AN ABSTRACT OF THE THESIS OF

CHUN-WING LI (Name) for the MASTER OF SCIENCE (Degree)

in CHEMISTRY (Organic) (Major) presented on July 1, 1969 (Date)

Title: THE SYNTHESIS AND CYCLIZATION OF 2,4-DIAMINOBUTYRIC ACID

Redacted for privacy

Abstract approved: Dr. Bert E. Christensen

The objectives of this study were to isolate 2,4-diaminobutyric acid and to attempt cyclizing the acid and its disulfonamide derivative. The original synthetic method by Adamson was followed but a modified isolation procedure for the acid was employed.

Following the synthesis, the acidic solution was passed through Dowex-3 ion-exchange column twice to remove sulphuric acid and any unreacted glutamic acid completely. The eluate was then concentrated to obtain the 2,4-diaminobutyric acid or excess hydrochloric acid was added to ensure obtaining the dihydrochloride salt. Excess acid, which was used for the isolation however, left an unknown residue in the alcohol, possibly as the ethyl ester of the dihydrochloride.

The 2,4-diaminobutyric acid was characterized by preparation of the dipicrate.

Cyclization of the free acid was attempted with thionyl chloride

and phosphorus oxychloride respectively. However, the resulting dark and sticky resin did not give any cyclization product. Nevertheless, when acetic anhydride was employed, I-acetyl-3-acetamido-2-pyrrolidone was obtained in very low yield.

The disulfonamide derivative was synthesized. Treating this compound with either thionyl chloride or phosphorus pentachloride gave the lactam, 1-p-toluenesulfonyl-3-(p-toluenesulfonamido)-2-pyrrolidone.

The Synthesis and Cyclization of 2,4-Diaminobutyric Acid

by

Chun-Wing Li

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1970

APPROVED:

Redacted for privacy

Professor of Chemistry

In charge of major

Redacted for privacy

Chairman of Department of Chemistry

Redacted for privacy

Dean of Graduate School

Date thesis is presented July 1, 1969

Typed by Clover Redfern for Chun-Wing Li

ACKNOWLEDGMENT

I wish to express my sincere gratitude to my major professor, Dr. Bert E. Christensen, for his supervision, understanding and guidance during the course of this study and in the preparation of this manuscript.

I am also grateful to my laboratory partner, Mr. B. E. Landberg, for his painstaking effort in the carbon-hydrogen analysis and encouragement during some of the most difficult times.

I also wish to thank Mrs. Redfern for typing the whole manuscript and corrections therein.

TABLE OF CONTENTS

LIST OF FIGURES

THE SYNTHESIS AND CYCLIZATION OF 2, 4-DIAMINOBUTYRIC ACID

INTRODUCTION

Historical

Much of the interest in the investigation of 2, 4-diaminobutyric acid stems from its discovery, in 1948-59, as the major constituent of the polymyxins and aerosporins both of which are produced by Bacillus polymyxa and Bacillus aerosporus (9, 17).

In 1901, Fischer (12) reported the earliest preparation of 2, 4-diaminobutyric acid via the β-phthalimido ethylmalonic ester.

By condensing sodium malonic ester with bromoethylphthalimide, followed by bromination with elementary bromine, the α-bromo-γ-phthalimidomalonic ester (Figure 1, I) was obtained. This in turn was decarboxylated, and aminated to the intermediate (Figure 1, II) which was then hydrolyzed and treated with aqueous oxalic acid. The racemic 2, 4-diaminobutyric acid was isolated in low yield as the crystalline oxalate, m. p. = 219° C.

Twenty-five years later, Karrer et al. (19) found that N-acetyl-glutamine (Figure 2, III) was converted into 2, 4-diaminobutyric acid by Hofmann's degradation reaction with bromine in aqueous barium hydroxide solution. The product was obtained as the oxalate in 18% yield, m. p. = 205° C.

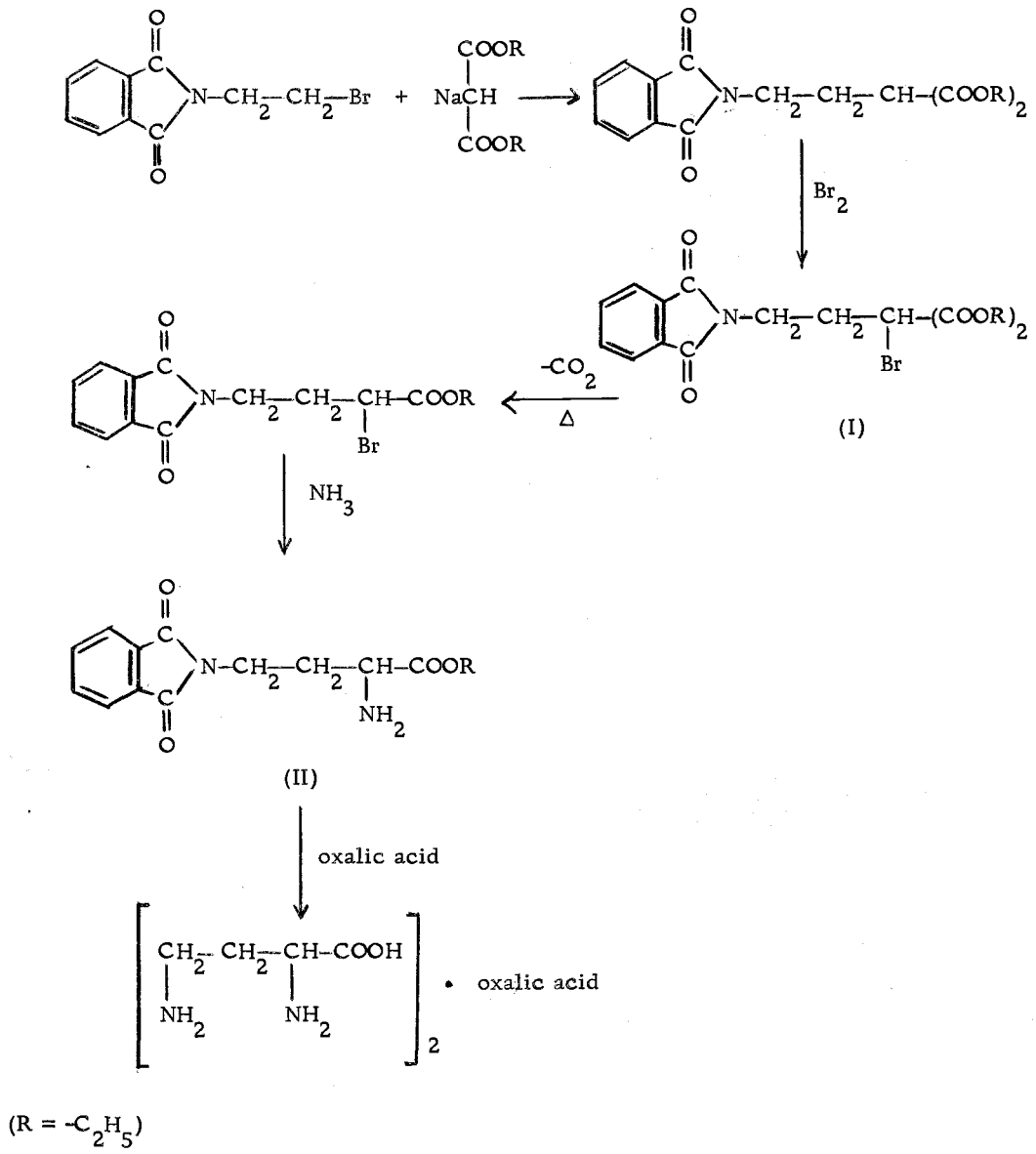
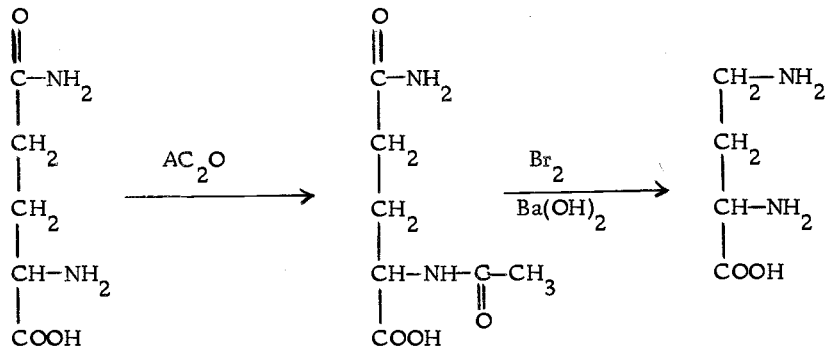


Figure 1. Synthesis of 2,4-diaminobutyric acid from β-phthalimido ethylmalonic ester by Fischer (12).



(III)

Figure 2. Synthesis of 2, 4-diaminobutyric acid from N-acetylglutamine by Karrer et al. (20).

Akabori and Numano in 1939 (3) adopted a slightly modified procedure. Glutamic acid was converted by potassium cyanate to α-carbamylglutamic acid (Figure 3, IV) and then with acid to the hydantoinpropionic acid (Figure 3, V). The acid was esterified in ethanol with hydrogen chloride, converted with hydrazine to the hydrazide (Figure 3, VI) and with nitrous acid to hydantoinpropionic acid azide (Figure 3, VII). Treating the azide with ethanol in turn gave the corresponding urethane (Figure 3, VIII) which in boiling barium hydroxide gave 2, 4-diaminobutyric acid. The final yield, however, was rather low, less than 20%.

