 grams (58%). Recrystallization from aqueous ethanol gave a product that melted at 79-80°. Waters (50) reported 79-80°.

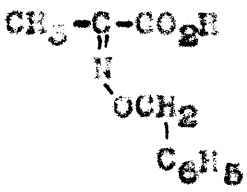
α-Benzyloximinobutyric acid

In a 500 ml., two-neck, round bottom flask fitted with a reflux condenser bearing a drying tube, and a dropping

funnel, was placed a solution of 5.0 grams of α-oximinobutyric acid (.04 mol.) in 200 ml. of absolute ethanol containing .06 equivalents of sodium ethoxide. The flask was heated on a water bath to reflux temperature and 7.9 grams of benzyl chloride was added quickly. Refluxing was continued for three hours, after which time the mixture was concentrated under vacuum to a viscous paste. Then 15 ml. of 5 per cent NaOH and 100 ml. of water were added and the mixture was extracted with ether. The alkaline layer was separated and carefully neutralized in the presence of crushed ice. The precipitate was collected washed with water and dried in air and in a desiccator. It had a slight greenish color. The product was taken up in 1N NH_4OH(50 ml), treated with charcoal and reprecipitated by the addition of acid. The dried product was white, weighed 7.8 grams (87.5 per cent) and melted at 86.5°. Weaver (78) reported 86°.

α-Benzyloximinopropionic acid

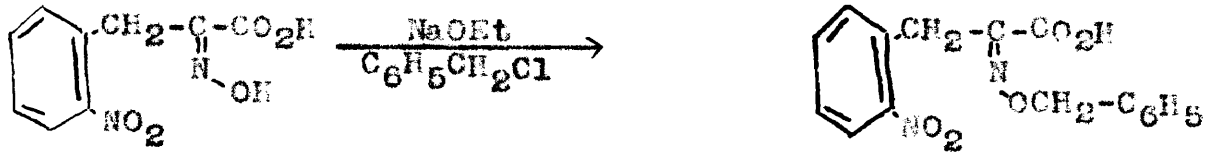




Seven grams (.05 mol.) of ethyl α-oximinopropionate prepared by Waters (80) was dissolved in 100 ml. of absolute

ethanol containing .075 equivalents of sodium ethoxide. To the refluxing mixture was added 9.0 grams (.075 mol.) of benzyl chloride and the reflux temperature maintained for an additional three hours. The excess alcohol was removed under vacuum and 100 ml. of 5 per cent NaOH was added and the ester was saponified by heating at 85° for 45 minutes. After cooling, the alkaline solution was extracted with ether and the benzyloximino acid carefully precipitated in the usual manner. Reprecipitation of the dried α-benzyloximinopropionic acid and drying in an evacuated desiccator over P_2O_5, yielded 8.2 grams (78.5 per cent yield) of product melting at 73-74°. Weaver (78) reported 73-74°.

β-(o-Nitrophenyl)-α-benzyloximinopropionic acid



Five grams (.022 mol.) of β-(o-nitrophenyl)-α-oximinopropionic acid was benzylated in the manner described above. The yield was 6.2 grams (89 per cent). After three recrystallizations from ethyl acetate-petroleum ether, a pale yellow, micro-crystalline compound was obtained which melted at 110-111°. Neutral equivalent: calculated, 313; found, 309, 310.

E. Recrystallizations of α-benzyloximino acids

The compound was dissolved in a minimum amount of dry

ethyl acetate (dried over anhydrous magnesium carbonate and distilled) and ten volumes of anhydrous petroleum ether was added. The cloudy solution was filtered and cooled. Occasionally, it was necessary to evaporate some solvent to initiate crystallization. The filtration step usually removes the colored decomposition products of the α-benzyloximino acids.

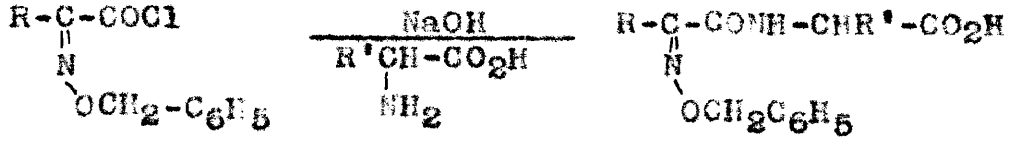
III. GENERAL METHOD FOR PREPARATION OF ACID CHLORIDES

In a 3-neck flask of 250 ml. capacity, fitted with an efficient mechanical stirrer, inserted through a tightly fitted collar of rubber tubing was placed .01 mol of the benzyloximino acid. To one neck of the flask was attached a calcium chloride tube in series with an attachment to the water pump. About 150 ml. of anhydrous ether was added to dissolve the benzyloximino acid. Stirring was begun and the water pump turned on. The evaporation of the ether under reduced pressure lowered the temperature of the flask to 5-10°. Thereupon, .011 moles of PCl_5 was added at once, as rapidly as possible, and the flask was stoppered to exclude moisture. Rapid stirring was continued for an hour during which time the HCl, $POCl_3$, and ether were being removed by distillation. The pump was then turned off and the mixture was allowed to stand at room temperature for one hour in the stoppered flask. It was filtered through a sintered-glass funnel to remove any unreacted phosphorous pentachloride and the filtrate was concentrated under the water

pump with gentle warming on a water bath to remove the last traces of HCl, and $POCl_3$. The straw-colored residual oil, which was substantially pure benzyloximinoacid chloride, was taken up in 100 ml. of anhydrous ether; this solution was a convenient form in which to use the acid chloride. Such solutions in glass-stoppered containers have been stored in the refrigerator for more than 21 days without appreciable deterioration. The acid chloride was not isolated because it decomposed partially upon distillation. The acid chlorides containing the amido group can not be distilled at all.

IV. METHODS FOR PREPARATION OF PEPTIDE INTERMEDIATES

A. Sodium hydroxide method



Into a 250 ml. round-bottom, three neck flask fitted with an efficient mechanical stirrer, and a dropping funnel, 75 ml. of 2N NaOH and 0.04 moles of the amino acid was introduced. When stirring was begun, the amino acid went into solution. The solution was cooled (5°) while 100 ml. of an etheral solution containing 0.02 moles of the acid chloride was added over a period of thirty minutes. After the addition, the mixture was well stirred for 1.5 hours and allowed to come to room temperature. Care should be taken that the

mixture remains slightly alkaline.

The etheral layer was separated and extracted with three 50 ml. portions of 0.1N NH_4OH. The ammoniacal solution of the amide was well cooled and then added dropwise with constant stirring to a beaker containing crushed ice and 10 ml. of concentrated HCl. An amorphous precipitate immediately formed.

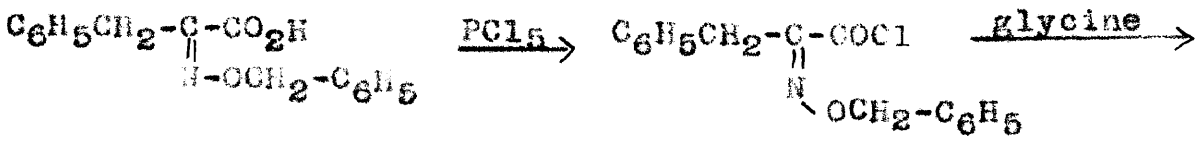
In some instances, however, the product separated out as a white viscous material which solidified to an amorphous solid after standing overnight in the cold.

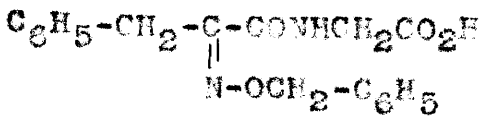
B. Magnesium oxide method.

Another procedure which gave excellent results was suggested by Sheehan and Frank (35).

A solution of 0.02 moles of the acid chloride in 50 ml. of dry ether was added dropwise during thirty minutes to a stirred, cooled (5°) suspension of 0.04 moles of the amino acid and 2 grams of magnesium oxide in 75 ml. of water. The mixture was stirred for 1.5 hours. The mixture was then carefully acidified with hydrochloric acid. The etheral layer was separated and the aqueous layer was further extracted with two additional portions of ether. The combined etheral extracts were shaken with three 50-ml. portions 0.1N NH_4OH and precipitated as described above under method A.

