THE SYNTHESIS OF α-AMINO ACIDS

By

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INTRODUCTION

General

It is not unusual that the chemistry of amino acids should occupy an important position in modern biochemistry. As the "building blocks" of the protein molecule, amino acids are widely distributed throughout both the plant and animal kingdoms. Because of this universal prevalence, many applications of protein chemistry had been made long before the distinctive characteristics of this class of compounds had been recognized. For example, processes for the manufacture of dairy products, clarification of turbid solutions such as wines, tanning of hides, preparation of glue and the cooking of food are ancient applications of protein chemistry.

Despite these well known operations, the chemical story of the proteins and amino acids is one of slow but steady progress, and at the present is assuming accelerated motion. Like many phases of scientific endeavor, some of the discoveries came about as a result of chance, while others followed from long and painstaking research. Progress of the future will depend largely on the discovery of new chemical tools.

Discovery of Amino Acids

Since it is not within the scope of this paper to give a detailed history of the discovery of the many amino acids, only the outstanding advances will be considered. Table I

shows the amino acids with the dates and names of the original discoveries and investigators.

Recognizing the distinguishing characteristics of the protein molecule so common to the plant and animal cells, Mulder, a Dutch chemist, introduced the term, "protein". The etymology of the word may be traced to the Greek adjective, proteios, (primarius) meaning to hold first place. Since the amino acids are now recognized as of chief concern in the constitution of proteins, one may consider Braconnot's chance discovery of glycine in 1820 as the starting point of modern protein chemistry. In an attempt to find out whether gelatin would, like the various carbohydrates, yield sugars on hydrolysis, Braconnot subjected it to the action of sulfuric acid and isolated a crystalline material having a sweet taste. Without discovering that the product contained nitrogen, he named it "sucre de gelatin" which became the German "Leimzucker". The exact composition was not determined until 1858, when Mulder carried out a complete analysis.

Two amino acids, whose discovery, however, did not come about by a purposeful splitting of the protein molecule, were found prior to 1820. Wollaston isolated cystine from a urinary calculus in 1810, and Proust, while investigating the fermentation process involved in cheese making, obtained leucine.

For some fifty years after the observation of Braconnot that proteins can be hydrolyzed by acids into simple crystalline substances, the amino acids discovered were more or less

chance products of fractional crystallization from the hydrolysates. It remained for Ritthausen, in 1868, to introduce a new experimental method into protein chemistry. This technique involved the precipitation of the barium or calcium salt of the amino acids from an alcoholic solution.

The next significant step came in the use of enzymatic action to split the protein molecule. Although it was observed as early as 1859 that pepsin could be used to hydrolyze proteins partially, it was not until 1875 that Kühne was able to show that trypsin would carry the cleavage to the amino acid stage. An examination of tissues in which proteolytic splitting had taken place, revealed new amino acids.

No real advances in the systematic isolation of all the products of protein hydrolysis were made until Emil Fischer, in 1901, showed that most of the neutral and acid amino acids formed esters which could be separated without appreciable decomposition by fractional distillation in vacuo. A further innovation was introduced by Dakin, who fractionally crystallized neutral solutions of protein hydrolysates from butyl alcohol.

With the isolation of threonine from oat protein as recently as 1926, it is evident that the search for new naturally occurring amino acids is by no means closed.

The Historical Aspects of Protein Chemistry

The development of protein chemistry began with a so-called descriptive stage. This period was marked by the

TABLE I

Historical Data on the Amino Acids[1]

Amino Acid	Isolation Through Protein Hydrolysis			Synthesis	
	Investigator	Protein	Date	Investigator	Date
Alanine	Weyl	Silk fibroin	1888	Strecker	1850
Arginine	Hedin	Fibrin	1895	Erlenmeyer. Lipp	1882
Aspartic Acid	Ritthausen	Legumin	1868	Piutti	1887
Cystine	Mörner	Horn	1899	Erlenmeyer	1903
Glutamic Acid	Ritthausen	Gliadin	1866	Wolff	1890
Glycine	Braconnot	Gelatin	1820	Perkin. Duppa	1858
Histidine	Hedin. Kossel	Sturin	1896	Pyman	1911
Hydroxy- glutamic Acid	Dakin	Casein	1919	Dakin	1919
Hydroxy- proline	Fischer	Gelatin	1902	Leuchs	1905
Iodogorgoic Acid	Drechsel	Coral	1896	Wheeler. Jamieson	1905
Isoleucine	Ehrlich	Fibrin	1904	Bouveault. Locquin	1905
Leucine	Braconnot	Muscle fibre	1820	Limpricht	1855
Lysine	Drechsel	Casein	1889	Fischer. Weigert	1902

[1]The data concerning the history of the amino acids and proteins were compiled from material available in modern textbooks on Physiological Chemistry (1,2,3,4,5,6).

TABLE I (cont'd.)

Historical Data on the Amino Acids

Amino Acid	Isolation Through Protein Hydrolysis			Synthesis	
	Investigator	Protein	Date	Investigator	Date
Methionine	Mueller	Meat infusion	1921	Barger. Coyne	1928
Norleucine	Thudichum	Neuro-plastin	1901	Hüfner	1870
Phenylalanine	Schulze	Squash seed	1881	Erlenmeyer. Lipp	1882
Proline	Fischer	Casein	1901	Willstätter	1900
Serine	Cramer	Silk gelatin	1865	Erlenmeyer	1902
Threonine	Schryver. Buston	Oat protein	1926	Carter	1936
Thyroxine	Kendall	Thyroid gland	1915	Harington. Barger	1927
Tryptophane	Hopkins. Cole	Casein	1901	Ellinger. Flamand	1907
Tyrosine	Liebig	Casein	1846	Erlenmeyer. Lipp	1882
Valine	Fischer	Casein	1901	Lipp	1880

findings of the middle eighteenth and early nineteenth century chemists, who isolated proteins from both animal and vegetable sources. Thus the earliest account reveals the isolation of gluten from wheat flour by Beccari in 1747.

It was in 1859, however, that actual progress was made in the field. Ritthausen at this time began an extended series of investigations on vegetable proteins. Although his contributions were inadequate owing to the lack of any definite knowledge of protein structure, Ritthausen laid the foundations for the more accurate work to follow.

Making use of these investigations, a newcomer brought about even greater advances in protein chemistry. Thomas B. Osborne, studying the problem from 1895 until his death in 1929, is recognized as an outstanding authority in this field of research. To Osborne are credited many improvements in the methods of isolation, purification and analysis of vegetable proteins.

Following the isolation of proteins in pure form, came studies on their composition. Through early inaccurate analyses, certain erroneous concepts had been advanced for the protein molecule. With the introduction of a method for the more exact determination of nitrogen in organic compounds, Dumas and Cahours, in 1842, were able to shed new light on protein composition and refute earlier misconceptions.

The now accepted peptide concept of the protein molecule, that is, union of the amino acids by amide linkage, had its

origin in certain clues established by Schaal in 1871 and Curtius in 1883. As a result of these preliminary clues, Emil Fischer and his pupils in 1901 began a long and brilliant series of researches on the synthesis of a great many peptides. This work established beyond a doubt the amide linkage in protein structure.

During the nineteenth century, organic chemistry dominated chemical science. Toward the end of that period, however, physical chemistry began to assume a more prominent position. It was during this period that the application of physico-chemical methods to the study of protein behavior appeared. Among the outstanding men of this phase are Hardy, who introduced the conception of "isoelectric point", Bjerrum, who developed the "zwitterion" theory, and Sörenson, who demonstrated the full importance of hydrogen ion concentration in its relation to proteins.

Thus, the coordinated effort of all branches of the chemical sciences has served to carry protein chemistry to its present high state of development.

The Term "Amino Acid"

Defined by the organic chemist, an amino acid is any organic acid having one or more substituent amino groups. It also may be considered as a substituted ammonia containing one or more organic residues. It is quite evident that this definition, when applied generally, includes a tremendous number of possibilities. However, the term "amino acid", as

used in biochemistry and nutrition, applies to a limited group of compounds. This group includes the amino acids which are constituents of proteins, i.e., the twenty-three generally accepted amino acids, and those acids whose existence in proteins has been reported but not verified. Certain postulates have been set forth as fundamental criteria on which the acceptance of an amino acid should be based. The amino acid must be isolated from protein hydrolysates; the isolation must be confirmed by an investigator other than the discoverer; the constitution of the amino acid must be proved by synthesis; and the synthetic product must be identical with that from natural sources.

Of the compounds indicated by this restriction in the definition of "amino acid", all have the basic nitrogen alpha to the carboxyl and are usually designated as " α-amino acids". As stated before, the proteins are considered to be composed of amino acids combined through an amide linkage into peptides. These chains of amino acids are then held in chemical union by some force, the nature of which is unknown.

Classification and Constitution of the Accepted Amino Acids

At this date, twenty-three amino acids have been accepted. By reference to Table II, which gives the structural formulas of the accepted amino acids, it is evident that all are alpha amino derivatives of relatively short chain aliphatic acids. Proline and hydroxyproline may be considered as cyclized derivatives of α-amino-valeric acid. By inspection of their

structural formulas, it is apparent that many of the amino acids are derivatives of alanine. Serine, threonine, tyrosine, phenylalanine, diiodotyrosine, tryptophane, histidine, thyroxine and cystine can all be considered as alanine derivatives. With the exception of glycine, all of the accepted amino acids have at least one center of asymmetry; cystine, hydroxyglutamic acid, hydroxyproline, threonine and isoleucine contain two asymmetric carbon atoms.

When classified according to their structure, the amino acids appear under the following groupings:

  I. Aliphatic Amino Acids

   A. Monoaminocarboxylic Acids

   B. Monoaminodicarboxylic Acids

   C. Diaminomonocarboxylic Acids

   D. Sulfur-containing Amino Acids

  II. Aromatic Amino Acids

  III. Heterocyclic Amino Acids

The fundamental step in determining the constitution of the products from protein hydrolysis is the purification of the unknown compound. This step is accomplished in a variety of ways. Contaminating inorganic salts may be removed by precipitating the amino acid as a heavy metal derivative or by recrystallization from an appropriate solvent. A derivative, from which the original acid can be regenerated, is often used to purify the compound. In some cases, purification is accomplished by fractionation at reduced pressures of the amino acid ester or by selective extraction by means of solvents.

TABLE II
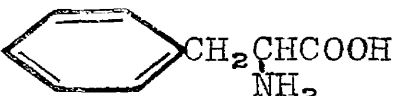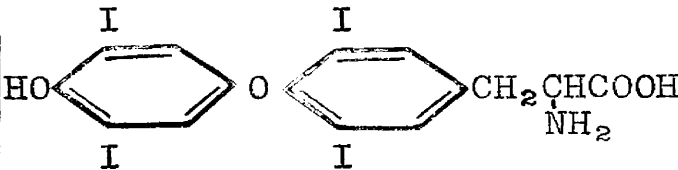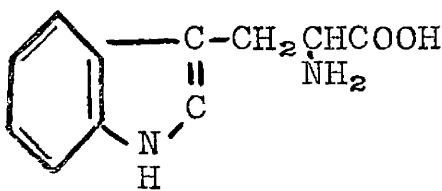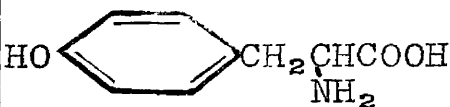
Names and Structural Formulas of the Amino Acids

Amino Acid	Structural Formula	Chemical Name
Alanine	$CH_3\overset{\displaystyle |}{\underset{\displaystyle NH_2}{C}}HCOOH$	α-amino-propionic acid
Arginine	$NH_2-\overset{\displaystyle \|}{\underset{\displaystyle NH}{C}}-NHCH_2CH_2CH_2\overset{\displaystyle |}{\underset{\displaystyle NH_2}{C}}HCOOH$	α-amino-δ-guanidino-valeric acid
Aspartic Acid	$HOOCCH_2\overset{\displaystyle |}{\underset{\displaystyle NH_2}{C}}HCOOH$	aminosuccinic acid
Cystine	$HOOC\overset{\displaystyle |}{\underset{\displaystyle NH_2}{C}}HCH_2S\!-\!SCH_2\overset{\displaystyle |}{\underset{\displaystyle NH_2}{C}}HCOOH$	di-α-amino-β-thiopropionic acid
Glutamic Acid	$HOOCCH_2CH_2\overset{\displaystyle |}{\underset{\displaystyle NH_2}{C}}HCOOH$	α-aminoglutaric acid
Glycine	NH_2CH_2COOH	aminoacetic acid
Histidine	$\begin{array}{c} NH-\overset{\displaystyle \|}{C}-CH_2\overset{\displaystyle |}{\underset{\displaystyle NH_2}{C}}HCOOH \\ CH \underset{N}{\diagdown\!\!\diagup} CH \end{array}$	α-amino-β-imidazoleprop-ionic acid
Hydroxy-glutamic Acid	$HOOCCH_2\overset{\displaystyle |}{\underset{\displaystyle HO}{C}}H\overset{\displaystyle |}{\underset{\displaystyle NH_2}{C}}HCOOH$	α-amino-β-hydroxyglutaric acid
Hydroxyproline	$\begin{array}{c} HO-CH - CH_2 \\ CH_2 \diagdown\underset{N}{}\diagup CHCOOH \\ H \end{array}$	γ-hydroxy-pyrrolidine-α-carboxylic acid
Iodogorgoic Acid	$\begin{array}{c} I \\ HO\!\!\diagdown\!\!\bigcirc\!\!\diagup\!\!CH_2\overset{\displaystyle |}{\underset{\displaystyle NH_2}{C}}HCOOH \\ I \end{array}$	3,5-diiodo-tyrosine
Isoleucine	$CH_3CH_2\overset{\displaystyle |}{\underset{\displaystyle H_3C}{C}}H\overset{\displaystyle |}{\underset{\displaystyle NH_2}{C}}HCOOH$	α-amino-β-methylvaleric acid
Leucine	$CH_3\overset{\displaystyle |}{\underset{\displaystyle CH_3}{C}}HCH_2\overset{\displaystyle |}{\underset{\displaystyle NH_2}{C}}HCOOH$	α-aminoiso-caproic acid

TABLE II (cont'd.)