In 1939, Synge (36) modified Karrer's (19) method further. N-acetylglutamine was treated with sodium hypobromite under the usual Hofmann procedure. The 2, 4-diaminobutyric acid was then precipitated by phosphotungstic acid. The precipitate was in turn decomposed by baryta, after which the barium was removed by excess

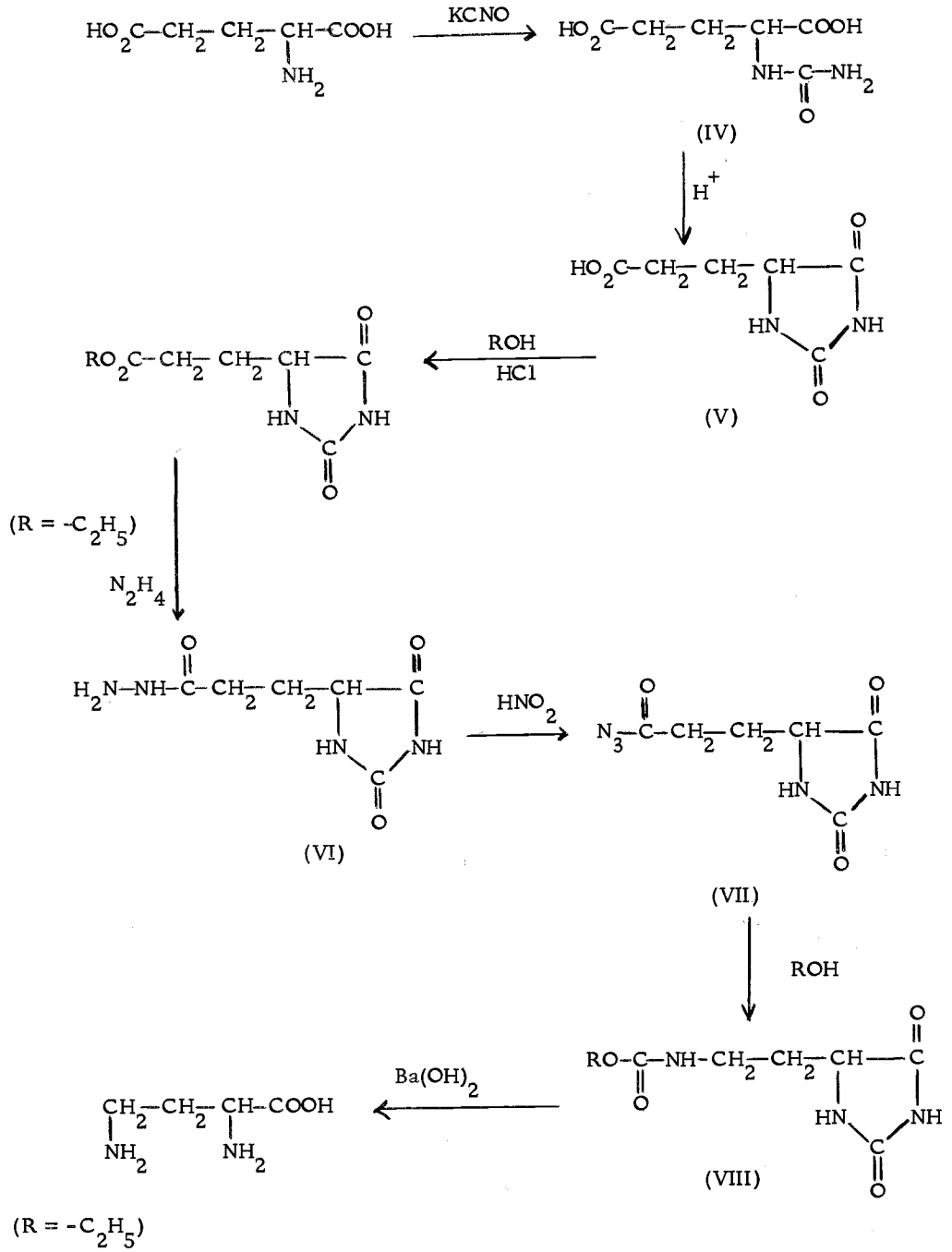


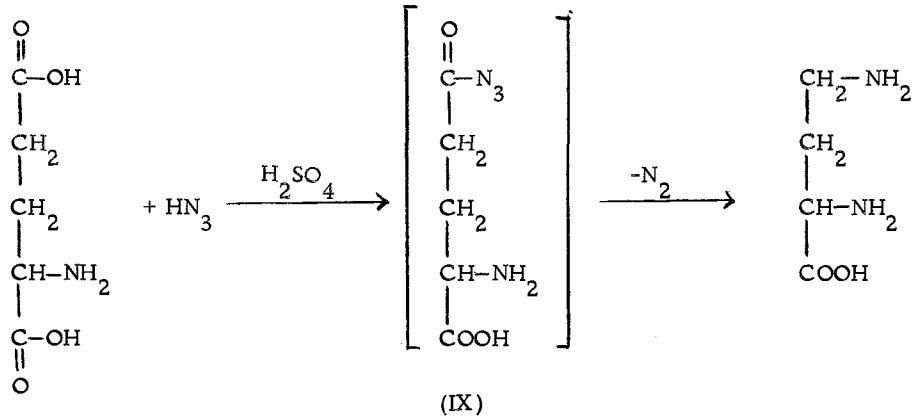
Figure 3. Synthesis of 2, 4-diaminobutyric acid from glutamic acid by Akabori and Numano (3).

sulphuric acid. On treating the resulting solution with flavianic acid, the crystalline 2, 4-diaminobutyric acid diflavianate was obtained in about 50% yield. Conversion of this salt to the oxalate, however, yielded three modifications in which the proportions of oxalic acid were 0. 5, 1. 0 and 1. 5 moles per mole of the 2, 4-diaminobutyric acid. This observation was the first reported discovery that three different salts of 2, 4-diaminobutyric acid exist.

Further improvement in the synthesis of 2, 4-diaminobutyric acid was reported shortly thereafter in the same year by Adamson (1). Considering the ease of converting carboxylic acids to the corresponding amines by the Schmidt reaction, coupled with the known stability of monoaminocarboxylic acids towards such treatment, the method was applied to α-amino-dicarboxylic acids, with a view to prepare basic amino acids which had been hitherto hard to synthesize. It was assumed that for these dicarboxylic acids, the protective effect of the α-amino group would be confined to the adjacent carboxyl group, leaving the other carboxyl group free to react with hydrazoic acid. Adamson thus successfully synthesized 2, 4-diaminobutyric acid and its higher homologs, such as ornithine and lysine. It was found that in general longer chain di-acids afforded better yields.

The method involved treating previously dried glutamic acid, which was dissolved in concentrated sulphuric acid, with a chloroform solution of hydrazoic acid. At a temperature of 43-6° C, the

intermediate azide (Figure 4, IX) rearranged to the diamine compound with loss of nitrogen. The sulphuric acid was then removed by hot saturated baryta and 2,4-diaminobutyric acid precipitated as the oxalate, m.p. = 216° C, or the dipicrate, m.p. = 180-1° C in 42% yield. The latter compound could in turn be converted to the dihydrochloride, m.p. 195-6° C.



(IX)

Figure 4. Synthesis of 2,4-diaminobutyric acid from glutamic acid and hydrazoic acid by Adamson (1).

The academic interests in 2,4-diaminobutyric acid received a tremendous impetus when, during the period 1948-59, several new amino acids, including 2,4-diaminobutyric acid itself, were discovered to be present in natural products and lower organisms. 2,4-Diaminobutyric acid was found to occur as the major constituent of polymyxins and aerosporins and related substances (9, 17, 31); in a number of other antibiotics (18, 22, 26, 27, 28) and in higher plants (13). In quick successions, 2,4-diaminobutyric acid was found to possess some extraordinary biological properties, e.g., it is being

concentrated specifically by certain cells and it is neurotoxic (10, 11, 31, 18).

A number of new and novel synthetic approaches to the new amino acid were immediately undertaken by a few investigators during the ensuing several years.

The existing preparative methods up to 1949 were, in most cases, tedious and afforded only poor yields. To overcome some of these difficulties, Carter et al. (8) first proposed a rather general synthetic procedure which could not only afford large scale preparation of 2, 4-diaminobutyric acid but also could be extended for the synthesis of many varieties of α, γ-diamino acids. The yields in all cases were very high.

The method involved coupling a diazoalkane (Figure 5, X) with an α-β-unsaturated ester (Figure 5, XI), giving a pyrazoline-3-carboxylic ester (Figure 5, XII) which was then reduced with hydrogen under high pressure in the presence of a Raney nickel catalyst.

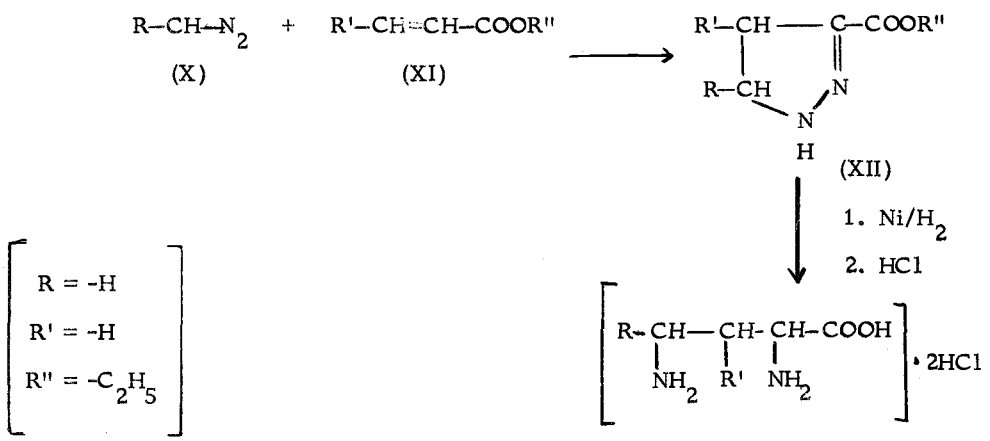


Figure 5. Synthesis of 2, 4-diaminobutyric acid by Carter et al. (8).