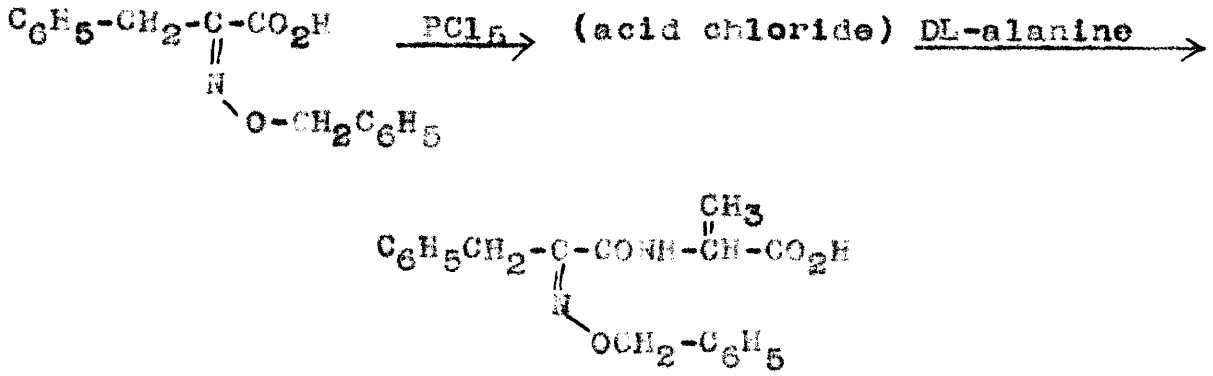
<u>β-Phenyl-α-benzyloximinopropionylglycine</u>





Three grams (.011 mol.) of β-phenyl-α-benzyloximinopropionic acid was treated with phosphorous pentachloride in the manner described above. The ethereal solution was allowed to react by the sodium hydroxide method (Method A described above) with the alkaline solution of 3.0 grams (.043 mol.) of glycine. The yield was 2.8 grams (77.5 per cent) after recrystallization from aqueous ethanol and desiccation. Using method B, the yields were increased to 90-92 per cent. The compound melted at 97°C. Weaver (78) reported 96-97°C.

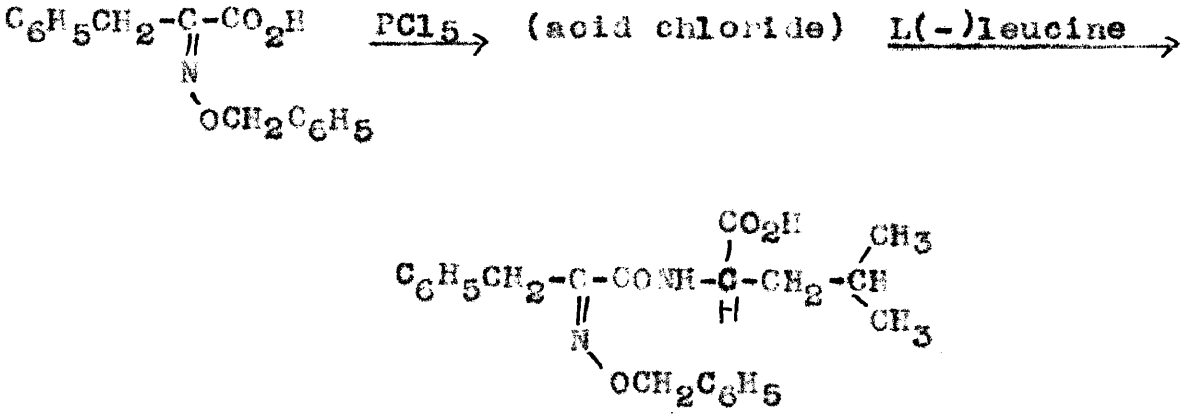
β-Phenyl-α-benzyloximinopropionyl-DL-alanine



Five grams (.019 mol.) of β-phenyl-α-benzyloximinopropionic acid was converted to the acid chloride in the usual manner. Using the sodium hydroxide method, the acid chloride was allowed to react with an excess of DL-alanine (5.0 grams, .056 mol.). The product obtained, weighing 5.4 grams (85.5

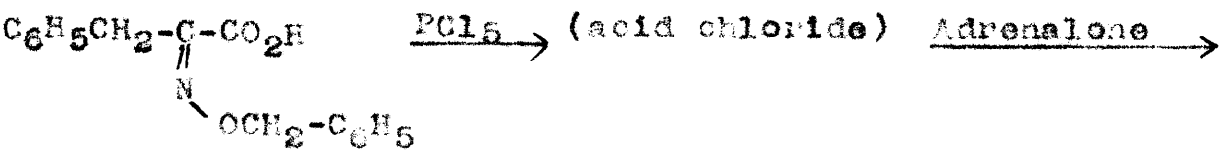
per cent), was recrystallized from ethyl acetate-petroleum ether and melted at 112°. Weaver (78) reported 112°.

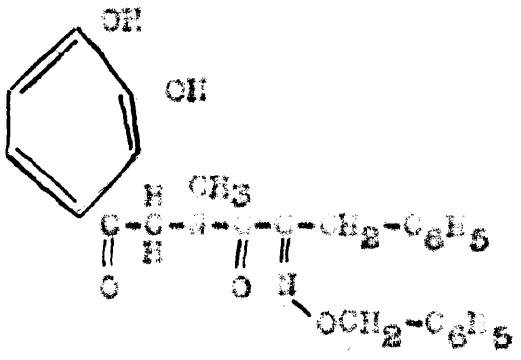
β-Phenyl-α-benzyloximinopropionyl-L(-)leucine



Using the general method, 5.0 grams (.019 mol.) of β-phenyl-α-benzyl-oximinopropionic acid was converted to the acid chloride and was allowed to react by Method B with an excess of L(-) leucine (4.5 grams, .034 mol.). A yellow viscous material separated which solidified after standing overnight in the cold. After recrystallization from ethyl acetate-petroleum ether the white amorphous product weighed 6.1 grams (84 per cent) and melted at 86-87°. Weaver (78) reported 86-87°.

N-(β-phenyl-α-benzyloximinopropionyl)-adrenalone





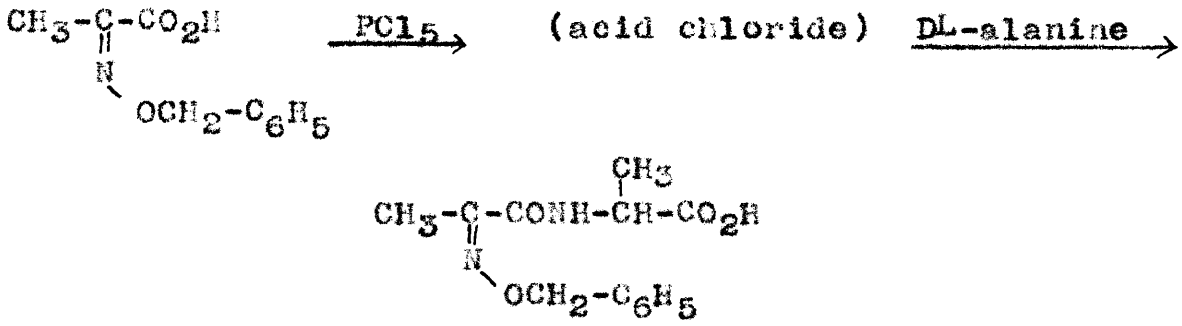
Three grams (.011 mol.) of β-phenyl-α-benzyloximinopropionic acid was converted in the usual way to the acid chloride. To a 250 ml. three-neck, round bottom flask, equipped with an efficient stirrer and dropping funnel were added 4.3 grams (.02M) of adrenalone hydrochloride (obtained through the courtesy of Dr. J. M. Sprague), 50 ml. of water and sufficient 1N NaOH to neutralize both the amine hydrochloride and dissolve the adrenalone. The solution turned a deep yellow. The flask was well-cooled externally and the ethereal solution containing the acid chloride was added dropwise with rapid stirring. After one hour 1N HCl was added slowly to the cooled mixture until the yellow color was discharged. The product was extracted with ether, and the ether solution was dried over anhydrous sodium sulfate. Removal of the solvent left a viscous greenish residue which was taken up in warm ethyl acetate, treated with Norit, and precipitated by the addition of petroleum ether in the cold. After three recrystallizations from ethyl acetate-petroleum ether a white microcrystalline solid was obtained (2.5 grams, 52.6 per cent yield) which melted sharply at 170°.