Names and Structural Formulas of the Amino Acids

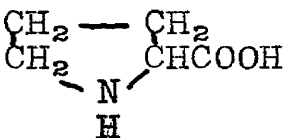| Amino Acid	Structural Formula	Chemical Name
Lysine	$NH_2CH_2CH_2CH_2CH_2\underset{NH_2}{C}HCOOH$	α-amino-ϵ-aminocaproic acid
Methionine	$CH_3SCH_2CH_2\underset{NH_2}{C}HCOOH$	α-amino-γ-methylthiol-butyric acid
Norleucine	$CH_3CH_2CH_2CH_2\underset{NH_2}{C}HCOOH$	α-amino-caproic acid
Phenylalanine	$CH_2\underset{NH_2}{C}HCOOH$	α-amino-β-phenylpropionic acid
Proline		pyrrolidine-α-carboxylic acid
Serine	$HOCH_2\underset{NH_2}{C}HCOOH$	α-amino-β-hydroxypropionic acid
Threonine	$CH_3\underset{HO\ \ NH_2}{C}HCHCOOH$	α-amino-β-hydroxybutyric acid
Thyroxine		β-[3,5-diiodo-4-(3',5'-diiodo-4'-hydroxyphenoxy)-phenyl]-α-amino-propionic acid
Tryptophane		α-amino-β-indolepropionic acid
Tyrosine		α-amino-β-(p-hydroxyphenyl)-propionic acid
Valine	$CH_3\underset{H_3C\ \ NH_2}{C}HCHCOOH$	α-amino-isovaleric acid

The melting point of an amino acid varies considerably under different experimental conditions as also does its crystalline form. Thus, the melting point and crystalline structure are not reliable criteria of purity. In place of the tedious method of recrystallization until the solubilities, specific rotations or specific conductivities are constant, the relative purity of a sample is more rapidly established by titration in glacial acetic acid with perchloric acid or by formal titration by means of the glass electrode.

The next step in determining the constitution of an amino acid, is to establish the empirical formula. No particular difficulty is encountered here as this can be accomplished by the usual ultimate analytical procedures.

The final and most difficult step is to determine the spatial arrangement of the atoms formed by analysis. Usually, certain degradations and chemical reactions give rise to definite clues. A study of the products resulting from the reaction of the amino acid with nitrous acid, permanganate, chlorine, hydrogen peroxide, alkalies, reducing agents and molds reveals the structural formula. After such deductions have been made, final proof must be supplied by synthesis. The synthetic compound should be identical with the racemized natural product; or, one of the optical forms of the synthetic should be the same as the natural amino acid in its chemical, physical and physiological properties.

With reference to the nomenclature now accepted for the

optically active forms of the amino acids, a significant

change has been made. In 1937, the following statement was

made by Sherman (7):

> "It has been customary to denote the naturally
> occurring form of the various amino acids by d- or l-,
> accordingly as they are dextro- or levorotatory. How-
> ever, recent studies have revealed the interesting
> fact that all of the amino acids which occur in pro-
> teins are stereochemically related, that is, there is
> an identical spatial arrangement of the groups about
> the alpha carbon atom in the natural form of the dif-
> ferent amino acids. For this reason, there is an
> increasing tendency among some investigators to desig-
> nate as the l-form that form of the amino acid which
> occurs in nature; to indicate the optical activity, a
> plus or minus sign is employed. For example, the new
> designation of naturally occurring alanine and serine
> would be l-(+)-alanine and l-(-)-serine, instead of
> the formerly d-alanine and l-serine, respectively."

The Importance of Amino Acids

Every living cell has protein as one of its components.

Thus all living material must have a supply of nitrogen

available. The green plant by virtue of its photosynthetic

capacities, converts inorganic into organic nitrogen. Mem-

bers of the animal kingdom form the proteins characteristic

of their own tissues but cannot build them up from simple

inorganic substances. Since proteins are essential for the

construction and repair or maintenance of their tissues, the

animals must depend upon the digestion products obtained from

the proteins of their food. Man is absolutely dependent upon

the plant kingdom for the amino acids which he needs for the

synthesis of his own characteristic proteins. Of course, some

of the ingested amino acids may have been derived from the

proteins of animals used by man as foods, but in the final

analysis, they were composed of amino acids obtained from plants.

The utter dependency of the vertebrates upon the plant kingdom is best illustrated by the fact that they cannot even remove the hydroxyl group from the para position of tyrosine to form phenylalanine. Animals feeding upon a diet complete with tyrosine showed deficiency symptoms when there was no phenylalanine in the diet.

Since the dietary proteins are apparently completely degraded to amino acids or to very simple peptides, prior to their conversion into tissue proteins, the question as to which of the component fragments can or cannot be synthesized by the body must be considered. In a series of brilliant researches, Rose has shown by feeding known mixtures of amino acids that at least ten amino acids derived from food sources are necessary for the growth of the white rat. These ten amino acids are lysine, valine, tryptophane, histidine, phenylalanine, leucine, isoleucine, threonine, arginine and methionine. To conduct an experiment on man similar to those conducted by Rose on rats would involve at the present time a prohibitive cost. The difficulty in proving which amino acids are essential and which are non-essential for the human organism lies in the trouble in securing foodstuffs in which the presence in adequate amounts or the total absence of the particular amino acids can be proven.

Two pronounced characteristics with their secondary effects are apparent in amino acid deficiencies. An extreme

lack of an essential amino acid in the diet leads to loss in body weight. This loss, which at first is rapid, continues until the animal dies.

The second aspect of amino acid deficiency is the decrease in food consumption. Apparently an animal confronted with a dietary shortage, automatically limits its total food to correspond metabolically with the restricted supply of the one indispensable factor. These conditions seem to result in a picture of partial starvation. However, although general symptoms of a debilitated state are manifested, demonstrations of irreparable tissue change and rapid physical change characteristic of certain vitamin deficiencies are notably absent.

Since the amino acids bear such a tremendous relationship to the nutrition and biochemistry of the normal individual, it is evident that any new information concerning these compounds is of great interest. An infinite number of unanswered problems indicates that the field of amino acid investigation is a fertile one and merits all the efforts of modern research chemistry.

Literature Survey of Methods of Synthesis of Amino Acids

Cyanohydrin Synthesis

The reaction of an aldehyde with ammonia, the subsequent treatment of the aldehyde-ammonia with hydrocyanic acid to form the aminocyanhydrin, and the hydrolysis of this compound to the amino acid was first carried out by Strecker (8) in 1850. The Strecker method, used to prepare alanine, glu-

tamic acid, glycine, serine, valine, phenylalanine, methionine, leucine, isoleucine, and norleucine, can be shown by the following outline equations:

$$RCHO + NH_3 \rightarrow R\underset{NH_2}{C}HOH \xrightarrow{HCN}$$

$$R\underset{NH_2}{C}HCN \xrightarrow[HBr]{H_2O} R\underset{NH_2}{C}HCOOH$$
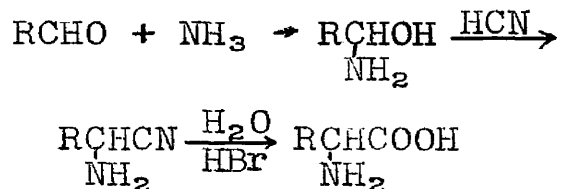
In a thorough study of the Strecker method, Cocker and Lapworth (9) in 1931, reported syntheses of several amino acids in yields averaging 70% of the theoretical.

Schmidt (5), in commenting upon the cyanohydrin synthesis, states the following:

"While the described syntheses of alanine, glycine, and serine are practicable procedures, the Strecker method has been little used for the preparation of other accepted amino acids. This may be explained by the technical difficulties involved in handling the low boiling, highly toxic, anhydrous hydrocyanic acid and the low yields which often result from the use of ammonium salts and alkali cyanides as employed in the modified processes for glycine and alanine. Furthermore, it is often difficult to obtain the required aldehyde and to prevent its polymerization to an unreactive resinous state."

Synthesis from α-Halogen Acids

Alanine, aspartic acid, glycine, isoleucine, leucine, norleucine and valine have been synthesized by a method first used by Perkin and Duppa (10), and Cahours (11) in 1858. This method, involving the conversion of α-halogen acids into the amino acids using ammonia, is illustrated by the following reactions:

$$RCH_2COOH \xrightarrow{Br_2 + P} R\underset{Br}{C}HCOOH \xrightarrow{NH_3} R\underset{NH_2}{C}HCOOH$$

A variation of this procedure uses ammonium carbonate as the aminating agent. Cheronis (12) made the important discovery that for the preparation of glycine, heating chloroacetic acid and ammonium carbonate in a molal ratio of 1:4 at 60 to 65° is superior to the method using ammonia in 1:60 ratio. While the Cheronis method is considered to be superior to any other for the synthesis of glycine, it is less satisfactory for the preparation of other amino acids.

Although these reactions are usually quite satisfactory, certain objections can be made for their use as a general method of synthesis. The amination step in this synthesis requires much investigation before optimum conditions are found and large ratios of ammonia to the halogen acids must be used.

The use of α-halogen acids in a synthesis involving potassium phthalimide was described in 1888 by Goedeckemeyer (13). A year later, a detailed study of the reaction was made by Gabriel and Kroseberg (14). The following equations indicate the steps in this synthesis:

$$\text{(phthalimide)}\begin{smallmatrix}CO\\CO\end{smallmatrix}\!\!>N\text{-}K \quad + \quad R\underset{Cl}{C}HCOOC_2H_5 \longrightarrow \text{(phthalimide)}\begin{smallmatrix}CO\\CO\end{smallmatrix}\!\!>N\text{-}\underset{R}{C}HCOOC_2H_5 \xrightarrow{KOH}$$

$$\text{(phthaloyl)}\begin{array}{c}\text{COOK}\\ \text{CONHCHCOOK}\\ \overset{|}{\text{R}}\end{array} \xrightarrow{\text{HCl}} \begin{array}{c}\text{RCHCOOH}\\ \overset{|}{\text{NH}_2}\end{array}$$
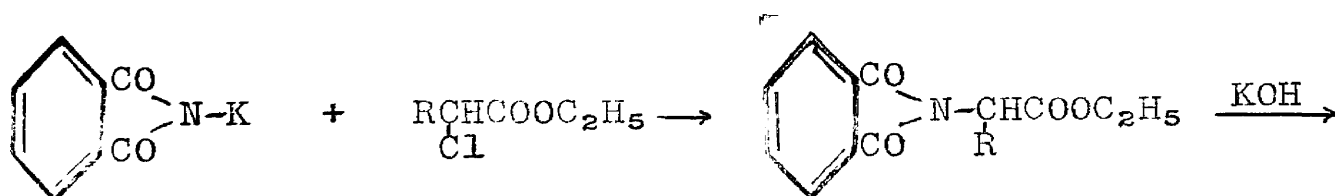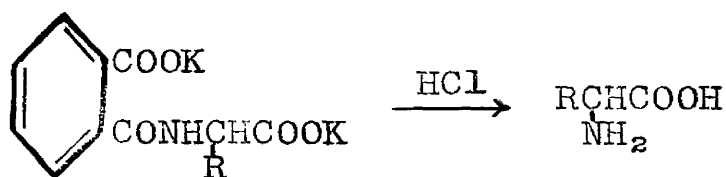
The phthalimide synthesis has its chief merit in the fact that the products are obtained in high yields and are free from impurities. However, the size of the runs is limited and the material expense is rather high. Also, potassium phthalimide having a high molecular weight must be used in relatively large quantities, thus increasing the cost of the process.