By catalytic hydrogenation of ethyl pyrazoline-3-carboxylate, which was prepared from ethyl acrylate and diazomethane followed by acid hydrolysis, 2, 4-diaminobutyric acid was isolated as the dihydrochloride in quantitative yield, m. p. = 200-1° C. During the process, a so-called sesquihydrochloride was encountered. This discovery again served to confirm fully the report by Synge (36) that three different oxalates of the diamino acid do occur.

In the same year, King and Kidd (20) refined Fischer's (12) procedure by first converting glutamine or glutamic acid to the N-phthalyl derivative (Figure 6, XIII) with phthalic anhydride in pyridine, followed by ring closure with acetic anhydride (Figure 6, XIV). Treatment with ammonia gave N-phthalylglutamine (Figure 6, XV) which by the usual alkaline hypobromite reaction produced α-N-phthalyl-2, 4-diaminobutyric acid (Figure 6, XVI). This compound, on acid hydrolysis, afforded 2, 4-diaminobutyric acid which could be isolated with picric acid as the dipicrate, m. p. = 184° C.

The next year, Le Quesne and Young (24) achieved another synthesis by converting glutamic acid to the γ-half-ester (Figure 7, XVII) in ethanol which was carefully purged with dry hydrogen chloride gas. This was followed by carbobenzyloxylation to N-carbobenzxyglutamic acid-γ-ethyl ester (Figure 7, XVIII). This in turn was converted to the γ-hydrazide, γ-azide and then through a Curtius degradation to the corresponding urethane (Figure 7, XIX). The

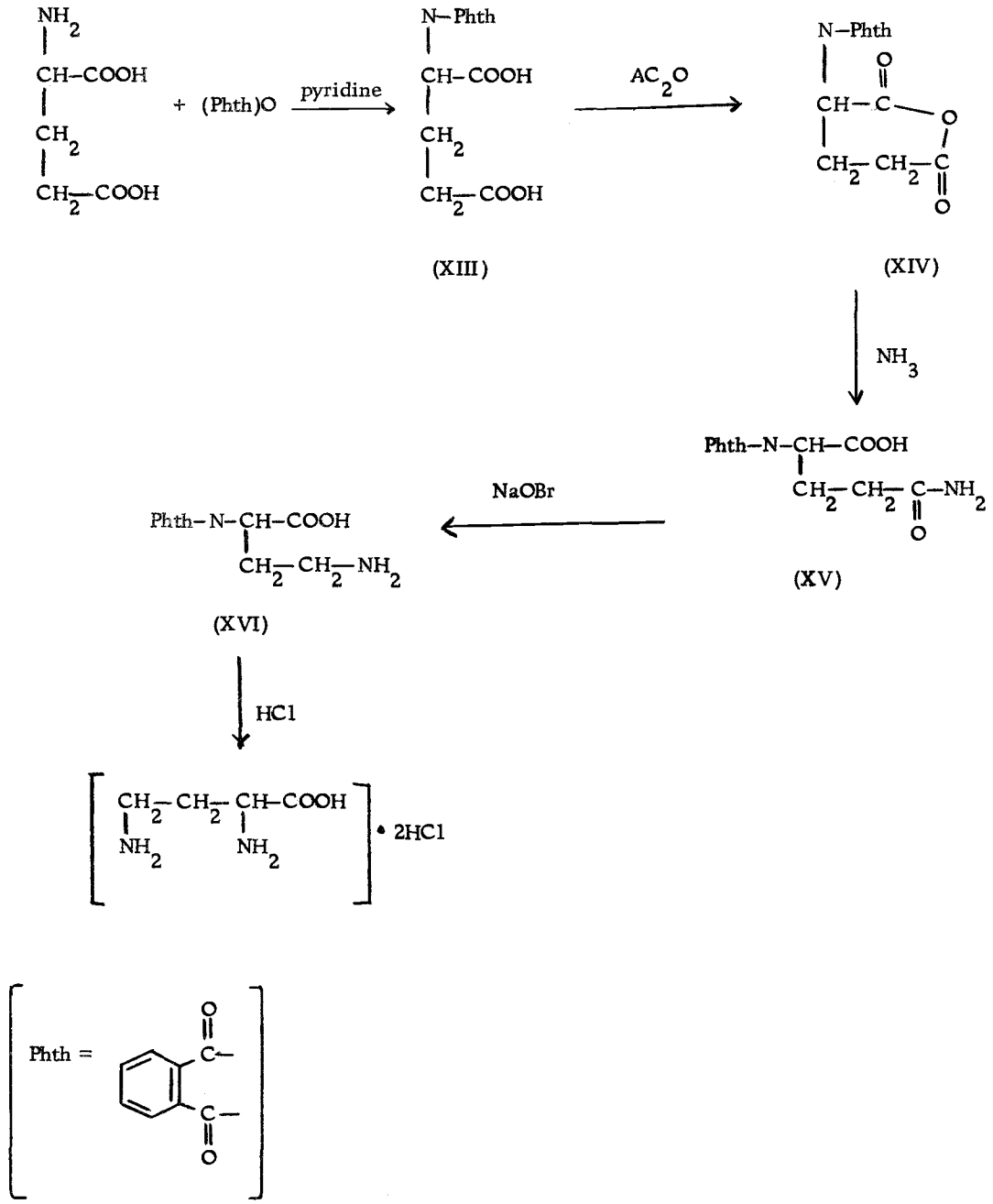


Figure 6. Synthesis of 2, 4-diaminobutyric acid from glutamic acid by King and Kidd (20).

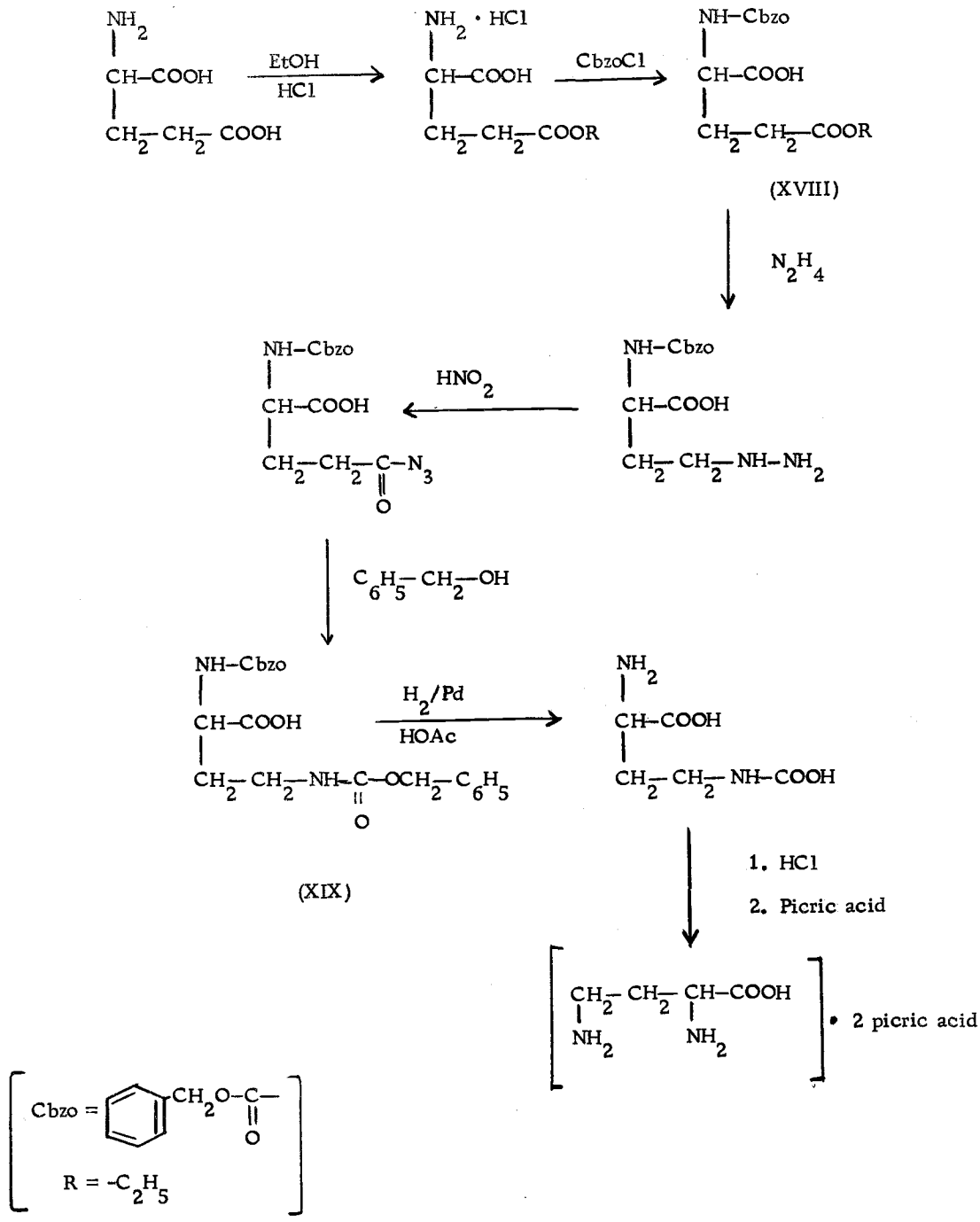


Figure 7. Synthesis of 2, 4-diaminobutyric acid from glutamic acid by Le Quesne and Young (24).

urethane was hydrogenated catalytically prior to acid hydrolysis. This sequence of reactions is illustrated in Figure 7. In spite of its novelty, the yield from this method was very low.

In 1951, the Schmidt reaction sequence, originally employed by Adamson, was again used to good advantage by Rothchild and Fields (32).

In this process, sodium ethyl malonate was condensed with β-chloropropionate. The resulting triester was then saponified to a tricarboxypropane (Figure 8, XX) which, on treatment with hydrazoic acid, yielded 2,4-diaminobutyric acid whose dipicrate melted at 187° C.