Presumably, the yellow color is due to the action of

alkali upon the free phenolic hydroxyls, accompanied by oxidation, with the formation of an ortho quinoid structure. Hence the amide prepared above should be insoluble in HCl and give a yellow-colored solution in the presence of alkali. The compound possessed these properties.

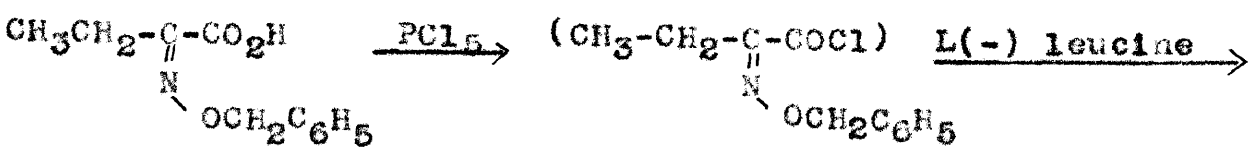
Analysis, % Nitrogen: calculated, $C_{25}H_{24}N_2O_5$, N, 6.47; found N, 6.28, 6.27%.

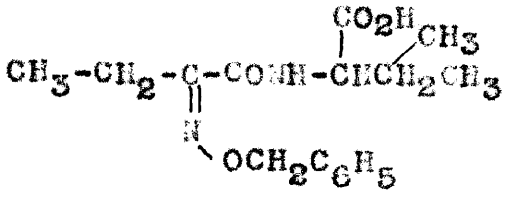
α-Benzyloximinopropionyl-DL-alanine



Five grams (.026 mol.) of α-benzyloximinopropionic acid was converted to the acid chloride in the usual manner. Using the sodium hydroxide method, the acid chloride was allowed to react with an excess of DL-alanine (5.0 grams, .056 mol.). The white product was recrystallized from aqueous ethanol after treatment with Nuchar. The yield was 6.2 grams, 91 per cent. It melted at 117°. Weaver (78) reported 118°.

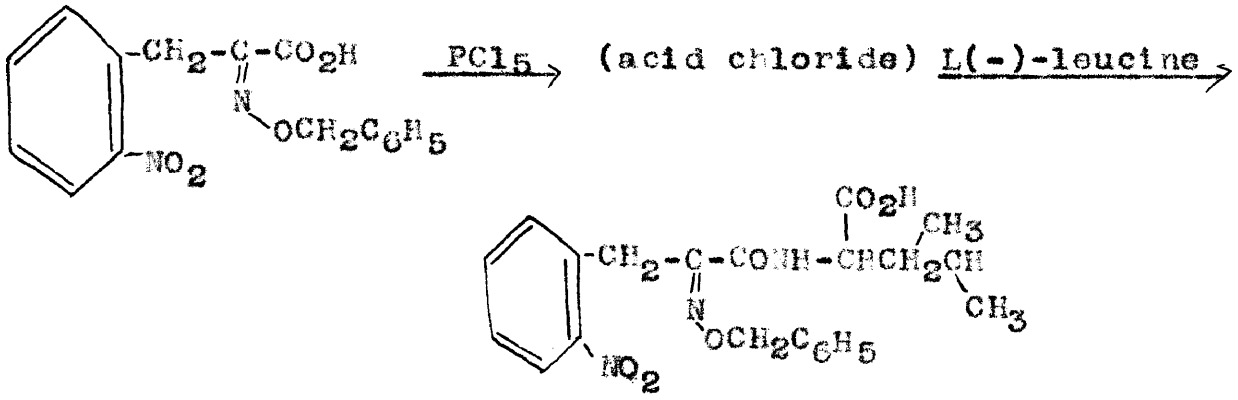
α-Benzyloximinobutyryl-L(-)leucine





Four grams (.019 mol.) of α-benzyloximinobutyric acid was converted in the usual way to the acid chloride. Using the MgO method, it was allowed to react with 4.0 grams (.03 mol.) of L(-)leucine. The product was recrystallized from ethyl acetate-petroleum ether, yield 4.8 grams (87.5 per cent). It melted at 87°. Weaver (78) reported 87°.

β-(o-Nitrophenyl)-α-benzyloximinopropionyl-L(-)leucine

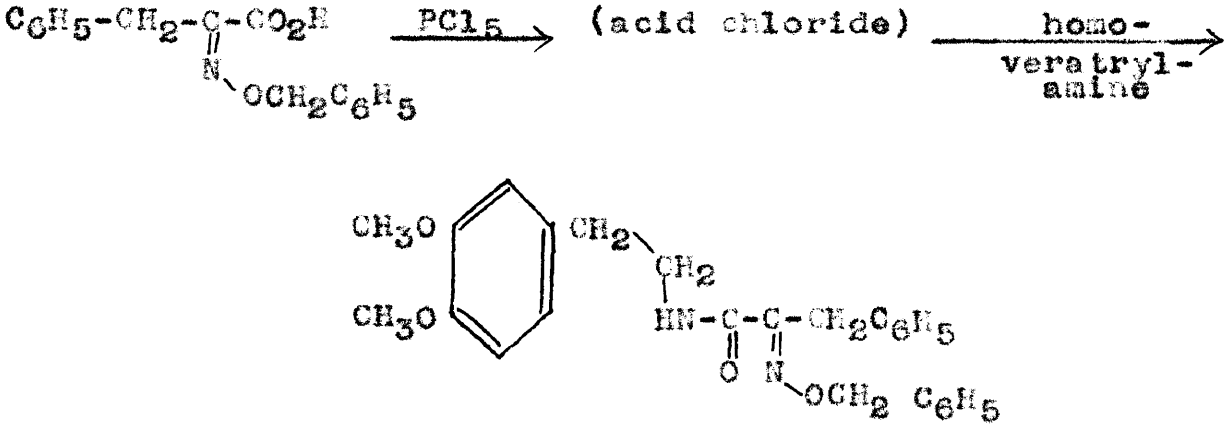


Three grams (.0095 mol.) of β-(o-nitrophenyl-α-benzyloximinopropionic acid was converted in the usual manner to the acid chloride. The ethereal solution containing the acid chloride was dropped slowly into a rapidly stirred, well-cooled solution of 3.0 grams (.023 mol.) of L(-)leucine in 50 ml. of 2N NaOH. A dark brownish tar was formed. It was taken up in ether and dried over anhydrous sodium sulfate. All attempts to crystallize the material failed. It was insoluble in acids and alkali.

It is assumed that the o-nitrophenyl group acts as an electron sink as does the α-benzyloximino group. It is well known that compounds such as $2NH_2-H_2C-CO_2H$ lose CO_2 spontaneously. Similarly, compounds of the type $C_6H_5-\underset{N}{C}=CH-CO_2H$ are readily decarboxylated. It is then reasonably safe to assume, that the β-(o-nitrophenyl)-α-benzyloximinopropionic acid was decarboxylated by the action of PCl_5 and the unstable 2-(o-nitrophenyl)-1-benzyloximinoethane formed, readily polymerized.

A repetition of this experiment using an acetone-Dry Ice bath to prevent decarboxylation yielded the same tar and an alkali soluble material. This acid melted at 92° and had a neutral equivalent of 307. Hence it was the original compound.