In the above uses of α-halogen acids, one fact is quite apparent. The syntheses are only applicable if the intermediate α-halogen acid can be obtained. For the aliphatic acids low in the series, these intermediates are readily obtainable but in other instances, their synthesis is difficult.

Synthesis from Halogenated Malonic Ester

The reaction of malonic ester with halides, bromination and subsequent amination with ammonia has been used for the synthesis of alanine, histidine, isoleucine, leucine, lysine, methionine, norleucine, hydroxyproline, phenylalanine, proline and valine. The outline equations may be represented as follows:

$$\begin{array}{c}\text{COOC}_2\text{H}_5\\ \overset{|}{\text{CH}_2}\\ \overset{|}{\text{COOC}_2\text{H}_5}\end{array} \xrightarrow[\text{RX}]{\text{NaOC}_2\text{H}_5} \begin{array}{c}\text{COOC}_2\text{H}_5\\ \overset{|}{\text{RCH}}\\ \overset{|}{\text{COOC}_2\text{H}_5}\end{array} \xrightarrow{\text{KOH}} \xrightarrow{\text{HCl}}$$

$$\underset{\overset{|}{COOH}}{\overset{COOH}{\overset{|}{RCH}}} \xrightarrow{Br_2} \underset{\overset{|}{COOH}}{\overset{COOH}{\overset{|}{RCBr}}} \xrightarrow[140^\circ]{\text{heat to}} \underset{\overset{|}{R}}{\overset{COOH}{\overset{|}{HCBr}}} \xrightarrow{NH_3} \underset{\overset{|}{R}}{\overset{COOH}{\overset{|}{HCNH_2}}}$$
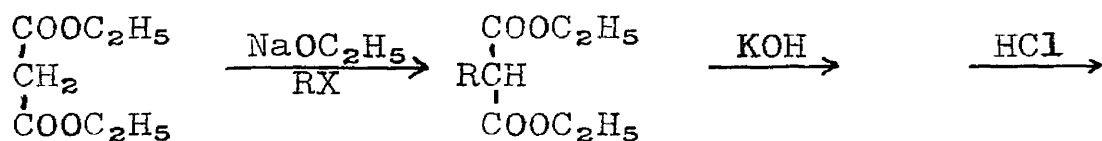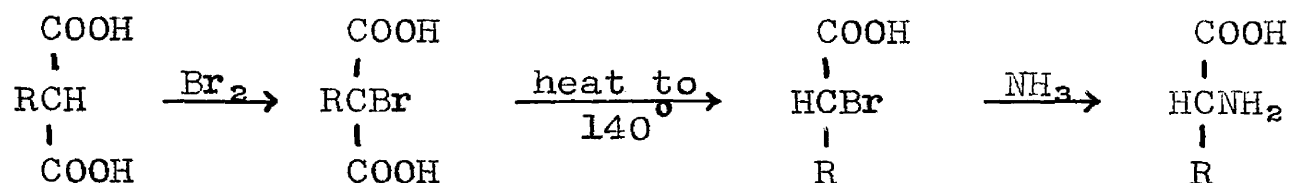
A combination of the above procedure with Gabriel's method was adapted by Sörensen (15,16,17) in 1903 for the synthesis of several amino acids. The equations for Sörensen's method are given below:

$$\underset{\overset{|}{COOC_2H_5}}{\overset{COOC_2H_5}{\overset{|}{CH_2}}} \xrightarrow[\text{in } CCl_4]{Br_2} \underset{\overset{|}{COOC_2H_5}}{\overset{COOC_2H_5}{\overset{|}{CHBr}}} \xrightarrow[\text{phthalimide}]{\text{potassium}} \text{(phthalimido)} N-\underset{\overset{|}{COOC_2H_5}}{\overset{COOC_2H_5}{CH}}$$

$$\xrightarrow[\text{in toluene}]{Na} \xrightarrow{RX} \text{(phthalimido)} N-\underset{\overset{|}{COOC_2H_5}}{\overset{COOC_2H_5}{CR}} \xrightarrow[(HBr)]{H_2O} \underset{\overset{|}{R}}{\overset{COOH}{\overset{|}{HCNH_2}}}$$
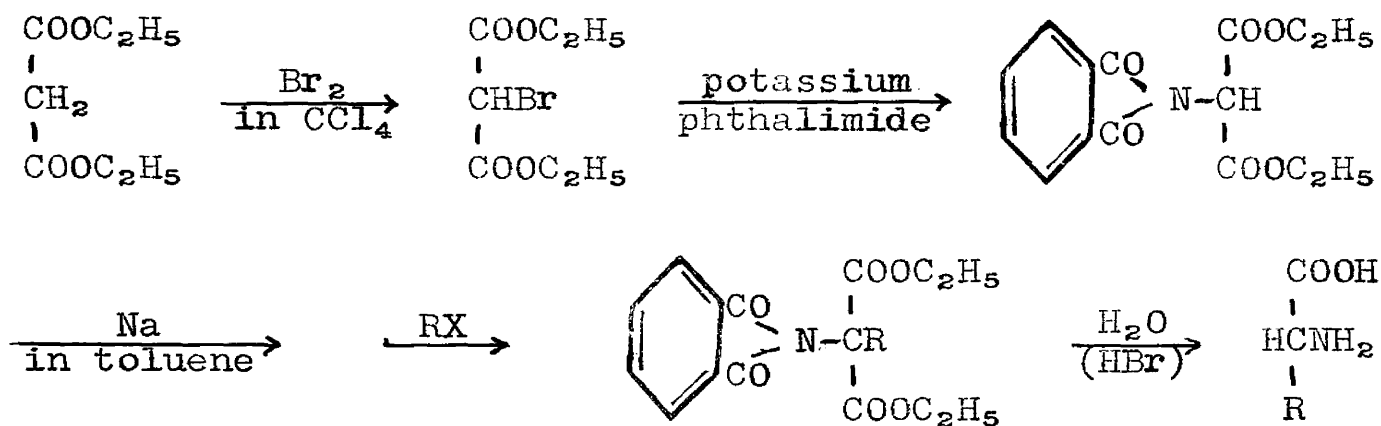
Although this synthesis has since been used to advantage in some cases, the method has certain outstanding objections as pointed out under Gabriel's procedure.

In 1939, Redemann and Dunn (18) reported a synthesis of α-amino acids using aminomalonic ester. This method involves the nitrosation of malonic ester and can be represented by these equations:

$$\underset{\overset{|}{COOC_2H_5}}{\overset{COOC_2H_5}{\overset{|}{CH_2}}} \xrightarrow[C_2H_5ONa]{C_4H_9ONO} \underset{\overset{|}{COOC_2H_5}}{\overset{COOC_2H_5}{\overset{|}{C=NOH}}} \xrightarrow[H_2]{Ni} \underset{\overset{|}{COOC_2H_5}}{\overset{COOC_2H_5}{\overset{|}{CHNH_2}}} \xrightarrow[\text{pyridine}]{C_6H_5COCl}$$

$$87\% \qquad 65\% \qquad 90\text{-}95\%$$

$$C_6H_5CONH-\underset{\underset{COOC_2H_5}{|}}{\overset{\overset{COOC_2H_5}{|}}{C}}-H \quad \xrightarrow[RX]{C_2H_5ONa} \quad C_6H_5CONH-\underset{\underset{COOC_2H_5}{|}}{\overset{\overset{COOC_2H_5}{|}}{C}}-R \quad \xrightarrow[(HBr)]{H_2O} \quad \underset{\underset{R}{|}}{\overset{\overset{COOH}{|}}{C}}HNH_2$$

60-90% 62-90%
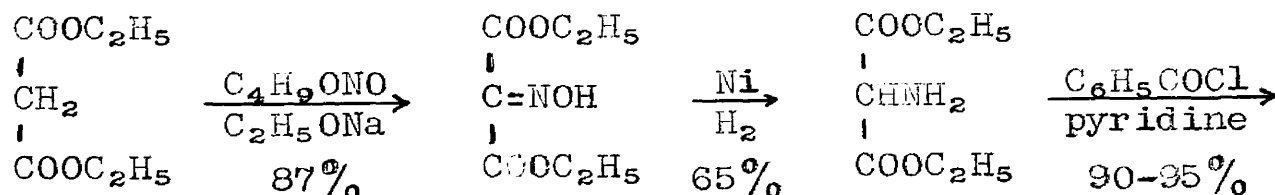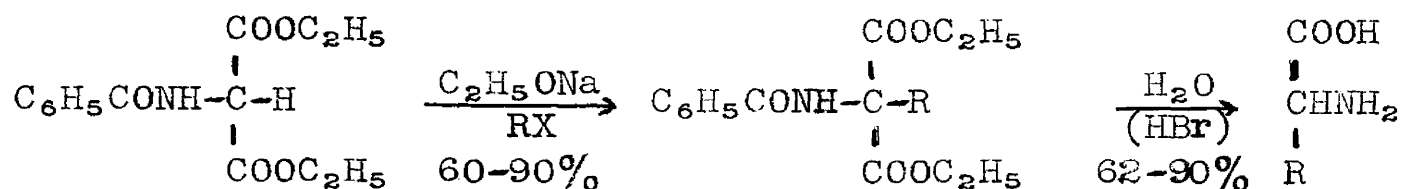
The procedure gives excellent results and is being used to
supply many of the biogenic amino acids. It seems reasonable,
however, to expect that the reduction step can be improved so
as to give better yields of aminomalonic ester.

Hydrazine Method

Analagous to his method for the preparation of primary
amines, Curtius (19,20,21,22) in 1884, converted an appro-
priately substituted malonic ester to the amino acid by means
of hydrazine. The reactions are shown below:

$$\underset{\underset{COOC_2H_5}{|}}{\overset{\overset{COOC_2H_5}{|}}{C}}HR \quad \xrightarrow[(C_2H_5OH)]{KOH + N_2H_4} \quad \underset{\underset{CONH-NH_2}{|}}{\overset{\overset{COOK}{|}}{C}}HR \quad \xrightarrow{HCl + NaNO_2}$$

potassium malonic
hydrazide

$$\begin{bmatrix} COOH \\ | \\ CHR \\ | \\ CO(N_3) \end{bmatrix} \longrightarrow \begin{bmatrix} COOH \\ | \\ CHR \\ | \\ NCO \end{bmatrix} \xrightarrow{H_2O} \underset{\underset{NH_2}{|}}{\overset{\overset{COOH}{|}}{C}}HR$$

malonazid
acid
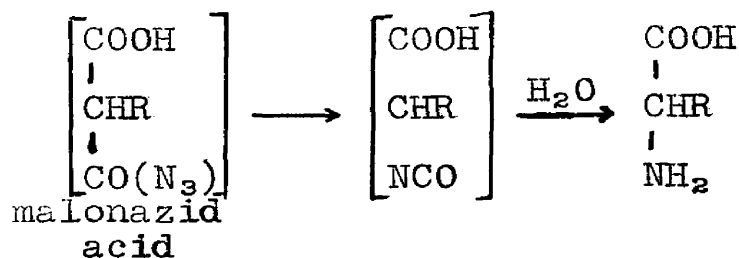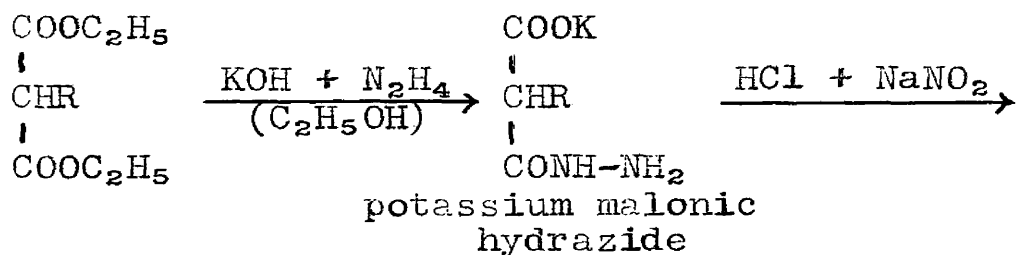
There has been little use made of this procedure probably
because of the instability of the intermediate hydrazides
and also the inconvenience of using hydrazine. Recently,
the synthesis of glycine (23) and α-aminobutyric acid (24)

by this method have been reported.