A year later, Akinson and Poppelsdorf (4) studied the phthalimido ester pathway and reported yet another synthetic procedure. Acrolein was condensed with phthalimide in Triton B to form β-phthalimidopropionaldehyde (Figure 9, XXI). This was then converted by the usual Strecker sequence to the amino nitrile (Figure 9, XXII). Refluxing with hydrochloric acid then afforded 2,4-diaminobutyric acid mono hydrochloride in 43% recovery.

In view of the success of acetamidocyanoacetic ester and related esters in simple amino acids synthesis, Kurihara and Ro (22) attempted to couple this versatile method with the equally well established phthalimido pathway. Accordingly, ethyl acetamidocyanoacetate was condensed with β-bromoethylphthalimide. Nonetheless, on

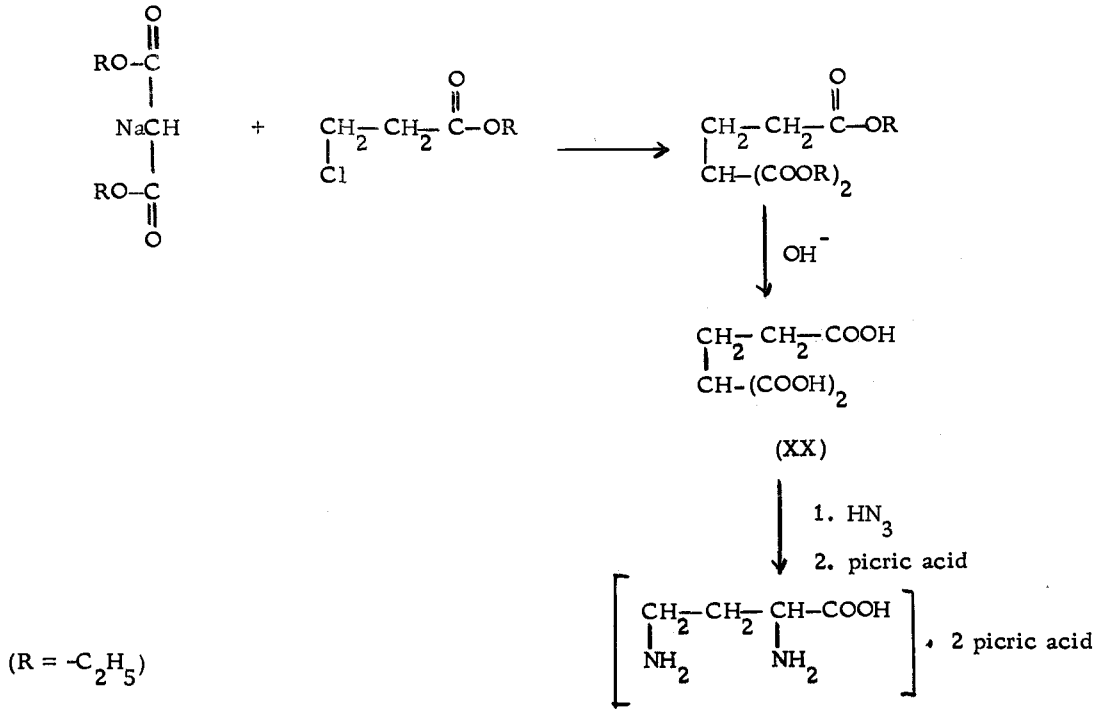


Figure 8. Synthesis of 2, 4-diaminobutyric acid by Rothchild and Fields (33).

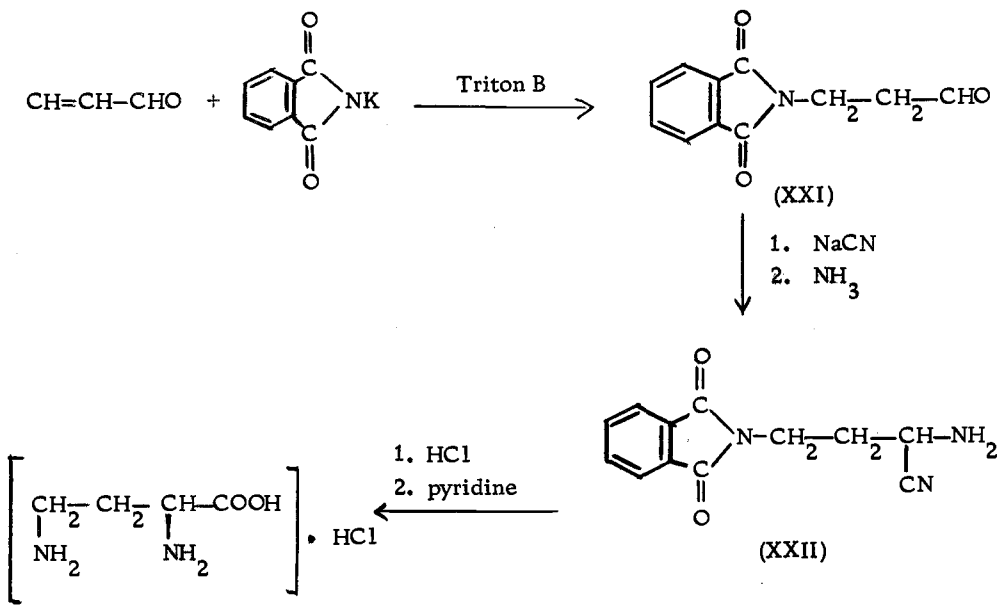


Figure 9. Synthesis of 2, 4-diaminobutyric acid from acrolein by Atkinson and Poppelsdorf (4).

subsequent hydrolysis, the expected 2, 4-diaminobutyric acid was obtained in very poor yield only.

It is interesting to note that at about this same period, the first successful resolution of racemic 2, 4-diaminobutyric acid into its optical enantiomorphs was achieved by Fu et al. (6, 15). This method involved the conversion of the sesquihydrochloride by chloroacetyl chloride into the racemic α, γ-dichloroacetyldiaminobutyric acid. Resolution of this compound was accomplished by an enzymatic route using acylase I. The precipitated γ-chloroacetyl-L-2, 4-diaminobutyric acid was then hydrolyzed with hydrochloric acid yielding the monohydrochloride of L-2, 4-diaminobutyric acid in 80% yield.

The corresponding D-α, γ-dichloroacetyl-2, 4-diaminobutyric acid was recovered from the resolution mother liquor through an ion-exchange process and then similarly hydrolyzed, giving the monohydrochloride of D-2, 4-diaminobutyric acid in also 80% yield.

In 1950, Watson et al. (39) successfully synthesized poly-L-glutamine from methyl α-poly-L-glutamate. Treatment with alkaline hypobromite, however, yielded 2, 4-diaminobutyric acid in only a 20% recovery.

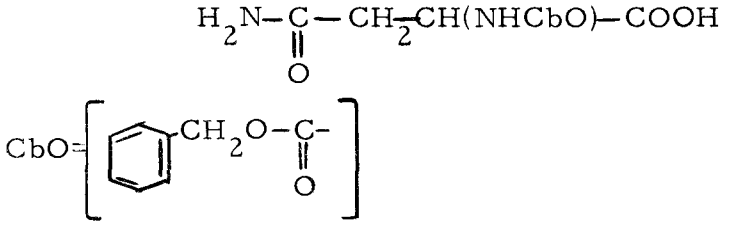
Three years later, Bruckner et al. (7) studied a similar system, poly-D-glutamic acid which occurs naturally. He reported virtually the same results.

Since 2, 4-diaminobutyric acid occurs in antibiotics, interest

continued to grow concerning its role in peptide chemistry.

In 1956, Ressler (30) first reported its formation from asparagine containing peptides. This prompted attempts to explain the presence of the diamino acid in nature via asparagine, which may be converted to an intermediate nitrile. Subsequent hydrolysis thus may give rise to 2,4-diaminobutyric acid.

This observation was closely studied in 1957 by Zoaral (41). The model then was to develop a route to peptides of polyfunctional amino acids by introducing into the peptide chain an intermediate which could be later converted to the desired end groups by suitable reactions. Using N-α-benzyloxycarbonyl-L-asparagine



it was found that direct dehydration by p-tosyl chloride in pyridine produced a cyano acid which was readily hydrolyzed to L-2,4-diaminobutyric acid under high pressure.

Careful literature search revealed that up to this point, the synthesis of 2,4-diaminobutyric acid involved in general three types of reactions:

a) insertion of an amine group via phthalimido esters.

b) degradations procedures via the Hofmann, Curtius or the Schmidt reactions.

c) the versatile method via pyrazoline-3-carboxylic ester.

Making use of the fact that γ-butyrolactone can be easily brominated (25), Talbot et al. (37) in 1958 proposed another method for the synthesis of 2, 4-diaminobutyric acid comparable in versatility as (c) above. This procedure afforded large scale preparation in over 80% yield.

The α-bromo-γ-butyrolactone was first treated with an equimolar quantity of phthalimide in boiling dimethylformamide. The cyclic 2-phthalimidobutyrolactone (Figure 10, XXIII) obtained was opened by a second equimolar quantity of phthalimide. Subsequent hydrolysis with concentrated hydrochloric acid produced the dihydrochloride of 2, 4-diaminobutyric acid which could be recrystallized from glacial acetic acid and hydrochloric acid.

Variations of the method were also reported by Frankel et al. (14). For example, (XXIV) could be converted to the acid bromide, esterified with ethanol and then refluxed with phthalimide in dimethylformamide, giving ethyl α, γ-diphthalimidobutyrate which on hydrolysis yielded the 2, 4-diaminobutyric acid.