N-(β-Phenyl-α-benzyloximinopropionyl)-homoveratrylamine



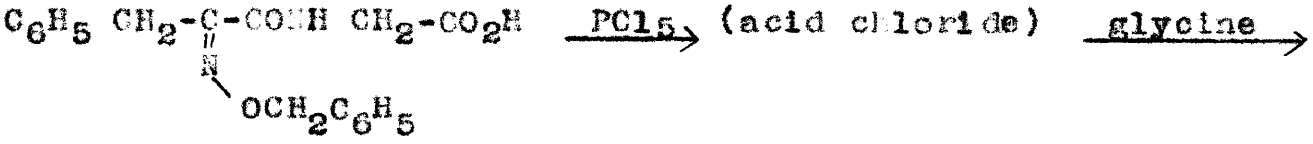
Three grams (.011 mol.) of β-phenyl-α-benzyloximinopropionic acid was converted in the usual manner to the acid chloride and was allowed to react directly with 10 grams (.055 mol.) of homoveratrylamine with cooling. After thor-

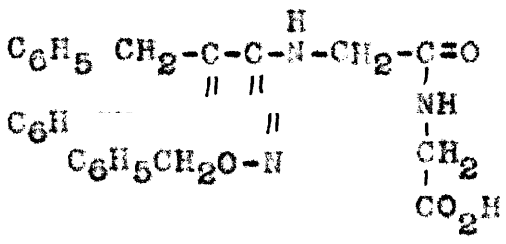
ough mixing, the tube was allowed to remain stoppered in the cold for one hour. Fifty ml. of water was added and the excess amine was neutralized with dilute hydrochloric acid with cooling. The ethereal layer was separated, dried over anhydrous sodium sulfate. After removal of the solvent, the viscous residue was taken up in ethyl acetate-petroleum ether (1:15) and allowed to crystallize slowly in the cold. Repeated recrystallizations yielded white needles, weighing 3.8 grams (79.5 per cent) melting at 38°.

Analysis, %N; calc., $C_{25}H_{28}N_2O_4$, N, 6.67; found N, 6.68, 6.58.

β-Phenyl-α-benzyloximinopropionylglycylglycine

Method A:

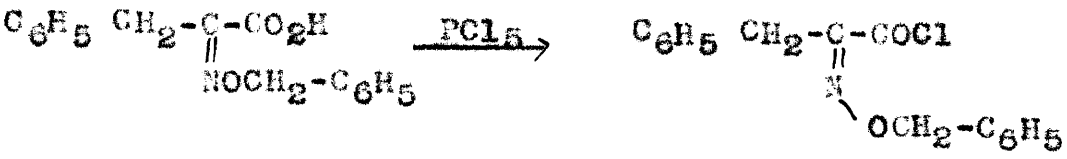




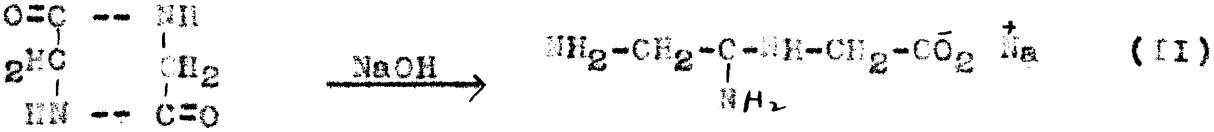
Four grams of β-phenyl-α-benzyloximinopropionylglycine was converted to the acid chloride. The acid chloride after removal of $POCl_3$ and HCl under vacuum, could be precipitated from an ethereal solution by the addition of petroleum ether (30-60°). However, because of the difficulty involved in the manipulation of the acid chloride - it becomes sticky

when in contact with the moisture of the air - it was found expedient not to isolate it, but rather to keep it in ether until used. Using the sodium hydroxide method, the acid chloride was allowed to react with 4.0 grams (.053 mol.) of glycine, care being taken to keep the temperature around 10-15° to prevent hydrolysis of the diamide formed. After completion of the reaction (one hour), the ether was removed by evaporation in a current of air and the mixture was cooled to 0°, and carefully neutralized with dilute hydrochloric acid. The amorphous precipitate was filtered, dried and recrystallized from ethyl acetate-petroleum ether. The diamide was not very soluble in cold ethyl acetate and this difference in solubility was used to remove any unreacted monamide that was present. The white diamide melted 182-184°, weighed 3.1 grams (66 per cent). Neutral equivalent (from ethanol) calculated: 383, found 379, 380.

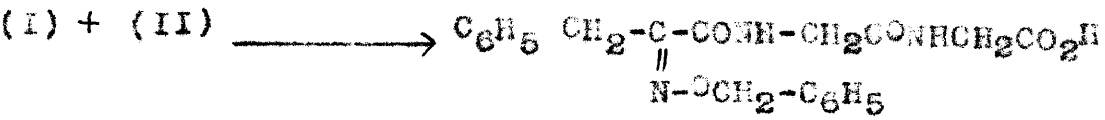
Method B:



(I)



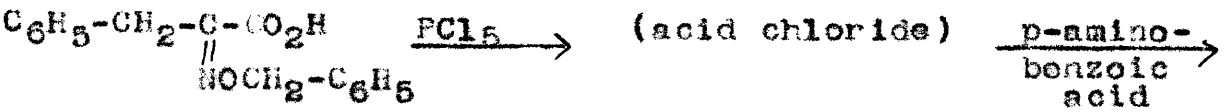
(II)

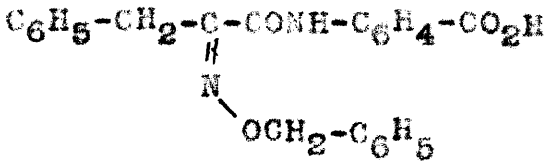


Three and five-tenths grams (.013 mol.) of β-phenyl-α-

benzyloximinopropionic acid was converted in the usual way to the acid chloride. Four grams (.035 mol.) of diketopiperazine was hydrolyzed according to Fischer's procedure (21) to the sodium salt of glycylglycine by allowing the diketopiperazine to stand at room temperature for fifteen minutes in the presence of 30 ml. of 2N NaOH. The acid chloride was allowed to react with glycylglycine, the product isolated and recrystallized in the manner described above. Yield 4.5 grams (90 per cent). Melting point 182-184°.

N-(β-phenyl-α-benzyloximinopropionyl)-p-aminobenzoic acid

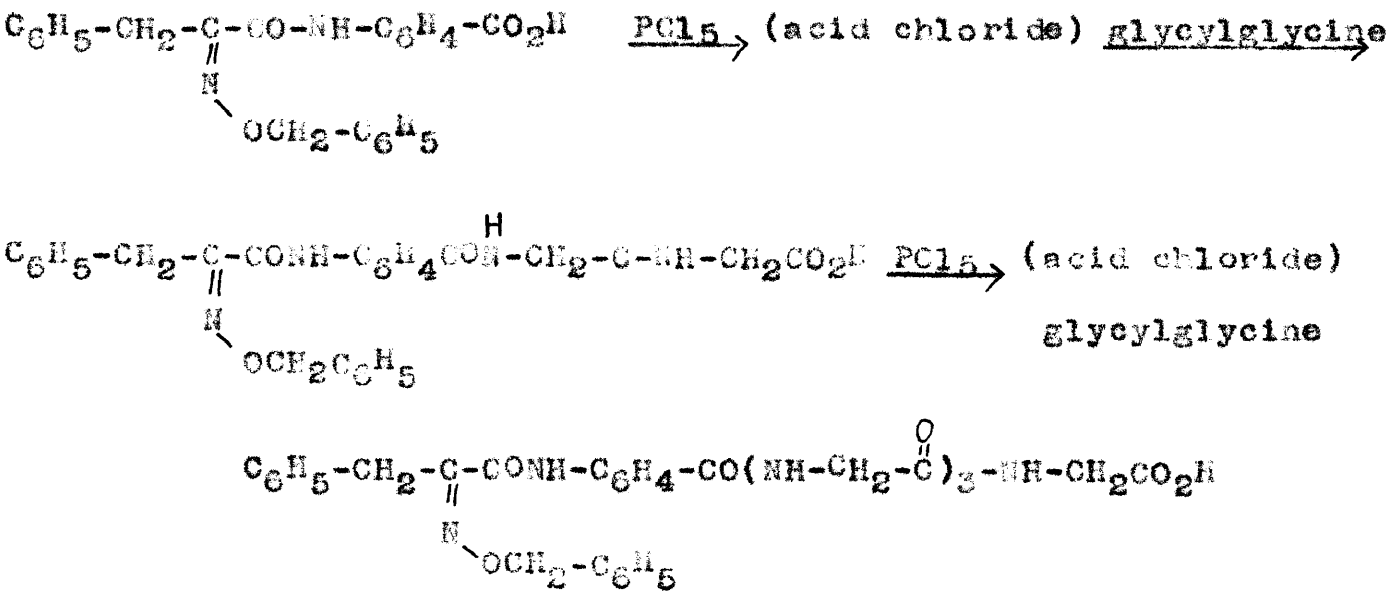




Four grams (.015 mol.) of β-phenyl-α-benzyloximinopropionic acid was converted to the acid chloride. Using the sodium hydroxide method, the acid chloride was allowed to react with 4.0 grams (.03 mol.) of p-aminobenzoic acid. Yield, 5.0 grams (86.5 per cent), melting point 86° upon recrystallization from ethyl acetate-petroleum ether. Neutral equivalent by back titration 375, 378; calculated, 388.