Condensation with Aldehydes

It was found by Pinner and Spilker (25) in 1889, that aldehydes would form hydantoin derivatives, which on treatment with red phosphorus and hydriodic acid would yield α-amino acids. Subsequent use of the hydantoin condensation has shown it to be satisfactory for the preparation of phenylalanine and tyrosine. The reactions involved are the following:

$$
\begin{array}{c}
\text{NH} - \text{CO} \\
| \qquad | \\
\text{CO} \qquad | \\
| \qquad | \\
\text{NH} - \text{CH}_2
\end{array}
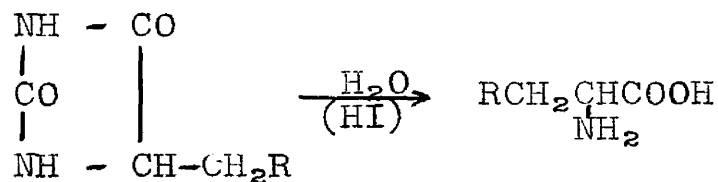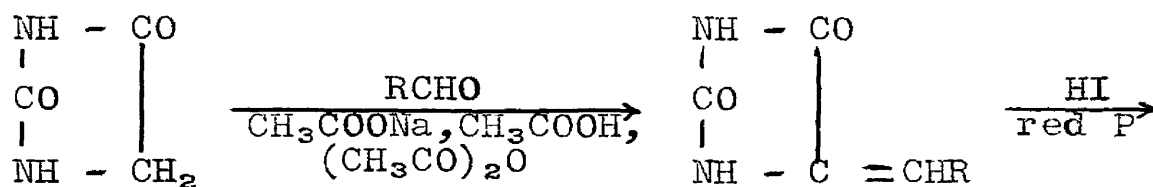\xrightarrow[\substack{\text{CH}_3\text{COONa, CH}_3\text{COOH,} \\ (\text{CH}_3\text{CO})_2\text{O}}]{\text{RCHO}}
\begin{array}{c}
\text{NH} - \text{CO} \\
| \qquad | \\
\text{CO} \qquad | \\
| \qquad | \\
\text{NH} - \text{C} = \text{CHR}
\end{array}
\xrightarrow[\text{red P}]{\text{HI}}
$$

$$
\begin{array}{c}
\text{NH} - \text{CO} \\
| \qquad | \\
\text{CO} \qquad | \\
| \qquad | \\
\text{NH} - \text{CH-CH}_2\text{R}
\end{array}
\xrightarrow[\text{(HI)}]{\text{H}_2\text{O}}
\begin{array}{c}
\text{RCH}_2\text{CHCOOH} \\
\quad\quad | \\
\quad\quad \text{NH}_2
\end{array}
$$

A second condensation of this type makes use of diketo-piperazine in place of the hydantoin used above. The condensation is similar (a modification of the Perkin reaction), as are also the reduction and hydrolysis steps. This method was proposed in 1921 by Sasaki (26) and is useful for the synthesis of phenylalanine and tyrosine.

The "azlactone method", the third of this series, involves the condensation of hippuric acid with an aldehyde and may be represented by the following:

$$\begin{array}{c} \text{COOH} \\ | \\ \text{CH}_2 \\ | \\ \text{NH--CO--C}_6\text{H}_5 \end{array} \quad \xrightarrow[\text{CH}_3\text{COONa} + (\text{CH}_3\text{CO})_2\text{O}]{\text{RCHO}} \quad \begin{array}{c} \text{RCH} = \text{C} - \text{CO} \\ \hspace{1.2cm} | \hspace{0.6cm} | \\ \hspace{1.2cm} \hspace{1cm} \text{O} \\ \hspace{1.2cm} | \hspace{0.6cm} | \\ \hspace{0.8cm} \text{N} - \text{C--C}_6\text{H}_5 \end{array}$$

$$\xrightarrow[\text{(NaOH)}]{\text{H}_2\text{O}} \quad \begin{array}{c} \text{RCH} = \text{C--COOH} \\ | \\ \text{NH--CO--C}_6\text{H}_5 \end{array} \quad \xrightarrow[\text{CH}_3\text{COOH}]{\text{HI} + \text{P}}$$

$$\begin{array}{c} \text{RCH}_2\text{CH--COOH} \\ | \\ \text{NH--CO--C}_6\text{H}_5 \end{array} \quad \xrightarrow[\text{(HI)}]{\text{H}_2\text{O}} \quad \begin{array}{c} \text{RCH}_2\text{CHCOOH} \\ | \\ \text{NH}_2 \end{array}$$
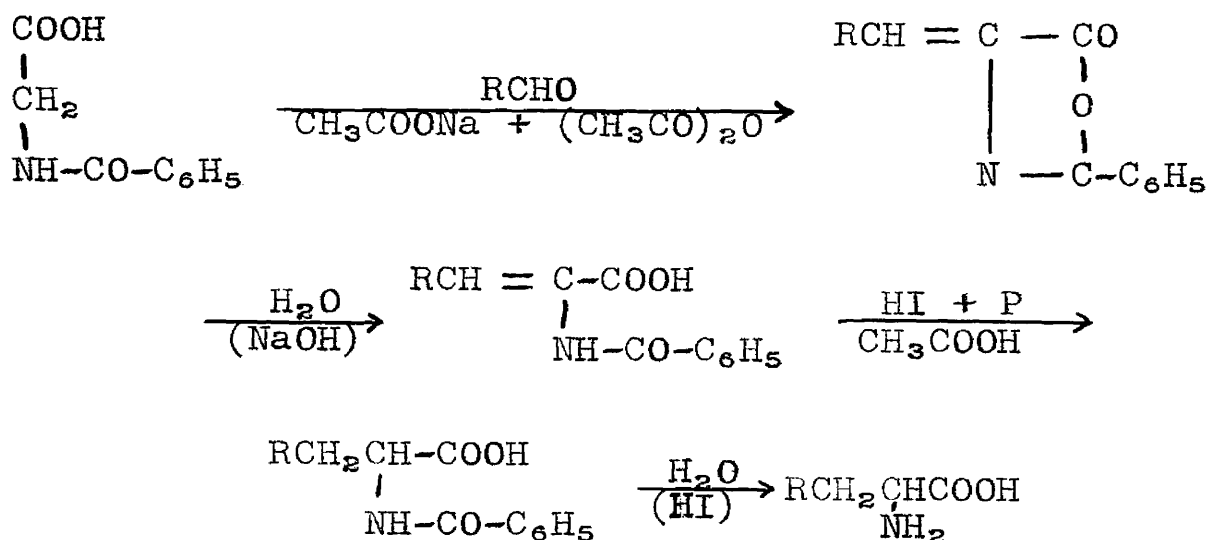
This method, first investigated in 1883 by Plöchl (27), was fully described by Erlenmeyer and Kunlin (28-34). With improvements made more recently, this method has been used to prepare leucine, tyrosine, histidine, tryptophane, phenylalanine and thyroxine. Although probably more useful than the other reactions of this type, the azlactone method has the limitations of the group. In all cases, glycine must be subjected to various reactions in order to obtain the preliminary hydantoin, diketopiperazine or hippuric acid. In addition to this limiting factor, the procedures have shown their value only in the synthesis of certain aromatic and heterocyclic amino acids.

α -Keto Acid Reactions

The reaction, $R.CO.COOH + H_2 + NH_3 + \text{catalyst} \rightarrow R.CH(NH_2).COOH + H_2O$, along with slight modifications, has been used to prepare glycine, alanine, aspartic acid, glutamic acid, and phenylalanine. However, it is relatively unimportant and may be dismissed for practical purposes because of low yields and

the difficulty in obtaining the keto acids.

Reduction of phenylhydrazones and of oximes of α-keto acids is becoming of greater importance. The phenylhydrazones have been investigated to some degree and show possible importance in the light of recent work by Feofilakov (35). This author formed the phenylhydrazones of α-keto acids by treatment of appropriately substituted acetoacetic or malonic esters with benzendiazonium salts. After rearrangement and reduction, the amino acid was obtained.
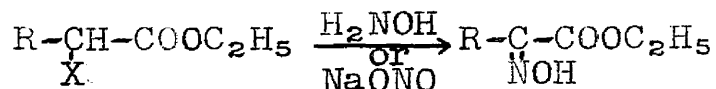
In consideration of the reduction of oximes, a review of the various methods of preparation of the α-oximino acids (or esters) will be discussed.

Placing an oximino or isonitroso group alpha to the carboxyl has been carried out in several ways. Briefly these methods may be grouped as:

(a) Reaction of α-keto acids and hydroxylamine:

$$R-\overset{O}{\underset{\|}{C}}-COOH + H_2NOH \cdot HCl \xrightarrow{\text{base}} R-\underset{NOH}{C}-COOH$$
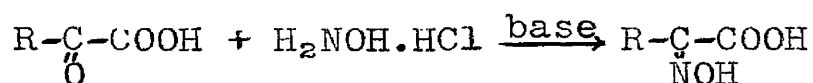
(b) Reaction of esters of α-halogen acids with hydroxylamine or inorganic nitrites:

$$R-\underset{X}{CH}-COOC_2H_5 \xrightarrow[\text{NaONO}]{\underset{or}{H_2NOH}} R-\underset{NOH}{C}-COOC_2H_5$$

(c) Nitrosation of substituted malonic esters:

$$R-\overset{COOC_2H_5}{\underset{COOC_2H_5}{\overset{|}{\underset{|}{C}}}}-H \xrightarrow[\text{RONO}]{NaOC_2H_5} R-\underset{NOH}{C}-COOC_2H_5$$

(d) Nitrosation of substituted acetoacetic esters:

$$CH_3-\underset{O}{\underset{\|}{C}}-\underset{R}{\underset{|}{C}}HCOOC_2H_5 \xrightarrow[\text{RONO}]{\text{NaOC}_2\text{H}_5} R-\underset{N\ OH}{\underset{\|}{C}}-COOC_2H_5$$

$$\xrightarrow{\text{HNO}_2} R-\underset{NOH}{\underset{\|}{C}}-COOC_2H_5$$

$$\xrightarrow{(NO)OSO_3H} R-\underset{NOH}{\underset{\|}{C}}-COOC_2H_5$$
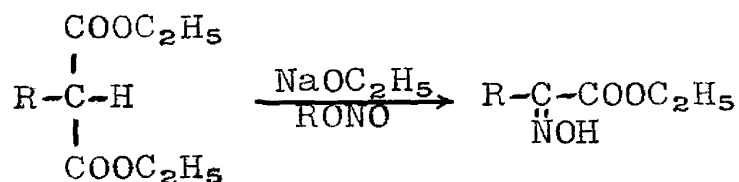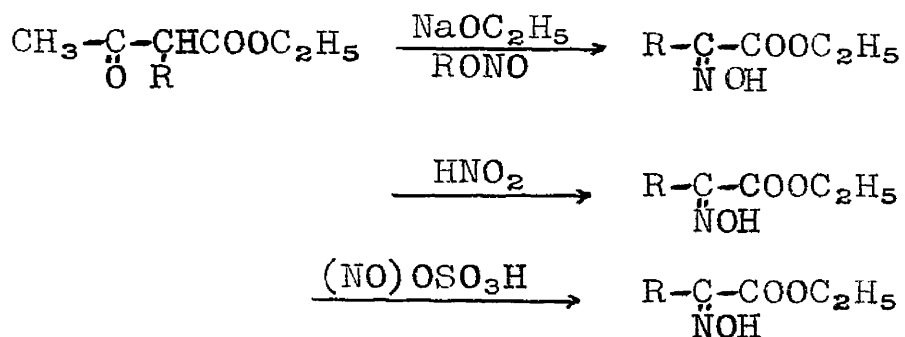
The action of hydroxylamine on an α-keto acid to form the corresponding α-oximino acid was investigated as early as 1882 by Meyer and Janny (36). These workers prepared the oxime of pyruvic acid. Using the same reaction, Cramer (37) in 1892, prepared α-oximino-acetic acid from glyoxalic acid. In the next few years other workers applied the procedure to obtain ethyl α-oximinoacetate, (38), α-oximinopropionamide (39,40) and α-oximinoisovaleric acid (41). The method has become quite general giving good yields. However, the real obstacle to adopting this as a general procedure is the difficulty in obtaining the α-keto acids.

Lepercq (42,43), in 1893, reported the reaction of esters of α-halogen acids with sodium nitrite to yield esters of α-oximino acids. Three years later, Hantzsch and Wild (44) replaced the nitrite with hydroxylamine to prepare α-oximino-acetic, -propionic and -butyric acids in this manner. No further reports have been found in the available literature relating to this procedure.