Zaoral (42) reported another interesting refinement the next year. γ-Butyrolactone was reacted with phthalimide to give α-phthalimidobutyric acid (A),

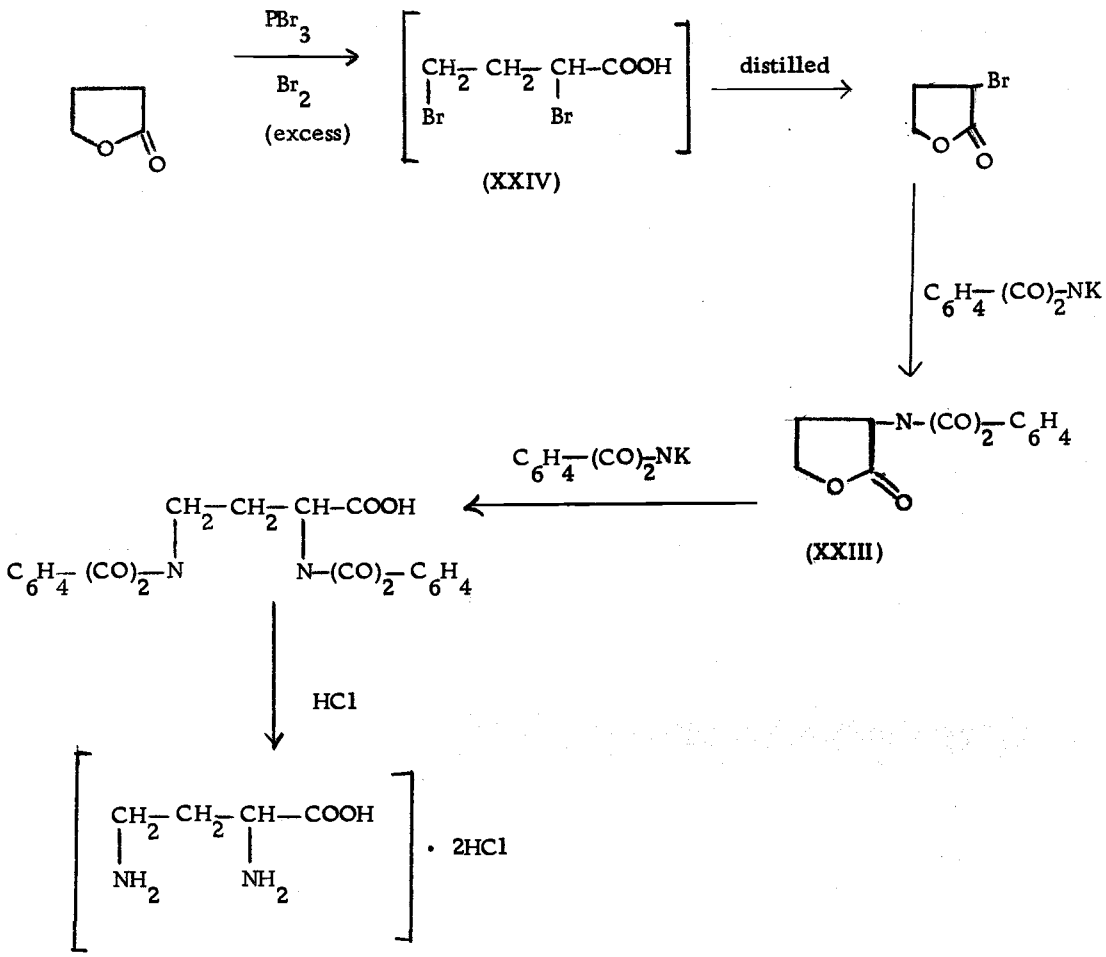
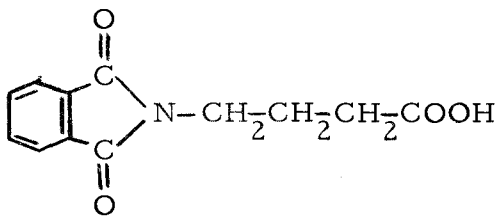
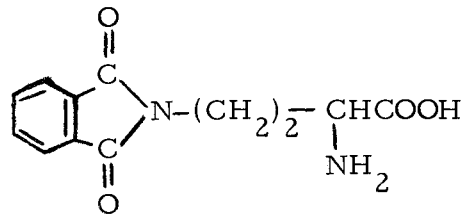


Figure 10. Synthesis of 2, 4-diaminobutyric acid from γ-butyrolactone by Talbot et al. (37).



(A)



(B)

which was brominated by the Gabriel method (16) and then to the corresponding α-bromo-γ-phthalimidobutyric methyl ester. The latter was easily converted to diphthalimido derivative. On acid hydrolysis, 2, 4-diaminobutyric acid was obtained in 40% yield as the dihydrochloride.

In turn, the brominated butyric acid above may be converted to the azido acid with sodium azide and then reduced to the amino compound (B) by hydrogenation over Adam's catalyst. Acid hydrolysis gave 44% yield of the 2, 4-diaminobutyric acid.

The realization by some workers in scattered reports that 2, 4-diaminobutyric acid could be cyclized to the corresponding 2-pyrrolidone led Smrt et al. (35) in 1959 to devise a scheme by which the relationship could be profitably utilized for a synthetic process.

The objective was to synthesize 3-amino-2-pyrolidone which could be easily decyclized to 2, 4-diaminobutyric acid.

Starting from α-aminobutyrolactone, α-amino-γ-bromobutyric acid was obtained by direct bromination. Reaction with hydroxylamine

produced the corresponding hydroxamic acid (Figure 11, XXV). Acetylation followed by hydrogenation effected ring-closure to 3-amino-2-pyrrolidone which was then hydrolyzed by hydrochloric acid to 2,4-diaminobutyric acid dihydrochloride in good yield.

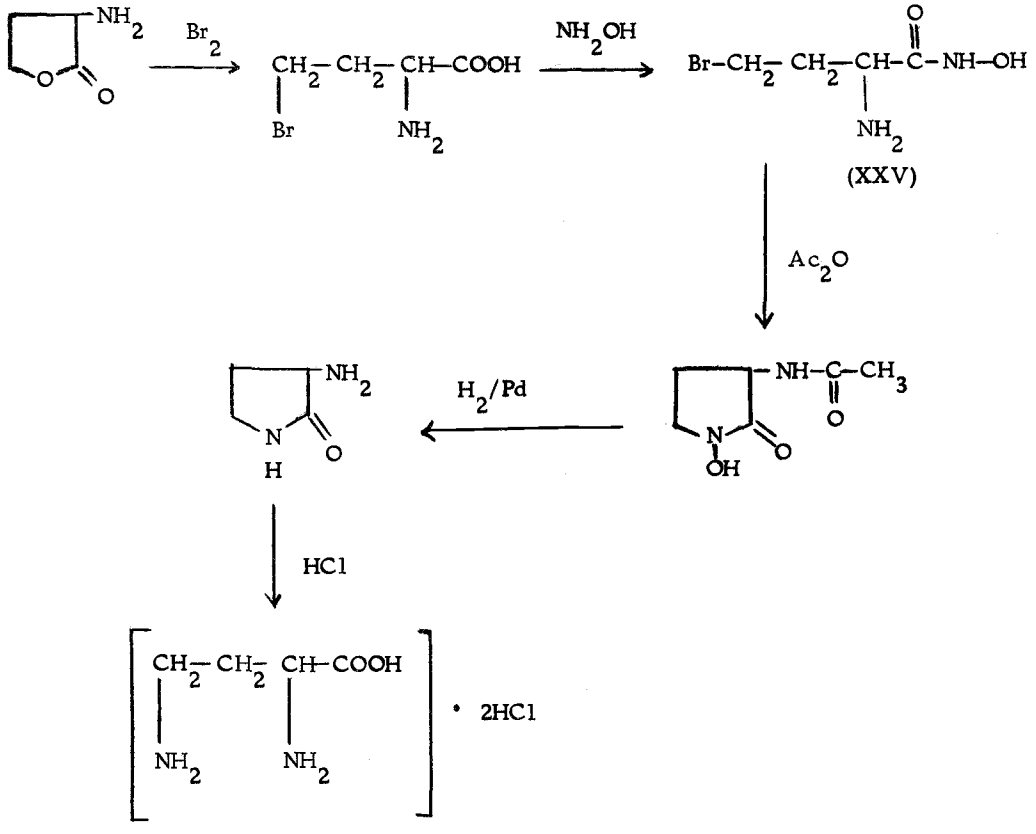


Figure 11. Synthesis of 2,4-diaminobutyric acid from α-amino-butyrolactone by Smrt et al. (35).

Sheradsky et al. (34) refined the procedure further. α-Bromo-butyrolactone was condensed with dibenzylamine to the

α-dibenzylamino-γ-butyrolactone. The latter compound was next decyclized by phthalimide and then hydrogenated. In this way, 2, 4-diaminobutyric acid was obtained in 85% yield.

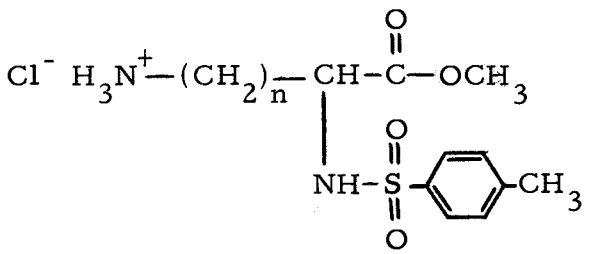
In 1943, during the investigation of the so-called lysine anhydride, Adamson (2) observed that, being the homolog of lysine, 2, 4-diaminobutyric acid could be cyclized to 3-amino-2-pyrrolidone by heating the methyl ester.

Six years later, Kurtz (23) reported similar cyclization reaction. When γ-phenylcarbamido-α-aminobutyric acid was evaporated to dryness with concentrated hydrochloric acid, 1-phenylcarbamyl-3-amino-2-pyrrolidone hydrochloride was isolated in 73% yield.