N-(β-phenyl-α-benzyloximinopropionyl)-p-aminobenzoyl-glycyl-glycine.

N-(β-phenyl-α-benzyloximinopropionyl)-p-aminobenzoyl-tri-glycyl-glycine.



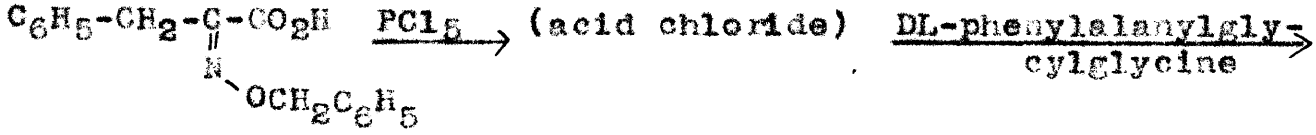
1. Four grams (.01 mol.) of N-(β-phenyl-α-benzyloximino-propionyl)-p-aminobenzoic acid was converted to the acid chloride in the usual manner. One hundred ml. of anhydrous ether containing the acid chloride was allowed to react by the sodium hydroxide method with 3.0 grams (.023 mol.) of glycylglycine, sodium salt, and the triamide was worked up as previously described. The product came down as a viscous material which slowly solidified. After recrystallization from ethyl acetate-petroleum ether, a white powder weighing 3.5 grams was obtained which melted at 61°.

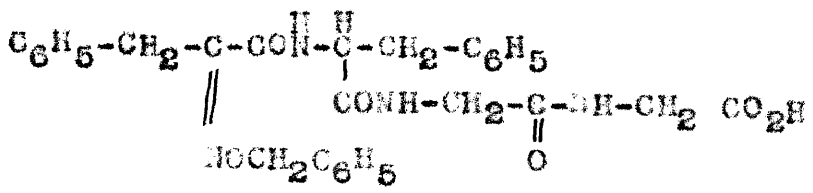
2. The above procedure was repeated using the same quantities. The product obtained melted at 62-65°. Repeated recrystallizations did not substantially alter the melting point. The neutral equivalent of the substance ob-

tained from Run 1 was 412, 415 (calc., 499). The neutral equivalent of the substance obtained from Run 2 was 420, 422. Because of the complexity of the molecule not too much can be ascertained from an examination of the N.E. It is possible that incomplete reaction of the acid chloride with the glycylglycine would result in a mixture of the original monamide with the triamide. Another possibility is the hydrolysis of the triamide to some extent. No solvent or combination of solvents was effective in fractionally crystallizing any of the components. The N.E. indicates that some triamide did form; it now becomes desirable to work out a method by which these poly-amides may be obtained in a pure state.

An attempt was made to prepare a pentamide, the forerunner of a hexapeptide, whose properties would be sufficiently different from the lower poly-amides to allow fractional separation. Accordingly, 2.0 grams (about .004 M) of the impure triamide was converted to the acid chloride and allowed to react by the sodium hydroxide method with 2.0 grams (.015 mol.) of glycylglycine, sodium salt. The product was worked up in the usual manner and melted over a range, 60-83°. Recrystallization from ethyl acetate-petroleum ether (1:1) yielded two fractions: Fraction A melted at 80-85°; Fraction B (ethyl acetate-petroleum ether insoluble fraction) softened 130-135° and melted at 166-168°. This type of behavior was found to be characteristic of impure poly-amides. Neutral equivalent of fraction B: calc., 610; found, 516, 523.

<u>β-phenyl-α-benzyloximinopropionylglycyl-glycyl-DL-phenylalanylglycylglycine.</u>





One and nine-tenths grams (.005 mol.) of β-phenyl-α-benzyloximinopropionylglycylglycine was converted to the acid chloride in the usual manner. Fifty ml. of an ethereal solution containing the acid chloride was allowed to react at 0° with 1.6 grams (.008 mol.) of DL-phenylalanylglycylglycine. The product obtained (1.5 grams) melted at 172-173°, 10 degrees lower than the original material. Recrystallization from ethyl acetate-petroleum ether yielded a compound that melted at 180-183°.

It would seem likely that the acid chloride was not obtained and further studies are necessary in order to devise improved methods for the preparation of acid chlorides of the higher poly-amides.

V. HYDROGENATION STUDIES

It has been assumed (84) that the reduction of the oximino group follows a stepwise course similar to that of the conversion of the nitrile to the free amine. Winans and Adkins (84) studying the hydrogenation of nitriles in neutral solution found that a mixture of primary and secondary amines is formed. They proposed the following explanation for the secondary amine formation:

$$RCN \xrightarrow{H_2} R\text{-}CH{=}NH \xrightarrow{H_2} RCH_2NH_2$$

$$R\text{-}CH{=}NH + RCH_2NH_2 \longrightarrow R\text{-}CH{=}N\text{-}R + NH_3$$

$$R\text{-}CH{=}N\text{-}R \xrightarrow{H_2} RCH_2\text{-}NH\text{-}R$$

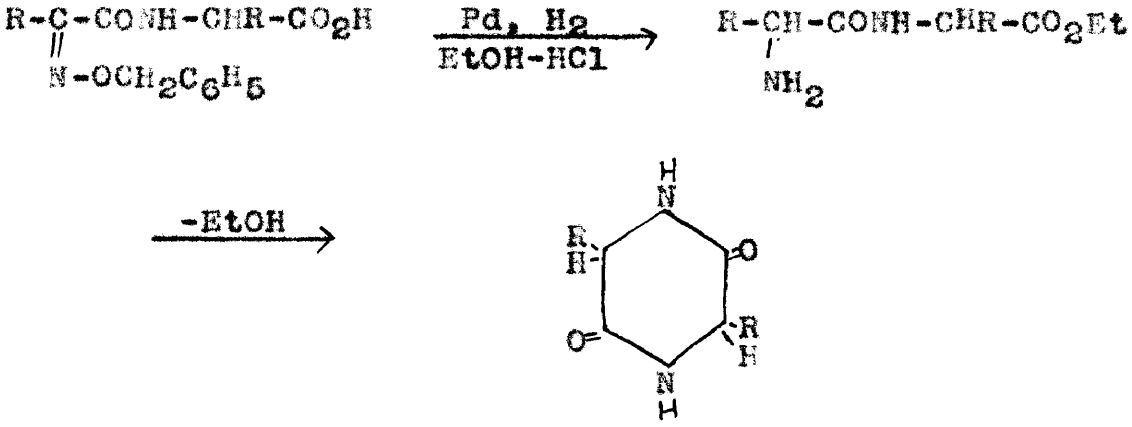
The aldimine reacts with the primary amine in the same fashion as an aldehyde, leading to a Schiff's base which then undergoes further reduction to the secondary amine.

Carothers and Jones (85) found that with platinum oxide the yield of the primary amine may be increased by using glacial acetic acid as a solvent. Hartung (74) obtained high yields of the primary amine by using palladium on Norite and adding one equivalent of hydrogen chloride to the alcohol used as the solvent. In their studies on the hydrogenation of oximes, Hartung and co-workers (74) recommended the addition of three or more equivalents of hydrogen chloride to the alcoholic solution of the oxime.