As early as 1864, Baeyer (45) reported the formation of oximinomalonic acid from oximinobarbituric acid. Dieckmann

and Groeneveld (46) treated methylmalonic ester with sodium ethoxide and ethyl nitrite and obtained ethyl α-oximino-propionate. In 1903, a practical procedure was given by Bouveault and Wahl (47) for preparing oximinomalonic ester using sodium ethoxide and methyl nitrite. Recently, Redemann and Dunn (18) improved this procedure to obtain an 87% yield.

The nitrosation of substituted acetoacetic esters has been investigated quite extensively. In 1878, Meyer and Zublin (48) treated methylacetoacetic ester, with aqueous potassium hydroxide and alcohol, added potassium nitrite and then acid-ified. When reaction was completed, the ethyl α-oximino-propionate was extracted with alkali. Dieckmann and Groeneveld (46) used sodium ethoxide and ethyl nitrite to prepare the same compound. By their method yields of from 65-75% were obtained. In 1904, Bouveault and coworkers published a series of articles (49,50,51,52,53) on the use of nitrosylsulfuric acid with alkyl substituted acetoacetic esters. They reported the synthesis of α-oximinoacetic, -propionic, -butyric, -valeric, -isovaleric, -isocaproic, -β-methylvaleric and -isoheptanoic acids in yields varying from 85-90%. Schmidt et al (54,55) made use of nitrous acid to obtain α-oximino-propionic acid from methylaceto-acetic ester. Wislicenus and Grützner (56) obtained diethyl α-oximinoglutarate using both the method of Bouveault and that of Dieckmann. These workers also obtained ethyl α-oximinophenylacetate from ethyl phenylacetate using ethyl nitrite with potassium ethoxide. In 1915, Hall, Hynes and

Lapworth (57), applied the method of Bouveault to obtain
α-oximino-β-phenylpropionic acid. Recently, the method
has received the attention of McIlwain and Richardson (58)
who prepared α-oximinoglutaric acid and α-oximino-δ-
chloro-γ-valerolactone to synthesize glutamic acid and
hydroxyproline. Godfrin (59) in 1939 also applied the
method for obtaining α-oximino acids. The work of Harington
and Randall (60), in 1931, in nitrosating diethyl β-keto-
glutarate by means of ethyl nitrite and hydrochloric acid,
indicates a wider application of such methods.

The reduction of these oximes was first reported in 1880
by Gutknecht (61) who converted α-oximinopropionic acid
into alanine using tin and hydrochloric acid. Aspartic acid
was synthesized by Piutti (62) in 1887 by the reduction of
the corresponding oxime using sodium amalgam. Wolff (63),
using tin and sulfuric acid, reduced the oxime of α-keto-
glutaric acid to glutamic acid. Bouveault (52,53) prepared
isoleucine by hydrogenating ethyl α-oximino-β-methyl-valer-
ate using zinc and hydrochloric acid, sodium amalgam, and
aluminum amalgam as reducing agents. Erlenmeyer (30,64,65),
Posner (66) and Knoop and Hoessli (67) reduced phenylpyruvic
acid oxime to phenylalanine. Tin and hydrochloric acid, zinc
and hydrochloric acid, aluminum amalgam, and sodium amalgam
were used to reduce the oxime.

Catalytic methods for the hydrogenation of oximes have also
been employed. Nickel was used by Wassiljew (68) but gave
both primary and secondary amines. Winans and Adkins (69)

attempted the reduction of α-oximinoacetoacetic ester using Raney nickel but obtained a pyrazine derivative. Adkins and Reeve (70) further investigated this reduction to show good yields of primary amine when the ether of the oxime is used. In 1934, Bauguess and Berg (71) reported the use of Raney nickel to reduce α-oximino-β-3-indole-propionic acid to tryptophane. This same catalyst employed by Levene and Schormüller (72) for the reduction of oximinomalonic ester also is used by Redemann and Dunn (18) who report 65°% yields of aminomalonic ester.

Platinum has had some application in the reduction of oximes and recently, Shemin and Herbst (73) used the Adams platinum oxide catalyst to reduce α-oximino acids to amino acids. McIlwain and Richardson (58), using platinum oxide with anhydrous sodium sulfate in acetic acid, reduced ethyl α-oximino-glutarate and α-oximino-δ-chloro-γ-valerolactone after shaking in an atmosphere of hydrogen for three days.

In 1908, Paal and Gerum (74) made use of palladium for the reduction of benzaldoxime. They recovered benzylamine, dibenzylamine, ammonia and benzaldehyde. Gulewitsch (75) found that reduction with palladium splits the oxime to yield hydroxylamine which is reduced to ammonia. Rosenmund and Pfankuch (76) successfully used the acetate of the oxime to prevent secondary amine formation, carrying out the reduction with palladium on barium sulfate. A report published by Hartung (77) fully described the use of palladium on charcoal as a catalyst for the hydrogenation of oximes. Alcoholic hydrogen

chloride was added to prevent the formation of secondary amines. Harington and Randall (60) using palladinized charcoal with alcoholic hydrogen chloride reduced ethyl α-oximino-β-ketoglutarate to hydroxyglutamic acid. McIlwain and Richardson (58) under these same conditions attempted the reduction of ethyl α-oximinoacetoacetate. They could not arrest the reduction at the hydroxy-acid stage and isolated only α-aminobutyric acid. Recently, Cocker (78) reported the reduction of ethyl α-oximinosuccinate. Platinum oxide, Raney nickel and palladium under various conditions were employed with no success. Aluminum amalgam finally yielded aspartic acid.

Research Aim

With biological investigation continuously widening our knowledge of amino acids, their role in nutrition and their metabolic products, the importance of these compounds is assuming greater proportions. This being the case, the demand for the individual, pure amino acids is constantly growing.

Although, as previously shown, many syntheses have been carried out, none is without limitations, and more generally applicable methods still remain to be developed. It was this thought that prompted the investigations reported below.
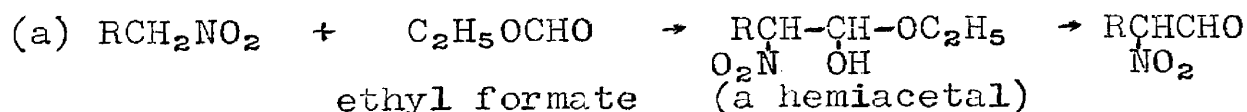
Possible Methods of Fulfillment

The problem is to find a procedure in which nitrogen may

be introduced alpha to a carboxyl group and which nitrogen
may readily be converted to a primary amino group. In look-
ing for a solution to the first phase, the following pro-
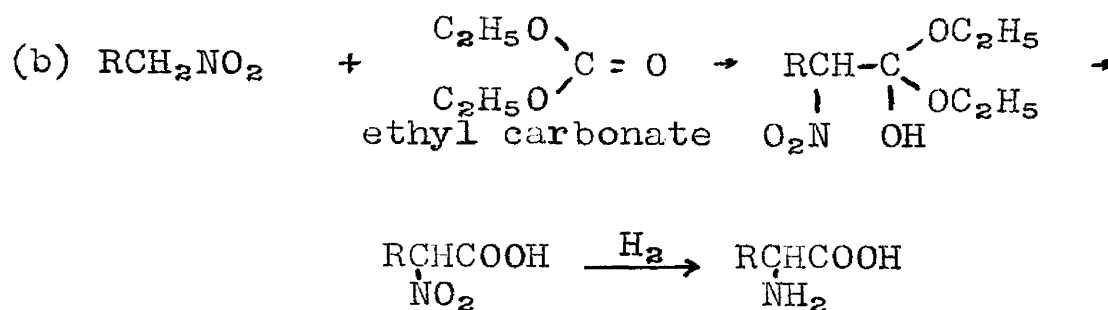posals came in for consideration:

Preparation of α-nitro acids. The aldehyde-nitroparaffin
condensation to give nitroalcohols was investigated as early
as 1895 by Henry (79). Because of the difficulty in obtain-
ing many of the nitroparaffins, the literature is still in-
complete. Recently, however, sufficient advances have been
made in the field of vapor-phase nitration, to make available
at low cost the complete group of nitroparaffins. In 1940,
Vanderbilt and Hass (80) made a systematic study of the con-
densation of nitroparaffin with a carbonyl group which pro-
ceeds according to this equation:

$$R-CH_2-NO_2 + R-CHO \rightarrow \underset{O_2N \quad OH}{R-CH-CH-R}$$
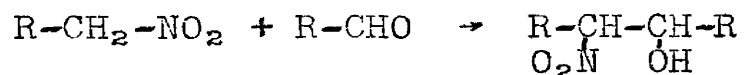
It was considered possible that the reactive carbonyl group
might also be supplied by an ester, e.g., ethyl formate or
diethyl carbonate in which case the reaction would be expected
to take the following course:

(a) $RCH_2NO_2 + C_2H_5OCHO \rightarrow \underset{O_2N \quad OH}{RCH-CH-OC_2H_5} \rightarrow \underset{NO_2}{RCHCHO}$

ethyl formate (a hemiacetal)
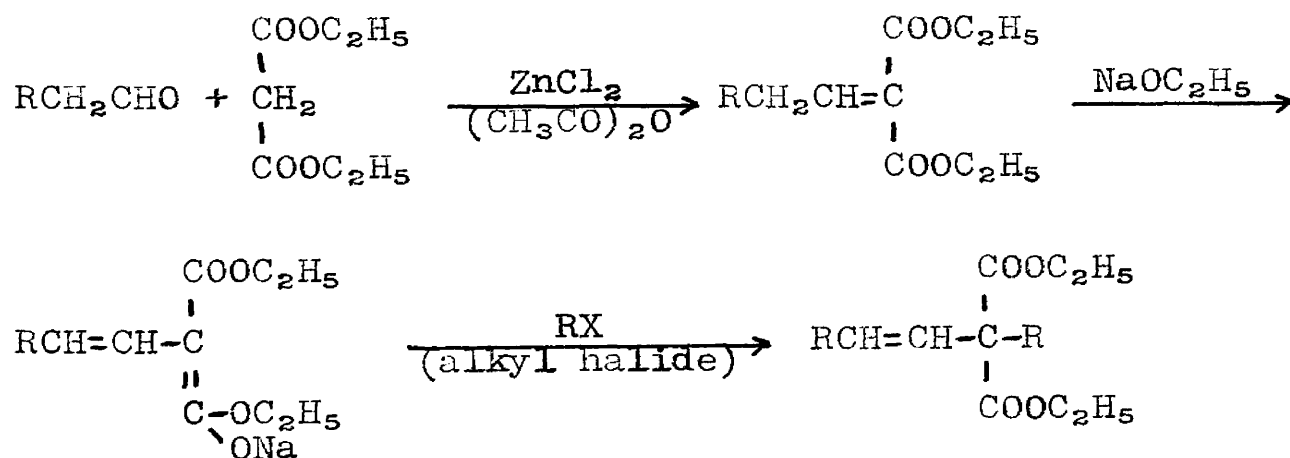
The aldehyde thus formed could be oxidized to the α-nitro
acid which in turn would form the amino acid on reduction.

(b) RCH_2NO_2 + $\begin{matrix} C_2H_5O \\ C_2H_5O \end{matrix} C = O \rightarrow RCH-C \begin{matrix} OC_2H_5 \\ OC_2H_5 \end{matrix} \rightarrow$

ethyl carbonate $\quad O_2N \quad OH$

$$RCHCOOH \xrightarrow{\ H_2\ } RCHCOOH$$
$$\ \ NO_2 \qquad\qquad\ \ NH_2$$

It was also considered that ethyl chlorocarbonate, in a similar way, would yield the nitroacid chloride. However, several attempts to carry out such condensations were unsuccessful.

Alkylidene malonic ester. A series of reactions investigated by Claisen and Crismer (81) and more recently by Cope and Hancock (82), served as a basis for a second plan. These reactions can be represented as:

$$RCH_2CHO + \begin{matrix} COOC_2H_5 \\ CH_2 \\ COOC_2H_5 \end{matrix} \xrightarrow[(CH_3CO)_2O]{ZnCl_2} RCH_2CH=C \begin{matrix} COOC_2H_5 \\ \\ COOC_2H_5 \end{matrix} \xrightarrow{NaOC_2H_5}$$

$$RCH=CH-C \begin{matrix} COOC_2H_5 \\ \\ C-OC_2H_5 \\ \ \ \ ONa \end{matrix} \xrightarrow[(alkyl\ halide)]{RX} RCH=CH-C-R \begin{matrix} COOC_2H_5 \\ \\ COOC_2H_5 \end{matrix}$$

If the alkyl halide were replaced by hydrogen chloride or water, it would be possible to obtain:

$$\begin{matrix} COOC_2H_5 \\ RCH=CH-CH_2 \\ COOC_2H_5 \end{matrix}$$
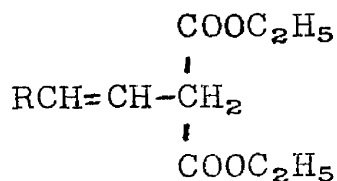
Such a compound could be decarboxylated and the resulting unsaturated acid would have the CH_2 group activated by the combined effect of a carboxy group and a double bond. It should react readily with an alkyl nitrite in acid medium to yield an oxime. Reduction would then produce an α-amino acid. Preliminary efforts, however, gave negative results.