In 1951, Wilkinson (40) reported another similar reaction. When treated with phosphorus pentachloride in cold ethereal solution, α, γ-dicarbobenzyloxyaminobutyric acid gave 1-carbobenzyloxy-3-(carbobenzyloxyamino)-2-pyrrolidone. This latter compound could be hydrogenated in the presence of platinum in glacial acetic acid to give 3-amino-2-pyrrolidone. In addition, the ethyl ester dihydrochloride of 2, 4-diaminobutyric acid also was found to cyclize easily to 3-amino-2-pyrrolidone on treatment with sodium ethoxide at 0° C.

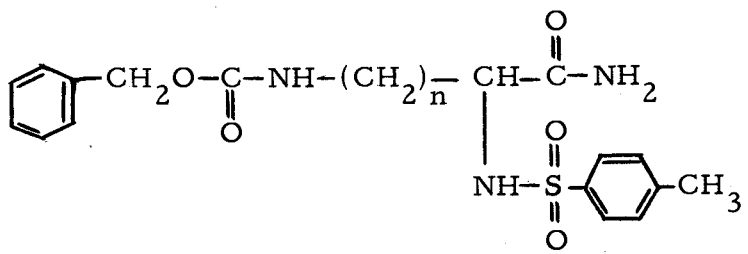
Rudinger (33) further substantiated the finding in 1954 by observing that the Curtius degradation of N-tosyl-L-glutamic acid γ-hydrazide led to the 3-tosyl lactam.

Finally, in 1957, Elmore and Barrass (5) found that the methyl ester hydrochloride of α-N-p-toluenesulfonyldiamino acids (C, n = 2, 3, 4)



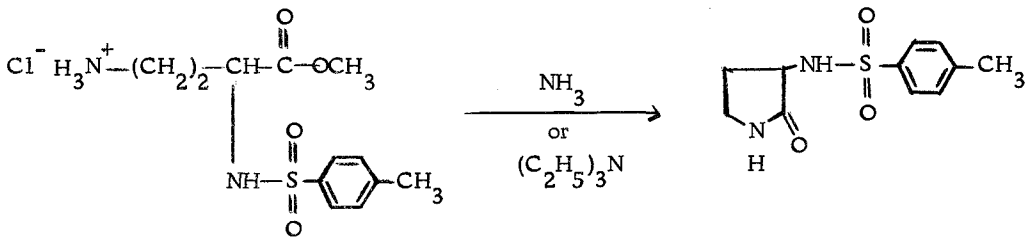
(C)

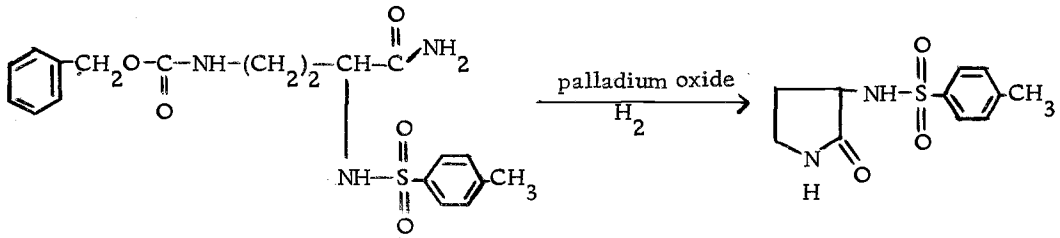
could be converted to the corresponding 3-tosyl lactams by reaction with ammonia or triethylamine. The lactams could also be obtained by hydrogenolysis of ω-N-benzyloxycarbonyl-α-N-p-toluenesulfonyl-diamino amides (D, n = 2, 3, 4) over palladium oxide in methanol containing a little acetic acid.



(D)

For example, when n = 2, the corresponding 2-pyrrolidone could be obtained.


Cl⁻ H3N⁺—(CH2)2—CH—C—OCH3
NH—S—CH3
NH3
or
(C2H5)3N
NH—S—CH3
N
H


CH2O—C—NH—(CH2)2—CH—C—NH2
NH—S—CH3
palladium oxide
H2
NH—S—CH3
N
H

DISCUSSION

The objectives of this study were to investigate a modified procedure to isolate the basic 2, 4-diaminobutyric acid and also to attempt cyclizing the acid itself and its disulfonamide derivative.

The major problem was to obtain pure 2, 4-diaminobutyric acid or its dihydrochloride salt. Most of the known methods to synthesize the amino acid were quite tedious and the yield obtained in most cases did not seem to justify the labor. Although the procedures of Carter et al. (8) and Talbot et al. (37) were very general synthetic approaches, operational difficulties hindered their employments. The method due to Carter involved high pressure hydrogenation while that due to Talbot involved the prior preparation of γ-bromobutyrolactone, the synthesis of which was a rather involved process in itself (25).

Of all the methods then, that due to Adamson (1) seemed the most promising for ready access to 2, 4-diaminobutyric acid. Adamson noticed that if a dicarboxylic amino acid was treated with hydrazoic acid, the α-amino group would protect the neighboring carboxyl group from being attacked while leaving the other more remote carboxyl group virtually free. During the Schmidt reaction, the rearrangement step took place without the necessity of isolating the intermediate.

Therefore, the synthesis of 2, 4-diaminobutyric acid was

carried out following the general procedure outlined by Adamson. It was necessary to maintain the temperature between 43-46° C since a higher temperature resulted in a decrease in yield (32).

In the original method, the removal of sulphuric acid and the isolation of 2, 4-diaminobutyric acid involved tedious and expensive precipitation procedures. Nowadays, it is possible to remove acids very readily by the proper choice of ion-exchange resins. Using such a modification, the isolation of 2, 4-diaminobutyric acid was accomplished in this laboratory.

Dowex-3 is a weakly basic resin which possesses an additional merit of being able to separate acidic amino acids such as glutamic acid and leaving the basic ones untouched. During the synthesis, 2, 4-diaminobutyric acid was obtained in sulphuric acid solution. By passing this solution through the column, the sulphuric acid and most of the unreacted glutamic acid would be removed. To ensure the complete removal of glutamic acid, the eluate was passed through another freshly prepared column. This new eluate could then be concentrated under vacuuo, azeotropic-distilled with benzene to remove the last traces of water and then freeze-dried to obtain the 2, 4-diaminobutyric acid. Or, the eluate could be converted to the dihydrochloride salt with concentrated hydrochloric acid.

Van der Horst (38) reported that 2, 4-diaminobutyric acid, under alkaline condition, was partly converted to the lactam, 3-amino-

2-pyrrolidone. Conceivably, during isolation and subsequent concentration, some of the very basic amino acid may be contaminated with the lactam. Nevertheless, thin layer chromatography indicated very little, if at all, any cyclization product.

Using such a method, 2, 4-diaminobutyric acid was isolated to yield 10 g batches of white solid, m. p. = 158-159^{o}C (capillary).

Interestingly, scattered reports indicated that 2, 4-diaminobutyric acid could give three different salts with either oxalic acid or hydrochloric acid. Synge (36) in 1939 first reported three different oxalates of the amino acid whereby the proportions of oxalic acid were 0. 5, 1. 0 and 1. 5 mole per mole of the 2, 4-diaminobutyric acid. Later, Carter et al. encountered a so-called sesquihydrochloride and observed that the 2, 4-diaminobutyric acid dihydrochloride could be converted to the monohydrochloride by repeated recrystallization from alcohol and water. Finally, in 1951, Wilkinson (40) reported that the dihydrochloride could be obtained by recrystallization from concentrated hydrochloric acid or from glacial acetic acid and concentrated hydrochloric acid. On careful recrystallization of the dihydrochloride from aqueous alcohol, the sesquihydrochloride was isolated. The monohydrochloride was prepared from the dihydrochloride on treatment with pyridine in aqueous alcohol.

Since 2, 4-diaminobutyric acid hydrochloride salt could exist in three modifications, a scheme was devised to obtain the pure

dihydrochloride salt. The column eluate was accordingly treated with a large excess of concentrated hydrochloric acid and the concentrated syrup obtained after evaporation under vacuuo was poured into hot absolute ethanol. By this method, 13.5 g of the dihydrochloride was isolated as a white crystalline solid. The use of excess of the acid, however, gave one drawback in that an unknown residue was retained in the alcohol. Since amino acids were known to esterify only in the presence of an equivalent of strong acid, it seemed plausible that the unknown might be the ethyl ester of 2,4-diaminobutyric acid dihydrochloride. A hydroxamate test showed the presence of ester although carbon and hydrogen analysis revealed deviations from the expected values.

In addition, to characterize the 2,4-diaminobutyric acid, the dipicrate was prepared.

Taking the melting points of all the products in capillary tubings probably accounted for their slight deviations from reported values.

The next major undertaking was to cyclize 2,4-diaminobutyric acid itself. Aside from Van der Horst, Poduska et al. (29) reported the interesting fact that extensive cyclization of the acid occurred in strong ammonia but only very slightly in 0.1 M ammonia at 80° C. The amide of 2,4-diaminobutyric acid was synthesized via the ester and was found to cyclize completely in 5 hours at 80° C.

In this laboratory, the cyclization was first attempted with thionyl chloride and phosphorus oxychloride, with an intention that the acid chloride intermediate would cyclize to the lactam, 3-amino-2-pyrrolidone. Contrary to expectation, in both cases, dark sticky resin was obtained which yielded no cyclized product on being sublimed.

Next, the cyclization was attempted with acetic anhydride. Wilkinson (40) reported a similar attempt in 1951 when 2, 4-diaminobutyric acid monohydrochloride was heated with acetic anhydride and sodium acetate. The resin obtained could be recrystallized from acetone and ether to give 1-acetyl-3-acetamido-2-pyrrolidone, m. p. = 132° C. The procedure was modified slightly in this laboratory by employing the dihydrochloride salt instead. However, the resin obtained could not be crystallized from acetone and ether, benzene or alcohol. It was sublimed and 1-acetyl-3-acetamido-2-pyrrolidone was recovered in very low yield. Similar observations were made when the 2, 4-diaminobutyric acid was employed for the cyclization reaction.