As was pointed out in the Introduction, Weaver (78)

attempted to hydrogenate amides of α-benzyloximino acids to the α-amino acids amides. Table I summarizes his methods and results:

From Weaver's hydrogenation experiments no consistent method could be ascertained. Thus in Experiment 2, using 10 per cent Pd on charcoal and glacial acetic acid as the solvent, no hydrogenation occurred. However, in Experiment 6, presumably under the same conditions there was taken up 66 per cent of that calculated for reduction to the dipeptides. Also, a comperison of Experiments 3 and 10, reductions that were likewise carried out under identical conditions, shows that results are not consistent: in Experiment 3 no hydrogenation occurred, whereas, in Experiment 10 a mixture of peptide and diketopiperazine is suspected. Nevertheless, Experiment 11 is of interest because of subsequent findings to be reported herewith. Weaver explained the formation of the diketopiperazines by way of the ethyl ester of the α-benzyloximino acid amides, as follows:



It was suggested that if it were possible to reduce

TABLE ONE

HYDROGENATION METHODS BY WEAVER (78)

Experiment	Catalyst	Solvent	Temp.	Pressure/ atmospheres	Results
1	Pd-C 10%	EtOH	20°	20	negative
2	Pd-C 10%	gl HAc	70°	20	negative
3	Pd-C 10% Pt.	EtOH-HCl	20°	20	negative
4	Pd-C 40% Pt.	EtOH-HCl	20°	20	produce assumed diketopiperazine
5	Pd-C 40%	EtOH	20°	20	negative
6	Pd-C 10%	gl HAc	20°	20	2/3 theory uptake of H_2
7	Pd-C 40%	gl HAc	20°	20	2/3 theory uptake of H_2
8	Raney Ni	EtOH	20°	20	negative
9	Raney Ni	EtOH	75°	96	theory H_2 uptake but products not peptide
10	Pd-C 10% Pt	EtOH-HCl	20°	20	no clear-cut products - presumed mixture of peptide and diketopiperazine
11	Pd-C 10% Pt	EtOH-HCl water(?)	20°	20	

the ester formation either by carrying out the hydrogenation in another solvent or by adding water to the reaction mixture, the amount of the free peptide formed would be increased. Experiment 11 indicated that this approach may be a fruitful one. Unfortunately the amount of water added was not reported.

In the light of these results, it now becomes desirable to study the effect of the solvent and the influence of the presence of water upon the course of the reaction. Another consideration is finding a suitable and practical condition under which the conversion of the benzyloximino group to the amino group may be effected. High temperature and pressures are to be avoided, if possible, to prevent any alteration of the free peptide molecule. It was therefore decided to study the hydrogenation primarily at room temperature and first at 50 pounds pressure on a Parr apparatus.

The use of various organic solvents, to be described below, was unfruitful. After repeating some of Weaver's experiments, it was decided that a new attack was necessary. Winans and Adkins (84) and Bowk (87) studied the conversion of nitrites to primary amines, using a nickel catalyst. Since with nickel acid could not be employed to minimize secondary amine formation, they carried out the reduction as quickly as possible and dissolved some anhydrous ammonia in the solvent to suppress the action of the aldimine with the already-formed primary amine. Using this line of attack, it was reasoned that aqueous alkali could be used as a

solvent and ammonia added to suppress secondary amine formation. The alkaline pH of the solution must be maintained in order to keep the α-benzyloximino acid in solution. Also, diketopiperazine formation would be unlikely. In addition the isolation of the peptides would be greatly facilitated as indicated below.

The work of Cohn (88) and of Schmidt and co-workers (89) has shown that almost every amino acid has one pK between pH 2.0 and pH 2.5, and another between pH 9 and pH 10. The simple amino acids have isoelectric points near pH 6, while those containing an additional amino or carboxyl group have isoelectric points at different pH values.

According to the classical theory the isoelectric point corresponds to minimum dissociation. Another theory, the amphion (zwitter ion) theory based upon the work of Debye and Hückel (89), considers the isoelectric point to correspond to maximal charge, since the amphion is bivalent while the anion and cation are univalent. Thus, according to Cohn, the pH of the maximal charge, except for histidine (where it is 3.9), lies between 5 and 7 for the amino acids. This may be of physiologic significance since it is close to the pH of the tissues. The exact expression is given:

$$pM' = \frac{(pK_1 + pK_2)}{2}$$

From a study of the pK' values of peptides, Simms (90) and Greenstein (91) found that the pK' values of the pep-

tides are closer together than the corresponding amino acids. The distance between the functional groups is a factor which influences their dissociation. The ionization of peptides, for example, takes place within a narrower range of pH than is the case for the individual components. Hence in solution the peptides will be minimally dissociated, or maximally charged, midway between the pK' of the particular amino and carboxyl groups.

In the isolation of the free peptides we have assumed the following sequence. The reduction of the benzyloximino acid amides is effected in approximately 0.1N NH_4OH solution:

$$R\text{-}C(=N\text{-}OCH_2C_6H_5)\text{-}CO_2^- \; \overset{+}{N}H_4 \xrightarrow[H_2]{Pd\text{-}C;\ H_2} R\text{-}CH(NH_3^+)\text{-}CO_2^- + NH_4OH + C_6H_5\text{-}CH_3$$

The pK' of the amino group of the free peptide is about 8.5; the pK' of NH_4OH in 0.1N solution is 9.2. Thus, the pK' values approach each other, and an equilibrium must exist:

$$R\text{-}CH(NH_3^+)\text{-}CO_2 + NH_4OH \rightleftharpoons R\text{-}CH(NH_2)\text{-}CO_2^- \; \overset{+}{N}H_4 + H_2O$$

However, the equilibrium is shifted to the left as the solution is concentrated and the ammonia evaporated leaving the free peptide as a residue. This is the basis for the isolation procedure of the free peptides used in this work.

The completeness of a hydrogenation experiment was determined by interrupting the reaction, withdrawing 5 ml. of

the mixture, filtering and acidifying with a few drops of concentrated hydrochloric acid. If no cloudiness or precipitate formed, it was assumed that the reaction was complete.

In comparative hydrogenation studies, using definite amounts of catalyst, starting material, solvent and length of time, the reaction mixture was quantitatively transferred to a beaker, filtered, precipitated, dried and weighed. The precipitate in all cases was the unreacted starting material as determined by the melting point. It has been anticipated that some debenzylated product would be present, since debenzylation is assumed to occur readily and to be the first stage in the reduction (50). Hence, one can speculate on the possibility of the hydrogenation of the benzyloximino group in aqueous ammoniacal solutions, following a route other than the generally-accepted one. One path that is possible, but not proved, is as follows, based on the work of Jones and Major (58):

$$C_6H_5\text{-}CH_2\text{-}\underset{\underset{NO\text{-}CH_2\text{-}C_6H_5}{\|}}{C}\text{-}CONH\text{-}CH_2\text{-}CO_2H + \xrightarrow[Pd\text{-}C]{H_2} C_6H_5\text{-}\underset{\underset{OCH_2\text{-}C_6H_5}{|}}{\overset{NH}{CH}}\text{-}CONH\text{-}CH_2CO_2H$$

$$\xrightarrow{H_2} C_6H_5\text{-}CH_2\text{-}\underset{\underset{NHOH}{|}}{CH}\text{-}CONHCH_2CO_2H + CH_3C_6H_5$$

"A"

or

$$C_6H_5\text{-}CH_2\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}CONH\text{-}CH_2CO_2H + HOCH_2\text{-}C_6H_5$$

$$C_6H_5CH_2OH \xrightarrow[H_2]{Pd\text{-}C} C_6H_5CH_3 + H_2O$$

The benzyl alcohol is reduced in the presence of palladium on charcoal to toluene and water. The conversion of benzyl alcohol to toluene and water has been discussed in the Introduction, requiring more drastic conditions. It should be noted that the substance "A" would be soluble in acid and would not appear in the precipitate formed upon the addition of acid to the reaction mixture. Nevertheless, the amount of unreacted starting material could be used as a criterion for judging the efficacy of a catalyst in the hydrogenation experiments.

VI. PRELIMINARY REDUCTION EXPERIMENTS

All reduction experiments carried out in this work were performed on a Parr apparatus with a 3 to 4 lb., pressure drop (for .01 mol. of starting material) as measured on gauge attached to the apparatus.

1. Three grams (.011 mol.) of β-phenyl-α-benzyloximinopropionyl-glycine was dissolved in 100 ml., of absolute ethanol containing 3 ml. of concentrated HCl. Three grams of 10 per cent Pd-C catalyst was then added and the mixture shaken at 50 pounds pressure at room temperature for three hours. No uptake of hydrogen was noted. This experiment was repeated using a 20 per cent catalyst and still there was no pressure drop. The starting material was recovered by filtering off the catalyst and removing the solvent.