The successes of Harington and Randall (60) and Redemann and Dunn (18) in synthesis of amino acids by means of oximino intermediates indicated possibilities in this direction. Thus,

Nitrosation of α-halogen esters with inorganic nitrites. The use of various inorganic nitrites in the procedure of Lepercq (42,43) was planned. The method involves the reaction of α-halogen esters with a nitrite to yield the α-oximino ester. The results obtained are indicated in the experimental section.

Nitrosation of substituted acetoacetic esters. The work of Bouveault et al (49,50,51,52,53) on the nitrosation of sub-stituted acetoacetic esters in both acid and alkaline media to yield α-oximino esters was the basis of a plan for modifi-cation of such procedures.

The second phase of the problem, i.e., the conversion of the alpha nitrogen to a primary amino group, appeared to have its solution in the work of Hartung (77). Since an outstand-ing fault of the nitrosation procedures for the synthesis of amino acids lay in the final reduction step (83), it was believed that the use of palladium under appropriate condi-

tions would serve as an excellent catalyst for the hydrogenation of the oximino acids.

Finally, in order to supplement existing studies on the crystal habits of α-amino acids, it was planned to study those synthesized under the polarizing microscope and to make photomicrographs of them by means of a camera attachment. Also, no previous studies having been reported, a similar investigation of the α-oximino acids was planned.

EXPERIMENTAL

General

All temperatures recorded, unless otherwise specified, are uncorrected. Final temperatures reported, when corrected, are designated by "(cor.)" and were determined by Anschütz "stem-immersion" thermometers.

When "absolute alcohol" was used, it was prepared from commercial absolute alcohol (about 97% ethyl alcohol). Ten liters of this was refluxed about twenty-four hours with three pounds of fresh, unslaked lime, broken into small pieces. The alcohol was distilled off and the operations repeated, the product from the second distillation being used.

Analyses for nitrogen were carried out using the Kjeldahl-Gunning method.

Syntheses

Preparation of 3-nitro-4-heptanol. Using the method of Vanderbilt and Hass (80), 45 gm. (0.25 moles) of 1-nitropropane, 25 cc. of 95% alcohol and 1 cc. of 10 N sodium hydroxide were placed in a 500 cc., three-neck flask equipped with a mechanical stirrer, reflux condenser and dropping funnel.
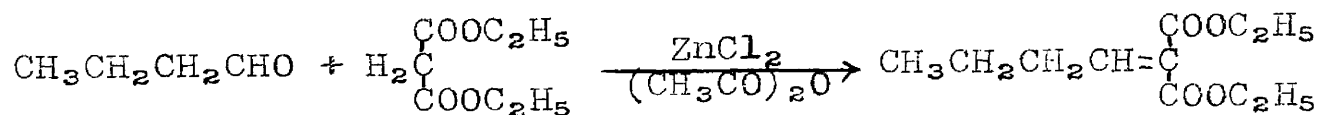
$$CH_3CH_2CH_2NO_2 \; + \; CH_3CH_2CH_2CHO \; \rightarrow \; CH_3CH_2CH_2\underset{HO}{C}H\underset{NO_2}{C}HCH_2CH_3$$
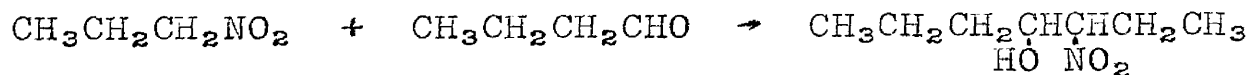
While stirring, 36 gm. (0.25 moles) of freshly distilled butyraldehyde was added slowly. The reaction temperature was maintained at 30-35°C. by external cooling. When approximately two-thirds of the butyraldehyde had been added, an additional 1 cc. of 10 N sodium hydroxide and 4 cc. of water were added. When addition was completed, the mixture was allowed to stand at 35°C. for four days. The alkali was then neutralized and the mixture distilled under slightly reduced pressure. After removal of the impurities, the pressure was reduced to 5 mm. and a 50$^o/_o$ yield (20 gm.) of 3-nitro-4-heptanol distilled over at 90-105°. Redistillation gave a boiling point of 76.5-77°/1 mm. (cor.).

α-Nitro acids. In an attempt to synthesize α-nitro-propionic acid, 50 gm. (0.66 moles) of nitroethane was treated with 50 gm. (0.66 moles) of ethyl formate in the manner described above. On the addition of the ester, no heat of reaction was evidenced. Distillation of the mixture, after allowing the prescribed period of time for reaction, yielded only the original reagents.

A similar run was carried out using ethyl carbonate with nitroethane. Here, also, there was no evidence of reaction.

Preparation of butylidene malonic ester. This compound was synthesized using a modification of a method by Cope and Hancock (82).

$$CH_3CH_2CH_2CHO + H_2C\begin{matrix} COOC_2H_5 \\ COOC_2H_5 \end{matrix} \xrightarrow[(CH_3CO)_2O]{ZnCl_2} CH_3CH_2CH_2CH=C\begin{matrix} COOC_2H_5 \\ COOC_2H_5 \end{matrix}$$

Into a one-liter, three-neck flask, fitted with a reflux condenser, mechanical stirrer and thermometer were placed 320 gm. (2 moles) of malonic ester, 180 gm. (2.5 moles) of freshly distilled butyraldehyde and 240 cc. of acetic anhydride. With vigorous stirring, 100 gm. of anhydrous zinc chloride was added all at once. After about two minutes and before complete solution of the zinc chloride, the color changed rapidly from colorless to almost black and the temperature rose rapidly to 120-125°C. Stirring was continued without external heating for seven hours longer and the mixture was allowed to stand overnight. The reaction product was then shaken with 1500 cc. of water. The aqueous layer was drawn off and discarded. To the non-aqueous portion was added 400 cc. of benzene and the resulting solution washed with water several times. Then the benzene and water were removed by distillation under partially reduced pressure. The residue was distilled at a pressure of 3 mm., the material distilling below 145° being collected. The distillate was fractionated, material boiling between 95 and 120°/2 mm. was collected. The final fractionation gave a 55% yield (235 gm.) of butylidene malonic ester with a boiling point of 101-102° (cor.) at 1 mm.

Hexene-3-oic acid. One-half mole (11.5 gm.) of sodium was dissolved in 375 cc. of absolute alcohol contained in a one-liter, three-neck flask equipped with reflux condenser, mechanical stirrer, dropping funnel and thermometer. Then, 100 gm. (0.47 moles) of butylidene-malonic ester was added dropwise

while the temperature was maintained below 0°C. by means of

an ice-salt bath. Slow stirring was required to maintain

homogeneity. After this addition was completed, 50 cc. of

water was added to the mixture. Next, 240 cc. of 50%

sodium hydroxide was added slowly while refluxing vigorously.

Refluxing was continued for five hours, the color slowly

changing from yellow to a deep red. After cooling, a 50%

solution of sulfuric acid was added until the mixture was

acid to litmus. A decided effervescence accompanied this

addition and a red oil separated above the precipitated

sodium sulfate. Refluxing was then continued for an addi-

tional one and one-half hours and the mixture allowed to

stand overnight. The liquid was decanted from the salt,

the residue being washed thoroughly with ether. The wash-

ings were combined with the decanted material and the whole

washed several times with water. The ether and water were

then removed under partially reduced pressure and distilla-

tion was begun at a pressure of 3 mm. There was ready de-

composition and difficulty was experienced in maintaining

the low pressure. A small quantity of liquid distilled

from 110-130°/3 mm. The residue was a thick, tarry mass

partially distilling above 170°/3 mm. Attempts to obtain a

neutral equivalent for the low boiling fraction were unsuc-

cessful. The material absorbed bromine readily but gave un-

reliable results as a quantitative estimation. The boiling

point of hexene-3-oic acid has been reported as 103°/8-10 mm.

(109). After these preliminary trials, this phase of investi-

gation was temporarily abandoned.

Preparation of ethyl α-bromopropionate. Following the procedure of Zelinsky (84), 200 gm. (2.7 moles) of propionic acid and 22 gm. (0.7 moles) of phosphorus were placed in a three-liter, three-neck flask. A mechanical stirrer, dropping funnel and reflux condenser provided with a gas trap were attached by means of rubber stoppers covered with tin foil. While stirring vigorously, 266 gm. (1.6 moles) of bromine was added dropwise.

$$3 \ CH_3CH_2COOH \ + \ PBr_3 \ \rightarrow \ 3 \ CH_3CH_2COBr \ + \ H_3PO_3$$

When the primary reaction was over, an additional 428 gm. (2.7 moles) of bromine was added slowly while heat was applied to maintain the temperature at 40-50°C. The reaction was considered finished when no more bromine vapors were seen in the reflux condenser. The acyl bromide was then decomposed by adding excess ethyl alcohol to the mixture while cooling.

$$CH_3CH_2COBr \ + \ Br_2 \ \rightarrow \ CH_3CHBrCOBr \ + \ HBr$$

$$CH_3CHBrCOBr \ + \ C_2H_5OH \ \rightarrow \ CH_3CHBrCOOC_2H_5 \ + \ HBr$$

The ester thus formed was separated, washed three times with water and distilled. The fraction boiling between 150-170° was collected as a water-white liquid. This represented a 60% yield (293 gm.) of ethyl α-bromopropionate based on the propionic acid used.

Preparation of ethyl α-bromocaproate. Using the procedure as described above, 348 gm. (3 moles) of caproic acid

was treated with 28 gm. (4.87 moles) of bromine. After reaction, the acyl bromide was treated with excess ethyl alcohol. The resulting ester was washed with water and a solution of sodium bisulfite. Distillation at 90-110°/4 mm. yielded 455 gm., 68% of the theoretical quantity (based on the caproic acid used), of ethyl α-bromocaproate. Redistillation gave a boiling point of 93-94°/1 mm. (cor.). Fichter and Pfister (85) report 103°/11 mm. for ethyl α-bromocaproate.

Preparation of ethyl α-oximinopropionate (α-halogenated ester method). Following the method of Lepercq (42,43), 100 gm. (0.55 moles) of ethyl α-bromopropionate was dissolved in 300 gm. of 95% ethyl alcohol and placed in a one-liter Erlenmeyer flask. A clear solution of 100 gm. (1.45 moles) of sodium nitrite in 300 cc. of water was added. The resulting homogeneous mixture was allowed to stand at room temperature over a period of thirty days. At this time, the material was evaporated to dryness over calcium chloride in a vacuum desiccator. The crystalline product was exhausted with ether (about 400 cc. were used) and the ether evaporated off. White crystals melting from 92-93° were obtained representing a 51% yield (37 gm.) based on the ester used. The material was further purified by crystallization from benzin, and melted at 96° (cor.). The melting point of ethyl α-oximinopropionate reported by Lepercq (42,43) is 94.4°C. The reaction is believed to follow this equation although no attempt was made to identify the inorganic products:

$$CH_3CHBrCOOC_2H_5 \; + \; 2 \; NaONO \; \rightarrow \; CH_3\underset{\overset{|}{NOH}}{C}COOC_2H_5 \; + \; NaBr \; + \; NaNO_3$$
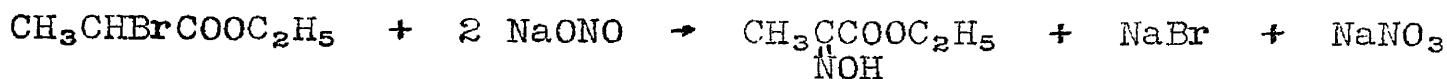
Preparation of ethyl α-oximinocaproate (α-halogenated ester method). Using the method outlined above, 135 gm. (0.6 moles) of ethyl α-bromocaproate, dissolved in 350 cc. of ethyl alcohol, was treated with 100 gm. (1.45 moles) of sodium nitrite in 300 cc. of water. The resulting mixture was not homogeneous. After a period of ten days (the liquids being thoroughly shaken once on each of nine days), 52 gm., 50% of the theoretical yield (based on the ester used), of white crystals was obtained on extraction. After recrystallization from benzin, the ethyl α-oximinocaproate melted at 56°C. (cor.). The reported melting point is 57°C. (86).