Other cyclization reactions involving derivatives of 2, 4-diaminobutyric acid have been reported (24, 2, 5). In these cases, either one or both of the amino groups of the acid was substituted or amides and esters of the acid were employed. In particular, Wilkinson (40) reported that α, γ-dicarbobenzyloxyaminobutyric acid

gave the corresponding lactam on treatment with phosphorus pentachloride. In this laboratory, 2,4-di-(p-toluenesulfonamido)butyric acid was prepared by reacting 2,4-diaminobutyric acid with p-toluenesulfonyl chloride in base. The resulting disulfonamide, when treated with thionyl chloride and phosphorus pentachloride, respectively, cyclized readily to 1-p-toluenesulfonyl-3-(p-toluenesulfonamido)-2-pyrrolidone in good yield.

EXPERIMENTAL

All melting points were determined with the aid of a Cole Parmer melting point apparatus and are uncorrected.

2, 4-Diaminobutyric acid was prepared using a modified scheme from the original method by Adamson (1).

Hydrazoic Acid

In a 250 ml three-necked flask equipped with a dropping funnel, mechanical stirrer and a thermometer inserted through a two-holed stopper, a paste was made from 16 gm of sodium azide and 16 ml of water. To the stirred paste was added 100 ml of chloroform. After cooling to 0° C, 6. 8 ml of concentrated sulphuric acid was added dropwise while the temperature was carefully controlled so as to remain below 10° C. At the end of the addition, 1 gm of anhydrous sodium sulphate was added and the solution allowed to stand in a refrigerator for 4 hours. The solution was then filtered and used immediately.

2, 4-Diaminobutyric Acid Dihydrochloride

L-glutamic acid was pre-dried in vacuuo for at least 24 hours. To 30 g of the dry acid dissolved completely in 55 ml of concentrated sulphuric acid which was well agitated, 100 ml of dry

chloroform was added, followed dropwise by the hydrazoic acid prepared previously. The temperature must be carefully controlled to 43-46° C. Nitorgen gas would evolve during the reaction. After the addition was completed, the stirring and temperature were maintained for a further 4 hours, during which all solid particles would dissolve. The chloroform was then separated with a separatory funnel. The sulphuric acid layer was carefully diluted to two liters with water in a large container.

A Dowex-3 ion-exchange column was prepared from a long glass tube fitted with a teflon stopper and at least 120 cm in length and 55 mm internal diameter. It was packed in the bottom with glass wool and 10 cm of Ottawa sand. Previously washed Dowex-3 resin (2 pounds) was then put into the column carefully to avoid any air bubble being trapped inside the resin. The top was then covered with a layer of sand. The resin was washed with water until relatively neutral. The aqueous solution prepared above was then added dropwise through a separatory funnel. The rate was carefully adjusted so that it equalled the rate of collection, about 5 to 10 ml per minute. The presence of the basic 2, 4-diaminobutyric acid was easily detected in the eluate by means of universal indicator paper (pH 8-12). After this removal of sulphate ions, the solution was passed through a freshly prepared column of Dowex-3 to remove any unreacted glutamic acid. The column was then washed with water at the end of the

elution until relatively free of the basic amino acid. During the elution, the collected eluate was evaporated in vacuuo. The bulk of the solution was brought to about 500 ml at which time 100 ml of concentrated hydrochloric acid was added. The still hot solution was decolorized with 8 g of norite, filtered, and concentrated to a syrup which was then azeotroped with 200 ml of dry benzene and immediately poured into 300 ml hot absolute ethanol. A white precipitate appeared and the mixture was agitated and then kept in a refrigerator overnight. The white crystalline 2, 4-diaminobutyric acid dihydrochloride was filtered and air-dried, giving a yield of 13. 5 gm, m. p. = 187-188° C (capillary). Adamson reported a value 195-196° C. The salt gave a strong bluish purple coloration reaction with alcoholic ninhydrin.

Calculated %:	C, 25. 1	H, 6. 28
Found:	C, 25. 2	H, 6. 40

The green alcohol filtrate from the preparation of the dihydrochloride was concentrated until cloudiness set in. The solution was cooled and the crystalline solid was filtered. The filtrate was reconcentrated and the solid again filtered. These steps were repeated often enough until finally a greenish yellow syrup remained. Concentration was then stopped and the thick syrup was put into a desiccator over phosphorus pentoxide under vacuum for at least three weeks. A greenish yellow, very hygroscopic and microcrystalline

solid was recovered at a yield of about 4 gm, m. p. = 184-186° C (melting block). The solid reacted with alcoholic ninhydrin to give a strong bluish purple color.

2, 4-Diaminobutyric Acid

The eluate from the Dowex-3 column, after the second elution, was concentrated in vacuuo to about 500 ml at which time it was decolorized with 8 g of norite and was then reconcentrated to almost dryness. At this stage, 200 ml of dry benzene was added portionwise to remove the remaining water by azeotropic distillation. The white solid obtained after this treatment was freeze-dried under vacuuo, filtered and kept in a desiccator over phosphorus pentoxide at reduced pressure. Alternatively, the white residue was put into a drying finger jacketed with boiling dioxane for five hours. The yield obtained was about 10. 9 gm, m. p. = 158-159° C (capillary) after initial softening at about 156° C. No literature reference value could be found. The compound gave a strong bluish purple coloration reaction with alcoholic ninhydrin.

Calculated %:	C, 40. 7	H, 8. 49
Found:	C, 40. 5	H, 8. 32

2, 4-Diaminobutyric Acid Dipicrate

One gram of the dihydrochloride dissolved in 5 ml of water was

mixed with 4 g of picric acid dissolved in 150 ml of water. The solution was allowed to stand overnight, after which the yellow crystals were filtered and recrystallized from a small amount of water, yielding 3 g of fine crystals, m. p. = 184-185° C (capillary). Adamson reported a value of 180-181° C.

1-Acetyl-3-Acetamido-2-Pyrrolidone

(A) From the dihydrochloride. A mixture of 4. 4 g 2, 4-diaminobutyric acid dihydrochloride, 40 ml of acetic anhydride and 2 gm of anhydrous sodium acetate was heated on a steam bath for 6-8 hours. After the heating, the deep brown solution was filtered and excess acetic anhydride was removed in vacuuo. The dark thick resin was then dried in vacuuo over phosphorus pentoxide for 4 hours, at the end of which the resin was transferred, with the aid of dry acetone, to a sublimation apparatus. After the removal of acetone by an air-jet, the resin was sublimed at 175° C and 3 mm of pressure, giving a white solid. The yield was 1. 7 gm, m. p. = 97-98° C (capillary). The reported value by Wilkinson (40) was 132° C. The product was very soluble in water and acetone but did not give a coloration reaction with alcoholic ninhydrin.

(B) From 2, 4-diaminobutyric acid. A mixture of 4 g, 2, 4-diaminobutyric acid, 30 ml of acetic anhydride and 2 gm of anhydrous sodium acetate was heated on a steam bath for 2 hours. After the

heating, following procedures analogous to (A) above, a white solid was obtained upon sublimation. The yield was 0. 52 gm, m. p. = 98-100° C (capillary).

Calculated %:	C, 52. 2	H, 6. 51
Found:	C, 52. 2	H, 6. 58

2, 4-Di-(p-Toluenesulfonamido)Butyric Acid

In a 125 ml flask, a solution of sodium hydroxide was made by dissolving 4. 5 gm of the pellets in 20 ml of water. To the cooled and stirred solution, 5 gm of 2, 4-diaminobutyric acid dihydrochloride was added, followed by a solution of 10 g p-toluenesulfonyl chloride in 50 ml of diethyl ether. The flask was then stoppered with a ground glass stopper which was well greased and wired across the top to make a good seal. The solution was stirred vigorously for about 12 hours. The ethereal layer was then separated from the aqueous layer which was cooled and acidified with 10% hydrochloric acid to about pH 4 with the aid of universal indicator paper. The inside wall of the container was scratched to induce the thick oil to crystallize. The mixture was then cooled in a refrigerator overnight, after which the white precipitate was removed by filtration, washed with cold water and air-dried. The crude yield was 9. 6 g. The derivative may be recrystallized from hot ethyl acetate. On cooling and addition of petroleum ether, the disulfonamide was obtained pure

white in over 90% recovery, m. p. = 146-147° C (capillary). Elementary analysis indicated the presence of sulphur. The product gave a bluish purple coloration reaction with alcoholic ninhydrin.

Calculated %:	C, 50. 8	H, 5. 17
Found:	C, 50. 9	H, 5. 14

1-p-Toluenesulfonyl-3-(p-Toluenesulfonamido)-2-Pyrrolidone

(A) Cyclization by thionyl chloride. 2. 9 g of the disulfonamide prepared previously was suspended in 30 ml of dry ethyl ether. From a dropping funnel, 10 ml of thionyl chloride was added dropwise to the stirred suspension, which was heated intermittently, until a clear solution resulted. Stirring was continued for an additional one hour, after which the ether was carefully evaporated with an air-jet to almost dryness. While still vigorously stirred, 50 ml of water was added to the solid. The resulting yellowish white mass was broken up and filtered, crude yield = 2. 6 g. The lactam may be recrystallized from ethyl acetate, followed by cooling and addition of petroleum ether, m. p. = 164-165° C (capillary). Elementary analysis indicated the presence of sulphur. The lactam gave no coloration reaction when treated with alcoholic ninhydrin.