2. Three grams of β-phenyl-α-benzyloximinopropionyl-glycine (.011 mol.) was dissolved in 100 ml. of methanol containing 3 ml. of concentrated HCl and 3 grams of 20 per cent Pd-C was added and hydrogenation carried out as above. No uptake of hydrogen was noted.

3. Using the above quantities of β-phenyl-α-benzyloximinopropionyl-glycine, catalyst (20 per cent) and hydrochloric acid in 50 per cent aqueous ethanol, no appreciable uptake of hydrogen was observed. The catalyst was removed by filtration and the solvent evaporated. The original material was recovered unchanged.

4. When the above experiment was repeated using 0.2 grams of Pt black prepared by the hydrogenation of chloro-

platinic acid *in situ*, no appreciable amount of hydrogen was taken up.

5. Following the method of Karrer and Heynemann (98), 3.8 grams of β-phenyl-α-benzyloximinopropionyl-glycylglycine (.01 mol.) was dissolved in 200 ml. of absolute ethanol, with the subsequent addition of 12 ml of .099 3N alcoholic hydrogen chloride. Two grams of Pd black was used as the catalyst. Hydrogen was diffused through the rapidly-stirred solution at 60° for 8 hours. After removal of the catalyst and concentration of the solvent to 25 ml. anhydrous ether was added. No precipitate was formed. Hence, no complete hydrogenation occurred, however, a faint odor of toluene was detected. Removal of the catalyst and solvent yielded a product melting at 178-180°. The presence of the debenzylated product as a contaminant would explain the depression of the melting point. Three and one-half grams of starting material, representing 92 per cent, was recovered.

6. Three and eight-tenths grams (.01 mol.) of β-phenyl-α-benzyloximinopropionyglycylglycine was dissolved in 100 ml. of dioxane containing 3 ml. of concentrated HCl. Three and one-half grams of 20 per cent Pd-C was added and the mixture was subjected to hydrogenation. There was no hydrogen uptake after three hours.

The above six reduction experiments were sufficient to indicate that the hydrogenation could not be carried out under the normal conditions of hydrogen pressure and temperature. Furthermore, these experiments, carried out as a

continuation of Weaver's study of the problem, confirmed what could have been anticipated from a theoretical approach. More and more it has been realized that the solvent has significant effect on the rate of reactions. Thus, Hugh S. Taylor (100) has shown kinetically that the rate of hydrogenation is proportional to the rate of chemisorption of hydrogen and the substance to be hydrogenated, on the catalyst and the rate of removal of the hydrogenated product from the surface of the catalyst. In this light we can visualize the poisoning of the catalyst and the slow rate of hydrogenation of peptides containing the benzyloximino group in a solvent such as absolute ethanol. The peptide-hydrochlorides are not too soluble in ethanol and as they are formed by the hydrogenation process, they are not readily desorbed. Thus the products inhibit further reduction by masking the active patches on the surfaces of the catalyst. Theoretically therefore, the presence of water should speed up the rate of the reaction since the lower molecular weight peptides are quite soluble in aqueous media and the desorption rate would increase. These considerations suggested the use of water and ammonia as the latter would (1) carry the starting material into solution and (2) counteract the formation of secondary amines as previously indicated.

VII. PREPARATION OF CATALYSTS

Palladium-charcoal Catalyst (74)

Nine grams of charcoal, previously heated with dilute nitric acid, washed free of acid with distilled water, was dried and powdered, added to 200 ml. of distilled water containing 1.0 gram of palladium chloride, C.P.* and 20 grams of sodium acetate. The mixture was hydrogenated at 50 pounds pressure on the Parr apparatus for one hour. The catalyst was filtered, washed free of chloride with distilled water, dried well and finely pulverized. It was stored in a tightly stoppered bottle and kept in a vacuum desiccator.

Palladium black (98)

Four and two-tenths grams of palladium chloride was added to 40 ml. of distilled water containing 3.0 ml of concentrated hydrochloric acid and the mixture was heated until complete solution was effected. It was then mixed with 25 ml. of 33 per cent formaldehyde and cooled to -10°. The mixture was then shaken with 50 ml. of 50 per cent KOH for ten minutes keeping the temperature between 0°-30°, at which time the palladium black precipitated. The flask was warmed for one-quarter hour at 60° and the supernatant liquid turned yellowbrown. The Pd was filtered, washed free of chloride, dried and finely pulverized.

Ammonia Catalyst

Nine grams of charcoal and 1.0 gram of palladium

*(The Coleman and Bell Co., Norwood, Ohio)

chloride, C.P. were suspended in 150 ml. of water, and 10 ml. of concentrated ammonia was added. The mixture was shaken on the Parr apparatus at 50 pounds pressure for two hours. The catalyst was filtered, washed free of ammonia, dried and pulverized.

Pd-Pt mixed catalysts

Hartung's procedure (74) was followed except for the addition of 0.10 to 0.15 grams of chloroplantinic acid, H_2PtCl_6 (Baker and Adamson) to the palladium chloride before hydrogenation.

Neutral Catalyst

The directions for the preparation of Hartung's catalyst (74) were followed except that no sodium acetate was added to the suspension. The hydrogenation was carried out for three hours.

PREPARATION OF PEPTIDES

DL-Phenylalanine

Three grams (.011 mol.) of β-phenyl-α-benzyloxiamino-propionic acid was dissolved in 100 ml. of distilled water by the dropwise addition of concentrated ammonia. After complete solution, 1.0 ml. of additional concentrated ammonia was added. Then 3.0 grams of Pd-C catalyst was added and the mixture hydrogenated for two hours on the Parr apparatus. A three pound drop in pressure occurred. (Theoretical: .75 liters, equivalent to 4#/liter x .75 liters = 3#). The suspended catalyst was removed by filtration and the clear aqueous solution had a strong odor of toluene. After distilling off the water, the solid remaining in the flask was taken up in methanol and precipitated by the addition of anhydrous ether. Yield 1.5 grams, 83 per cent. The compound melted at 263-265°. The melting point reported (92) 273°. The benzoyl derivative of DL-phenylalanine, prepared in the usual way melted 185-186° (reported (28) 186°).

DL-Phenylalanylglycine

Four grams (.0123 mol.) of β-phenyl-α-benzyloxiaminopropionyl - glycine was dissolved in 150 ml. of water containing 2.5 ml. of concentrated ammonia. Three and one-half grams of 10 per cent Pd-C catalyst was suspended in the solution and the mixture hydrogenated for two hours. The catalyst was removed by filtration and 1.0 ml. of the clear filtrate was pipetted out of the solution and acidified as a

test for complete reduction. Since there was no apparent cloudiness, the reaction was considered complete. The filtrate was transferred to a 250 ml. distilling flask, fitted with a side arm condenser and a receiving flask, and was concentrated under reduced pressure using a steam bath. The solid residue was triturated in methanol, and ether was added to assure complete precipitation. After collection of the product on a suction funnel, 0.05 grams was placed between two watch glasses and was tested for ammonium ion by means of moist litmus and concentrated sodium hydroxide. There was no visible change in color of the moist litmus and the material appeared to be the free peptide. Yield, 2.4 grams, 87.5 per cent.

After standing for a month in a stoppered container, a melting point was taken and found to be 250°. Analytical data was consistent with that calculated for the dihydrate. After drying carefully over P_2O_5, the compound darkened at 250-255° and melted at 271-273° to a dark red liquid. Fischer and Blank (92) reported darkening at 255° and a melting point 273°, turning to a dark red liquid. Analysis: calculated for $C_{11}H_{14}N_2O_3 \cdot 2H_2O$: N, 10.5 found 10.2, 10.4. Phenylalanylglycine, calculated for $C_{11}H_{14}N_2O_3$: N, 12.5 per cent, found 12.8, 12.5.

DL - Alanyl - DL - Alanine

Three and five-tenths grams (.013 mol.) of α-benzyloximinopropionyl - DL - alanine was reduced as above, using aqueous ammonia (100 ml. water, 3.0 ml. of concentrated

ammonia) and 3.0 grams of 10 per cent Pd-C catalyst. A 3.5 lb. drop in pressure was observed after one hour. The isolation procedure was the same as described under DL-phenylalanylglycine. Yield, 1.9 grams 90 per cent; sintering at 190° and melting 277-278° (uncorr.) Fischer and Kautzsch (93) reported 276° (corr.). Analysis: calculated for $C_8H_{12}N_2O_3$; N, 17.5; found: 17.0, 17.1 per cent.