Various inorganic nitrites were used in a series of experiments of this type. The halogenated ester reacted was ethyl α-bromocaproate and in all cases but the last, an aqueous-alcoholic solvent was employed. By varying the ratio of water and alcohol, homogeneity of the mixtures was approached but in no cases reached. Dioxane replaced the alcohol in the last run in order to effect complete miscibility. However, here again two layers resulted.

The different mixtures listed below were shaken vigorously, daily, except Sundays, over a period of four weeks. At this time evaporation of the solvents at room temperature was begun. The dried residue was extracted with ether and the ether in turn with 5% sodium hydroxide. The alkaline extract was acidified and the solution extracted with ether. After drying over anhydrous sodium sulfate, the ether was evapor-

ated, leaving behind the oxime.

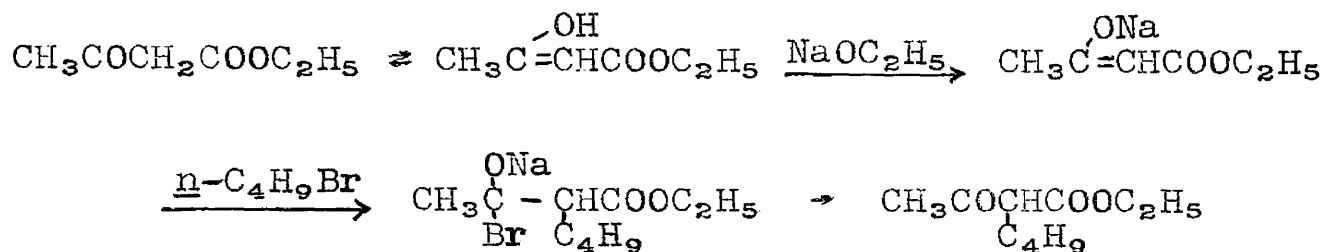The experiments run and the tabulated results are given below:

(1) 53.5 gm. (0.24 moles) of ester, 200 cc. of an 18% aqueous solution of ammonium nitrite (0.6 moles) and 200 cc. of 95% ethyl alcohol.

(2) 80 gm. (0.36 moles) of ester, 62 gm. (0.9 moles) of sodium nitrite, 200 cc. of 95% ethyl alcohol, and 150 cc. of water.

(3) 27 gm. (0.12 moles) of ester, 34.5 gm. (0.15 moles) of barium nitrite, 100 cc. of 95% ethyl alcohol, and 75 cc. of water.

(4) 53.5 gm. (0.24 moles) of ester, 51 gm. (0.6 moles) of potassium nitrite, 200 cc. 95% ethyl alcohol and 100 cc. of water.

(5) 53.5 gm. (0.24 moles) of ester, 41.5 gm. (0.6 moles) of sodium nitrite, 200 cc. of dioxane, and 100 cc. of water.

TABLE III

Action of Inorganic Nitrites on Ethyl α-Bromocaproate

Experiment	Product	% Yield
1	α-Oximinocaproic acid	0.15
2	Ethyl α-oximinocaproate	65
3	α-Oximinocaproic acid	9
4	Ethyl α-oximinocaproate	53
5	No oxime found.	

Preparation of ethyl n-butylacetoacetate. Following the general procedure in Organic Syntheses (87) for the preparation of this compound, the reaction was carried out in a three-liter, three-neck flask provided with a reflux condenser, a dropping funnel and a mechanical stirrer.

$$CH_3COCH_2COOC_2H_5 \rightleftharpoons CH_3\overset{OH}{C}=CHCOOC_2H_5 \xrightarrow{NaOC_2H_5} CH_3\overset{ONa}{C}=CHCOOC_2H_5$$

$$\xrightarrow{n-C_4H_9Br} CH_3\overset{ONa}{\underset{Br}{C}}-\overset{}{\underset{C_4H_9}{C}}HCOOC_2H_5 \rightarrow CH_3CO\underset{C_4H_9}{C}HCOOC_2H_5$$

To the reflux condenser was attached a calcium chloride tube. To one and one-half liters of absolute alcohol placed in the flask, was added 69 gm. (3 moles) of fresh sodium cut into small pieces. Cooling of the flask was necessary to speed up the addition and to prevent too vigorous refluxing. After final addition of the sodium, 390 gm. (3 moles) of ethyl acetoacetate was added through the separatory funnel in a fairly rapid stream. At this point, stirring was begun and heat applied by means of an oil-bath until the mixture was gently refluxing. To this, 450 gm. (3.28 moles) of n-butyl bromide was added dropwise. This addition required about two hours. Refluxing with stirring was continued until the mixture was neutral to litmus, about eight hours being necessary. During this period, large quantities of sodium bromide precipitated, requiring rapid stirring to prevent bumping.

The mixture was then cooled and the supernatant liquid washed with 50 to 100 cc. of absolute alcohol and the wash-

ings added to the main solution. The alcohol was then removed by distillation. The residue was subjected to distillation under reduced pressure, the fraction distilling over between 85 and $120°$C. at 5 mm. was collected. On redistillation a yield of 70% (390 gm.) of ethyl $\underline{n}$-butylacetoacetate boiling between 95-105$°$/5 mm. was obtained. The final corrected boiling point was found to be 85.5-86.0$°$/1 mm.

Preparation of ethyl methylacetoacetate. Following the procedure outlined above, ethyl methylacetoacetate [CH_3CO- the $CH(CH_3)COOC_2H_5$] was prepared from ethyl acetoacetate and ,CO-
$CH(CH_3)COOC_2H_5$] was prepared from ethyl acetoacetate and methyl bromide. Because of its high volatility, it was necessary to dissolve the methyl bromide in absolute alcohol (100 gm. of CH_3Br to 150 cc. of alcohol). By this procedure, a yield of 78% (224 gm. for a two-mole run) was obtained. When the less volatile methyl iodide was used, the yield was slightly lower, 71% of ethyl methylacetoacetate being obtained. The reaction is more rapid, only about five hours being required for refluxing for a two-mole run. The boiling point found was 57.5$°$/1 mm. (cor.). Auwers (88) reports 75.5-76.5$°$/12 mm.

Preparation of ethyl sec-butylacetoacetate. [CH_3CH_2- $CH(CH_3)CH(COCH_3)COOC_2H_5$] sec-Butyl bromide was used in the general method described above to prepare ethyl sec-butylacetoacetate. The condensation was extremely slow. Refluxing was continued over twenty-four hours for a two-mole run. The reaction mixture at the end of this time was still basic although

a 10% excess of the halide had been used. The yield was low, only 45% (165 gm.) of the theoretical (based on the acetoacetic ester) was obtained, the observed boiling point of the pure ethyl sec-butylacetoacetate being $82.5°/1$ mm. (cor.); that reported in the literature (89) is $102.5-104.1°/14.5-15$ mm.

Preparation of ethyl acetylsuccinate. $[C_2H_5OOCCH_2CH-(COCH_3)COOC_2H_5]$ The intermediate ethyl chloroacetate was first prepared. A mixture of 200 gm. (2.1 moles) of chloroacetic acid, 150 cc. of 95% ethyl alcohol and 25 gm. of conc. H_2SO_4 was refluxed for about four hours. The resulting ester was separated from the aqueous layer, the latter being washed with 50 cc. of ether. The ester plus the ether extract were washed with 100 cc. of water and finally with 50 cc. of 5% sodium bicarbonate solution. The ester was then distilled, a 60% yield (154 gm.) of ethyl chloroacetate coming over at $142-146°C$.

Using the halogenated ester, an acetoacetic ester condensation was carried out in the manner described above. The reaction went smoothly, a one-mole run becoming neutral after four hours of refluxing. A 65% yield (140 gm.) of ethyl acetylsuccinate was obtained. The boiling point is $106.5°$ (cor.) at 1 mm. as compared to that of $133-134°/8$ mm. reported by Ruhemann and Hemmy (90).

Preparation of ethyl α-acetylglutarate. $[C_2H_5OOCCH_2CH_2-CH(COCH_3)COOC_2H_5]$ The intermediate ethyl β-chloropropionate was first prepared. One hundred grams (0.78 moles) of $\beta -$

chloropropionyl chloride (E.K. Co.) was placed in a 250 cc.
Erlenmeyer flask under a reflux condenser. A gas trap to
collect evolved hydrogen chloride was provided and 95%
ethyl alcohol added slowly by means of a dropping funnel.
After primary evolution of hydrogen chloride was over, an
additional 50 cc. of alcohol was added and then the mixture
brought to boiling. It was then cooled and the ester washed
with cold water. Distillation yielded 36 gm., 90% of the
theoretical amount of ethyl β-chloropropionate. This was
then used in a 0.6 mole run with acetoacetic ester. The
condensation went in the usual manner, about five hours
being necessary to complete the reaction. The yield of
ethyl α-acetylglutarate was 62% (85 gm.), the boiling point
of the pure ester being $126.5^{\circ}/0.5-1$ mm. (cor.). Emery (91)
reports $162^{\circ}/11$ mm. as the boiling point of the compound.

Ethyl α-acetyl-δ-cyanovalerate. $NCCH_2CH_2CH_2CH(COCH_3)-$
$COOC_2H_5$ The procedure of Derick and Hess (92) for the prep-
aration, modifies the above general procedure for the
substitution of acetoacetic ester in two respects. They
recommend that the solution of sodium ethoxide be well cooled
during the addition of the acetoacetic ester and that one and
one-half equivalents of the ester be used to prevent disub-
stitution. Following this method, γ-chlorobutyronitrile
(E.K. Co.) was used in place of γ-bromobutyronitrile. Con-
densation was very slow, a one mole run requiring twenty-
four hours of refluxing. The solution was still basic after
this period. When the alcohol had been removed, 150 cc. of

benzene was added to the residue and this mixture was washed thoroughly with water. After drying over anhydrous sodium sulfate for one hour, the benzene was distilled off at partially reduced pressure. The residue was then distilled under a pressure of 1 mm. The fraction boiling between 120-140°/1 mm., weighing 69 gm., was collected. On fractionation, two cuts were obtained, 115-118°/1 mm. (cor.) and 136-141°/1 mm. (cor.). These results did not correspond with those obtained by Derick and Hess (92) who report an 82% yield of ethyl α-acetyl-δ-cyanovalerate boiling at 154°/2 mm. This phase was postponed for later investigation.

Preparation of ethyl benzylacetoacetate. [CH_3COCH-($CH_2C_6H_5$)$COOC_2H_5$] Again following the general procedure given by Organic Syntheses, benzyl chloride was reacted with acetoacetic ester in the presence of sodium ethoxide. Condensation for a two-mole run was complete after eight hours of refluxing. The yield of ester distilling between 143-146°/3 mm. was 71% (312 gm.). Redistillation gave a boiling point of 132.5°/1-2 mm. (cor.). Christ (93) reported the boiling point of ethyl benzylacetoacetate to be 164-165°/12 mm.
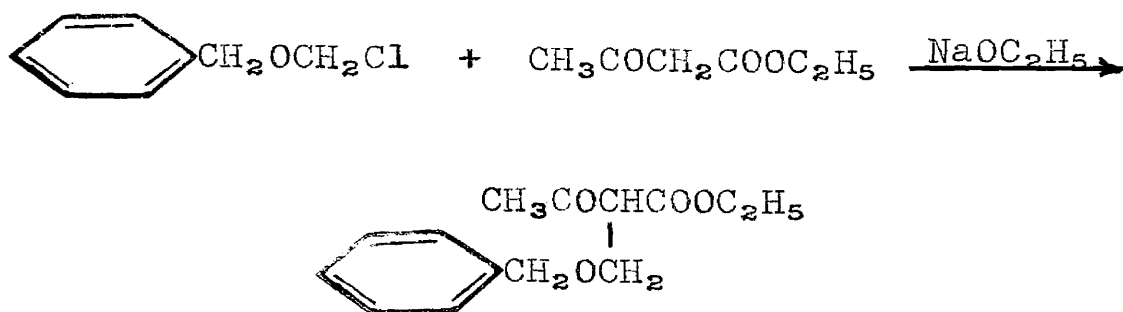
Preparation of ethyl p-methoxybenzylacetoacetate.