Calculated %:	C, 53. 0	H, 4. 91
Found:	C, 53. 0	H, 4. 97

(B) Cyclization by phosphorus pentachloride. One gram of the disulfonamide was suspended in 50 ml of dry ethyl ether. To the cold

and vigorously stirred suspension, 6 g of phosphorus pentachloride was added. The mixture was then brought to gentle reflux for 1 hour. The ether was then carefully evaporated by means of an air-jet. This was followed by 50 ml of water added dropwise. The white mass was broken up and filtered, crude yield = 0.75 g. The lactam may be recrystallized as in (A) above, m.p. = 164-165° C (capillary).

Calculated %:	C, 53.0	H, 4.91
Found:	C, 52.9	H, 4.98

SUMMARY

The objectives of this study were to isolate 2, 4-diaminobutyric acid and to attempt cyclizing the acid and its disulfonamide derivative. The synthetic procedure due to Adamson was followed except for the isolation steps where a modified method was employed.

A Schmidt reaction was carried out by the action of hydrazoic acid upon glutamic acid in the presence of sulphuric acid. The resulting solution containing the 2, 4-diaminobutyric acid was passed through Dowex-3 ion-exchange column twice. The weakly basic resin was chosen because it also preferentially removed the acidic amino acid. By this method, any unreacted glutamic acid would be removed completely. The free 2, 4-diaminobutyric acid could then be obtained by concentrating the eluate, azeotropic-distilled the residue with benzene and then freeze-dried the remaining solid. Following this method, 2, 4-diaminobutyric acid was obtained as a white solid, m. p. = 158-159° C (capillary).

To ensure obtaining the dihydrochloride salt, the concentrated eluate was treated with excess concentrated hydrochloric acid and the syrup resulting after further concentration in vacuuo was poured into absolute ethanol. The white crystalline dihydrochloride salt obtained melted at 187-188° C (capillary). Use of excess acid, however, left an unknown, impure and hygroscopic solid in the alcohol.

This solid may be the ethyl ester of the dihydrochloride salt.

The 2,4-diaminobutyric acid was characterized by making the dipicrate from the dihydrochloride salt.

Cyclization of the free acid was attempted with thionyl chloride and phosphorus oxychloride respectively. In both cases, the resulting dark and sticky resin yielded no cyclization product on being sublimed. When acetic anhydride was employed, the resulting dark resin gave 1-acetyl-3-acetamido-2-pyrrolidone in very low yield upon sublimation.

The disulfonamide derivative was synthesized and was obtained as a white solid, m. p. = 146-147° C (capillary). The compound, on treatment with thionyl chloride and phosphorus pentachloride respectively, cyclized readily to give the lactam, 1-p-toluenesulfonyl-3-(p-toluenesulfonamido)-2-pyrrolidone, a white solid, m. p. = 164-165° C (capillary).

BIBLIOGRAPHY

1. Adamson, D. New synthesis of basic amino acids and lysine. Journal of the Chemical Society, 1939, p. 1564-1568.

2. Adamson, D. The anhydrides of basic amino acids. Journal of the Chemical Society, 1943, p. 39-40.

3. Akabori, S. and Shinji Numano. Synthese von Imidazolderivaten aus α-Aminosauren. IV. Eine neue Synthese von Histamin. Bulletin of the Chemical Society of Japan II: 214-217. 1936.

4. Akinson, R. O. and F. Poppelsdorf. A new synthesis of α, γ-diaminobutyric acid. Journal of the Chemical Society, 1952, p. 2448.

5. Barrass, B. C. and D. T. Elmore. The formation of cyclic lactams from derivatives of basic amino acids. Journal of the Chemical Society, 1957, p. 4830-4834.

6. Birnbaum, S. M. et al. Specificity of amino acids acylases. The Journal of Biological Chemistry 194: 455-470. 1952.

7. Bruckner, V. et al. The synthesis of poly-glutamine and its Hofmann degradation thereof. Journal of the Chemical Society, 1953, p. 1512.

8. Carter, H. E. et al. A general method for the synthesis of α, γ-diamino acids. The Journal of Biological Chemistry 178: 325-334. 1949.

9. Catch, J. R. et al. The chemistry of polymyxin A. The Biochemical Journal 43: xxvii-xxviii. 1948.

10. Christensen, H. N. and Thomas R. Riggs. Structural evidences for chelation and Schiff's base formation in amino acid transfer into cells. The Journal of Biological Chemistry 220: 265-277. 1956.

11. Christensen, H. N. et al. Intense concentration of α, γ-diaminobutyric acid by cells. The Journal of Biological Chemistry 198: 17-22. 1952.

12. Fischer, E. Synthese der α, γ-Diaminobuttersaure. Berichte der Deutschen Chemischen Gessellschaft 34:2900-2906. 1901.

13. Fowden, L. and Mary Bryant. Nitrogenous compounds and nitrogen metabolism in the Liliacease. The Biochemical Journal 70:626-629. 1958.

14. Frankel, M. et al. Introduction of γ-amino and γ-hydroxyl groups into α -aminobutyric acid via γ-reactive related compounds. Bulletin of the Research Council of Israel 7A:173-178. 1958.

15. Fu, S. C. J. et al. Enzymatic resolution of α, γ-diaminobutyric acid. The Journal of Biological Chemistry 199:207-215. 1952.

16. Gabriel, S. Über einige synthetisch verwertbare Derivate des Glycino und Berichte der Deutschen Chemischen Gesellschaft 40:2647. 1907.

17. Hausman, W. and Lyman C. Craig. Polymyxin B. Journal of the American Chemical Society 76:4892-4896. 1954.

18. Inoue, M. et al. Glumamycin, a new peptide-type antibiotic. Bulletin of the Chemical Society of Japan 33:1014-1015. 1960.

19. Karrer, P. et al. Über die Konfiguration der d-Glutaminsaure, des d-Ornithins und d-Lysins. Helvetica Chimica Acta 9:301-323. 1926.

20. King, F. E. and D. A. A. Kidd. A new synthesis of glutamine and of γ-dipeptides of glutamic acid from phthalylated intermediates. Journal of the Chemical Society, 1949, p. 3315-3319.

21. Kovacs, J. et al. Über die Bindungsart der amidierten Reste der β-Aminodicarbonsauren in Eiweibbstoffen. Experentia 11: 96-97. 1955.

22. Kurihara, T. and Katsuo Ro. Synthesis of α, γ-diaminobutyric acid. In: Third annual report of the Tohoku College of Pharmacy. Sendai, Japan, 1956. p. 77-81.

23. Kurtz, A. C. Use of copper (II) ion in masking α -amino groups of amino acids. The Journal of Biological Chemistry 180:1253-1267. 1949.

24. Le Quesne, W. J. and G. T. Young. Amino acids and peptides. An examination of the use of carbobenzyloxy-L-glutamic anhydride in the synthesis of glutamylpeptides. Journal of the Chemical Society, 1950, p. 1954-1959.

25. Livak, J. E. et al. Synthesis of dl-methionine. Journal of the American Chemical Society 67: 2218. 1945.

26. Martin, J. and W. K. Hausmann. Isolation and identification of D-α-pipecolic acid, α(L), β-methylaspartic acid and β, γ-diaminobutyric acid from the polypeptide antibiotic aspartocin. Journal of the American Chemical Society 82: 2079. 1960.

27. Oda, T. et al. Studies on colistin I. Journal of the Pharmaceutical Society of Japan 74: 1246. 1954.

28. Petersen, D. H. and L. M. Reineke. The chemistry of circulin, chromatographic isolation of the amino acid constituents with powdered cellulose. The Journal of Biological Chemistry 181: 95-108. 1949.

29. Poducka, K. et al. Intramolecular aminolysis of amide bonds in derivatives of α, γ-diaminobutyric acid, γ, β-diaminopropionic acid and ornithine. Collection of Czechoslovak Chemical Communications 30: 2410-2413. 1965.

30. Ressler, C. Formation of α, γ-diaminobutyric acid from asparagine-containing peptides. Journal of the American Chemical Society 78: 5956. 1965.

31. Riggs, T. R. et al. Amino acid concentration by a free cell neoplasm. Structural influences. The Journal of Biological Chemistry 209: 395-411. 1954.

32. Rothchild, S. and Melvin Fields. The preparation of diamino-acids via the Schmidt reaction. Journal of Organic Chemistry 16: 1080-1081. 1951.

33. Rudinger, J. Some derivatives of 1-p-toluenesulphonyl-L-pyrrolid-5-one-2-carboxylic acid. Collection of Czechoslovak Chemical Communications 2: 365-374. 1954.

34. Sheradsky, T. et al. Synthesis of selectively protected homoserine and α, γ-diaminobutyric acid. Journal of Organic Chemistry 26: 1482-1487. 1961.

35. Smrt, J. et al. N-Hydroxypyrrolidon-(2), ein neuer Pyrrolidonderivat-Typ. Collection of Czechoslovak Chemical Communications 5: 1672-1676. 1959.

36. Synge, R. L. M. The Hofmann degradation of glutamine residues in gliadin. The Biochemical Journal 33: 671-678. 1939.

37. Talbot, G. et al. Synthesis of 4-aminobutyric acid and 2, 4-diaminobutyric acid from butyrolactone. Canadian Journal of Chemistry 4: 393-595. 1958.

38. Van der Horst, C. J. G. Artefacts of ornithine and α, γ-diaminobutyric acid found during chromatographic investigation of rumen liquid. Nature 196: 147. 1962.

39. Watson D. et al. Synthetic polypeptides. Part II. Polyglutamic acid. Journal of the Chemical Society, 1950, p. 3239.

40. Wilkinson, S. α, γ-Diaminobutyric acid. Journal of the Chemical Society, 1951, p. 104-108.

41. Zoaral, M. and J. Rudinger. A new synthesis of derivatives and peptides of ornithine and diaminobutyric acid. Proceedings of the Chemical Society, 1957, p. 176-177.

42. Zaoral, M. Preparation of DL-diaminobutyric acid and its derivatives from γ-butyrolactone. Collection of Czechoslovak Chemical Communications 4: 1314-1319. 1959.