DL-Phenylalanyl-DL-Alanine

Three and one-half grams (.01 mol.) of β-phenyl-α-benzyloximinopropionyl-DL-Alanine was reduced in 100 ml. of water containing 3.0 ml. of concentrated ammonia, using 3.0 grams 10 per cent Pd-C catalyst. The hydrogenation was complete in one hour. The product was isolated in the usual manner. Yield, 2.0 grams (87 per cent), m.p. 240-243° with decomposition.

Fischer and Blank (94) reported sintering at 230°, and melting at 241° with decomposition.

Analysis, calculated for $C_{12}H_{16}N_2O_3$: N, 11.9, found: 11.1, 11.3

DL-Phenylalanyglycylglycine

Three and eight-tenths grams of β-phenyl-α-benzyloximinopropionyl-glycylglycine (.01 mol.) was dissolved in 100 ml. of water containing 2.5 ml. of concentrated ammonia. Using 3.5 grams of Hartung's 10 per cent Pd-C catalyst, the hydrogenation took one and one-half hours. The isolation was similar to that previously described. The compound had

a slight yellow color and was dissolved in hot water and the solution was treated with charcoal, filtered and concentrated by evaporation of the water. Yield 2.2 grams (79 per cent). The product melted at 233-236° with decomposition. Fischer (96) reported 235° (Decomp.); Sigmund and Wesseley (97) reported 225-230° (d); Sheehan and Frank (35) reported 221-223° (d).

In another run using the ammonia catalyst on similar quantities of starting materials, the β-phenyl-α-benzyloximinopropionylglycylglycine was hydrogenated in 40 minutes (2-3# pressure drop). Yield 2.8 grams (90 per cent). Melting point 235° (d) Analysis: calculated for $C_{13}H_{17}N_3O_4$: N, 15.1; found: 14.5, 14.8.

In the conversion of the benzyloximino group to the free amino group by catalytic hydrogenation the following factors might be considered as influencing the rate of hydrogenation:

(1) Nature and structure of substituents on the carbon bearing the benzyloximino group,

(2) Nature of the catalyst,

(3) Solvent, pH.

An endeavor was made in the following experiments to study each of these factors separately. The compounds used and their symbols are given in the following table:

TABLE II

α-BENZYLOXIMINO ACIDS AND AMIDES

Compound		Symbol	M.W.
I	β-phenyl-α-benzyloximino propionic acid	PA	269
II	β-phenyl-α- benzyloximinopropionylglycine	PA.G	326
III	α-benzyloximinopropionyl-DL-Alanine	A-A	264
IV	β-phenyl-α-benzyloximino propionyl-L(-) Leucine	PA-L	375
V	β-phenyl-α-benzyloximinopropionyl-glycylglycine	PA.G.G	383

Experiment A

Keeping the catalyst, solvent and pH constant, the structure of the substance to be hydrogenated was varied. Using .01 moles of compounds I to V inclusive, the hydrogenation was carried out in the usual manner for 45 minutes using the ammonia catalyst. After the allotted time, the hydrogenation was stopped, the catalyst removed by filtration, and the filtrate acidified. The precipitate was carefully collected with minimum loss, dried and weighed. The results obtained are given in Table III.

From the results obtained, it is apparent that the presence of a benzyl group on the carbon-bearing the benzyloximino group decreases the rate of hydrogenation. Thus III AA was completely converted, whereas, PA was hydrogenated to a relatively small extent.

TABLE III

INFLUENCE OF STRUCTURE ON HYDROGENATION RATE

Compound		Amt(.01 mol.) (Start)	Amount Unreacted (Isolated)	Per Cent Hydrogenated
I	PA	2.7 grams	2.0	24
II	PAC	3.3	1.9	36
III	AA	2.6	0.0	100
IV	PAL	3.8	0.9	77
V	PAGG	3.8	0.8	79

Experiment B

In this experiment, the solvent, pH and the substance to be hydrogenated were kept constant while the catalyst, prepared three different ways, was varied. The results are summarized in the next table.

TABLE IV

INFLUENCE OF CATALYST ON THE HYDROGENATION RATE

Description of Catalyst (10%)	Substance	Amount (Start)	Amt.recov.on hydrogenation	% Hydrogenated
Neutral - Pd-C	AA	2.6 grams	1.6	38
Hartung's Pd-C	AA	2.6	0.7	73
Ammonia Pd-C	AA	2.6	None	100

It can be seen that the difference in activity between

Hartung's Catalyst and the Ammonia Catalyst is not too marked, while there is a significant difference between the neutral catalyst and the other two. Hence, the method of preparing the catalyst influenced the rate of the hydrogenation.

Experiment C

This experiment had as its objective, the study of the effect of the pH on the rate of hydrogenation. Three and three-tenths grams (.01 mol.) of PA.B. was dissolved in 35 ml. of 95 per cent ethanol and an equal volume of water was added. The solution became cloudy. Three grams of 10 per cent Pd-C catalyst was added. In the run containing 3 ml. concentrated ammonia, the solution was clear. Time, one hour.

TABLE V

EFFECT OF pH ON THE RATE OF HYDROGENATION

Medium 75ml. H_2O-Ethanol containing	Amount (Started)	Amt.Recovered unreacted	% Hydrogenated
---	3.3 grams	3.0 grams	---
3 ml. conc. HCl	3.3	3.2	---
3 ml. conc. NH_3	3.3	2.2	33

The use of aqueous ethanol was necessary to bring PA.C. into solution. It does not seem reasonable that the presence of ethanol in this experiment was the rate determining

factor, but rather the pH. It may also be argued that in the case of the neutral and acid media runs, the P.A.G. was not completely dissolved but in a colloidal state.

From the above experiments it may be concluded that optimum conditions for the hydrogenation of a benzyloximino group which is alpha to a carbonyl, carboxyl, or carbamido group would be: 1) hydrogenation in a solvent in which both the unhydrogenated and hydrogenated products are quite soluble, 2) use of a Pd-C prepared under alkaline conditions, 3) possibly an alkaline medium which might enhance the reactivity of Pd-C. No attempt was made to study the effects of temperature because of the lability of the peptide linkage towards hydrolysis. Studies involving increased pressures were not made.

Addendum

DL - Phenylalanyl - L(-) Leucine

Three and one-half grams (.009 mol.) of β-phenyl-α-benzyloximino-proplonyl-L(-) leucine was reduced using the ammonia catalyst (3.5 grams) in 100 ml. of water containing 3.0 ml. of a concentrated ammonia. The reduction took 75 minutes for a three-pound drop in pressure. Yield 2.3 grams (89%). The compound, after prolonged drying over P_2O_5, sintered at 180-182° and melted 225-227° (uncorr.). Fischer and Blank (94) reported two compounds for DL-phenylalanyl-leucine: "A" sintering at 186° and melting 196° (corr.);

"B" sintering at 210° and melting 224.5°. It would seem from our results that compound "B" corresponds to DL-phenylalanyl-L(-) leucine. Analysis, calculated for $C_{15}N_{22}N_2O_3$; N, 10.1 per cent; found: 9.5, 9.7 per cent.

SUMMARY

1. Studies have been carried out on the preparation of peptides without the isolation of the intermediate acid chlorides. A diamide was prepared.

2. A study of the hydrogenation of the benzyloximino group was carried out. It was found that the peptides containing the benzyloximino group can be hydrogenated in aqueous ammoniacal medium in the presence of 10 per cent Pd-C catalyst and 10 per cent Ammonia Pd-C at room temperature and 50 pounds pressure.

3. A method for isolating free peptides was devised involving the use of aqueous ammoniacal media and evaporation. An amino acid, a dipeptide, and a tripeptide were prepared.

4. Semi-quantitative studies on the rate of hydrogenation of the benzyloximino group revealed that the rate was dependent upon the medium, pH, and mode of preparation of catalyst. A high alkaline pH and use of Pd catalysts prepared in an alkaline media gave optimal results in the hydrogenation.

5. Attempts to prepare higher polypeptides were unsuccessful. An unsuccessful attempt was made to crystallize fractionally a mixture of polyamides.

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