[CH_3O⟨benzene ring⟩$CH_2CH(COCH_3)COOC_2H_5$] The intermediate p-methoxy-benzyl chloride was first prepared from the corresponding alcohol. Three-quarters of a mole (156 gm.) of phosphorus pentachloride was added to 200 cc. of ether in a one-liter,

round-bottom flask. A reflux condenser and a gas trap were attached. The p-methoxybenzyl alcohol, 60 gm. (0.43 moles) was added slowly by means of a dropping funnel. External cooling was necessary to control the rate of refluxing. After all the alcohol was added, heat was applied and refluxing continued until the evolution of hydrogen chloride had ceased. Cold water was dropped in carefully to decompose the excess phosphorus pentachloride and the phosphorus oxychloride formed. The ethereal layer was then separated, washed well with cold water and finally with 5 % sodium bicarbonate solution. The ether solution was dried over anhydrous sodium sulfate for one hour, the ether then removed and the halide distilled under reduced pressure. The fraction boiling from 90-95°/2 mm. was collected. This represented an 85 % yield (57 gm.) of p-methoxybenzyl chloride. The latter was combined in the usual way with acetoacetic ester, in a 0.3 mole run, requiring about three hours of refluxing. A benzene solution of the resulting ester was washed with water, dried and distilled. The yield of ethyl p-methoxybenzylacetoacetate based on the halide used was 59 % (44 gm.). Its boiling point is 160-161°/1 mm. (cor.), as compared to 172°/0.25 mm. reported by Goodall and Hayworth (94).

Ethyl benzoxymethylacetoacetate. It was hoped to carry out the synthesis of this compound according to the following reaction:

$$\langle\rangle CH_2OCH_2Cl \quad + \quad CH_3COCH_2COOC_2H_5 \quad \xrightarrow{NaOC_2H_5}$$

$$\begin{array}{c} CH_3COCHCOOC_2H_5 \\ | \\ \langle\rangle CH_2OCH_2 \end{array}$$

The benzylchloromethyl ether was prepared following the procedure of Marvel and Porter (95) for the synthesis of monochloromethyl ether:

$$\langle\rangle CH_2OH \quad + \quad HCHO \quad + \quad HCl \quad \rightarrow \quad \langle\rangle CH_2OCH_2Cl \quad + \quad H_2O$$
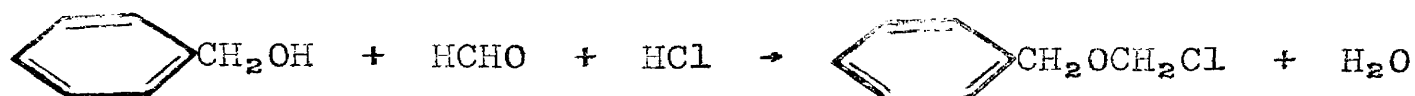
In a one-liter, round-bottomed flask, fitted with a stopper carrying a reflux condenser and a glass tube reaching nearly to the bottom of the flask, were placed 280 gm. (2.6 moles) of benzyl alcohol and 151 gm. of formalin containing 60.5 gm. (2 moles) of formaldehyde. A rapid stream of hydrogen chloride was run into the mixture, which was cooled by running water. This was continued over a period of about three hours to saturate the mixture completely. The ether layer was separated and the aqueous layer saturated with calcium chloride. Any ether separating here was added to the main body which was then dried over anhydrous calcium chloride. On distillation, a yield of 86% (269 gm.) of benzylchloromethyl ether was obtained, boiling at 97°/5-10 mm. This agreed with that of 102°/14 mm., reported by Sabetay and Schving (96).

Preliminary work has been done on the synthesis of ethyl

benzoxymethylacetoacetate using the halide prepared above with acetoacetic ester. However, to date, positive results have not been attained, and the problem is being reserved for later investigation.

The substituted acetoacetic esters synthesized are all water-white liquids. Table IV lists those prepared with their respective boiling points and the yields obtained.

TABLE IV

Substituted Acetoacetic Esters Synthesized

Ester	Boiling Point (cor.)	% Yield
Ethyl methylacetoacetate	57.5°/1 mm.	78
Ethyl n-butylacetoacetate	$85.5-86.0^{\circ}$/1 mm.	70
Ethyl sec-butylacetoacetate	82.5°/1 mm.	45
Ethyl acetylsuccinate	106.5°/1 mm.	65
Ethyl α-acetylglutarate	126.5°/0.5-1 mm.	62
Ethyl benzylacetoacetate	132.5°/1-2 mm.	71
Ethyl p-methoxybenzylacetoacetate	$160-161^{\circ}$/1 mm.	59

Preparation of n-butyl nitrite. The method of Noyes (97) (slightly modified) was used for the synthesis of this compound.

$$2\ C_4H_9OH + 2\ NaONO + H_2SO_4 \rightarrow 2\ C_4H_9ONO + Na_2SO_4 + 2\ H_2O$$

A three-liter, three-neck flask was provided with a strong mechanical stirrer, a thermometer and a dropping funnel leading to the bottom of the flask. The sodium nitrite, 380 gm.

(5.5 moles), was placed in the flask and 500 cc. of water plus 1 kilogram of crushed ice were added. An additional ice-salt bath provided external cooling. Next, 100 cc. of water, 136 cc. of concentrated sulfuric acid (sp. gr. 1.84) and 457 cc. (5 moles) of n-butyl alcohol were carefully mixed and cooled to 0°C. This solution was then introduced below the surface of the nitrite solution, the mass being slowly stirred. The crushed ice in place of the water used by Noyes permitted the rapid addition of the acid solution, the time being reduced from two hours to fifteen minutes.

The mixture was allowed to stand until the ester separated from the salt. The liquid was decanted and water added to the residue. Additional ester was separated and the whole yield washed twice with 50 cc. portions of a solution of 2 gm. of sodium bicarbonate and 25 gm. of sodium chloride in 100 cc. of water. The ester was then distilled and the fraction boiling between $75-78^{\circ}$ collected. The yield was 81% (417 gm.) of the pure compound.

Butyl nitrite may be conveniently stored in a refrigerator for as long as six weeks before distillation is again necessary.

α-Oximino Acids or Esters from Substituted Acetoacetic Esters

The procedure of Dieckmann and Groeneveld (46), using alkyl nitrites and sodium ethoxide with substituted acetoacetic esters, was repeated. Using ethyl methylacetoacetate,

a 48°/$_o$ yield of ethyl α-oximinopropionate was obtained.
Since the method was cumbersome and the yield reported by
Dieckmann (75°/$_o$) was not attained, this was not repeated.

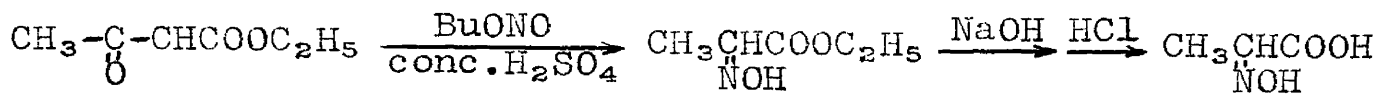Nitrosations following the method of Bouveault et al
(49-53) were carried out. The substituted acetoacetic
esters were dissolved in 85°/$_o$ sulfuric acid and nitrosated
by means of nitrosyl sulfate (Kahlbaum), dissolved in sul-
furic acid. The yields of oximino acid were excellent,
about 85°/$_o$ for the α-oximino-n-caproic acid and α-oximino-
β-phenylpropionic acid. These results agreed rather closely
with the reports of Bouveault.

However, nitrosyl sulfate is somewhat inconvenient to ob-
tain. Laboratory methods of preparation are available but
have decided disadvantages. Also commercially (when obtain-
able), the price of nitrosyl sulfate is very high. Therefore,
it seemed reasonable that the procedure could be improved by
substituting a more convenient nitrosating agent. After in-
vestigating various conditions such as nitrosating agent,
temperature and concentrations of solvent, a modified proce-
dure was devised. When butyl nitrite is used in the method
outlined below, higher yields and a more pure product can be
obtained. This was found particularly true for the aromatic
acetoacetic esters. Thus, the modified procedure provided a
general method for obtaining α-oximino acids (or esters) from
substituted acetoacetic esters.

Preparation of α-oximinopropionic acid. The procedure used
here, served as a general method for the preparation of all

oximino acids used and thus will be described in detail at this point.

$$CH_3-\underset{O}{\overset{}{C}}-CHCOOC_2H_5 \xrightarrow[\text{conc.}H_2SO_4]{\text{BuONO}} CH_3\underset{\overset{}{N}OH}{CHCOOC_2H_5} \xrightarrow{\text{NaOH}} \xrightarrow{\text{HCl}} CH_3\underset{\overset{}{N}OH}{CHCOOH}$$

Thirty grams of 85% sulfuric acid (about twice the weight of ester used) was placed in a 400 cc. beaker, surrounded by an ice-salt bath. Mechanical stirring was provided and the temperature of the acid was maintained at $-5-0°C$. At this point, 14.4 gm. (0.1 mole) of ethyl methylacetoacetate was added slowly, keeping the temperature of the mixture below $0°C$. to prevent hydrolysis of the ester by the concentrated acid. When the ester had been added, 11 gm. (0.1 mole plus a 5% excess) of butyl nitrite, prepared above, was added dropwise on the surface of the mixture. Here again, heat was generated but the temperature was controlled so as not to exceed $0°C$. Efficient cooling was produced if the ice-salt bath was stirred by means of a "wire-loop" stirrer.

After complete addition of the nitrite (too great an excess of nitrite decomposes the oxime and produces brown nitric oxide fumes), crushed ice was added to the acid mixture. At this point, a white, curdy mass of ethyl α-oximino-propionate precipitates. In this individual case, since the ester has a high melting point ($96°C.$), it was found more suitable to isolate the ester. However, generally, the mixture was next extracted with ether. The ethereal portion then was thoroughly extracted with 10% sodium hydroxide. To obtain the free oximino acid, the alkaline extract was heated

on a steam bath for ten minutes. After cooling and carefully acidifying with concentrated hydrochloric acid, a portion of the α-oximinopropionic acid precipitated. Because of the high solubility of this acid, it was necessary to salt out the product and extract well with ether. The free oximino acid then was obtained by removing the ether. The α-oximinopropionic acid, on recrystallization from ether and benzin, melted at 182° (cor.) with decomposition. Inglis and Knight (98) reported 180-181° with decomposition. Analysis of the oximino ester showed:

$C_5H_9O_3N$. Calculated N, 10.69%; found N, 10.91%. The yield, as the ester, was 11.5 gm., 88% of the theoretical. If carried through the hydrolysis step, the acid was obtained in 80% yields (based on the methylacetoacetate used).

Preparation of α-oximinobutyric acid. Using 15.8 gm. (0.1 mole) of ethyl ethylacetoacetate (E. K. Co.) nitrosation was carried out following the general method described above. The ester separated as an oil from the nitrosation mixture. After hydrolysis, crystalline α-oximinobutyric acid was obtained in a yield of 80% (9.4 gm.). The material was recrystallized from ether and benzin. The pure compound melted at 155° (cor.) with decomposition. The reported melting point is 154° (98). Nitrogen analysis gave the following results:

$C_4H_7O_3N$. Calculated N, 11.96%; found N, 11.72%.

Preparation of α-oximino-n-valeric acid. The usual procedure was followed for the nitrosation of 17.2 gm. (0.1 mole) of ethyl n-propylacetoacetate. α-Oximino-n-valeric acid was obtained in a yield of 85% (11.1 gm.). After recrystallization from ligroin, the melting point was 145.5° (cor.) with decomposition. The melting point found in the literature (99) is 143-144° with decomposition. An analysis of the free acid for nitrogen was made.

$C_5H_9O_3N$. Calculated N, 10.69%; found N, 10.45%.

Preparation of α-oximino-n-caproic acid. Nitrosation of 18.6 gm. (0.1 mole) of ethyl n-butylacetoacetate was carried out. The ester separated as a white, curdy precipitate. After hydrolysis, a yield of 89% (12.9 gm.) of α-oximino-n-caproic acid was obtained. The compound, purified by recrystallization from benzin, melted at 137° (cor.) with decomposition. The melting point was previously reported as 132° with decomposition (55). Analysis for nitrogen gave these results:

$C_6H_{11}O_3N$. Calculated N, 9.65%; found N, 9.56%.

Preparation of α-oximino-β-methyl-n-valeric acid. Using 18.6 gm. (0.1 mole) of ethyl sec-butylacetoacetate, nitrosation was carried out under the usual conditions. After hydrolysis, a 70% yield (10.2 gm.) of pure α-oximino-β-methyl-n-valeric acid was obtained. After repeated recrystallizations from ligroin, the compound melted at 145° (cor.) with

decomposition. Bouveault and Locquin (52) reported 164° as the corrected melting point. Analysis of the compound showed:

$C_6H_{11}O_3N$. Calculated N, $9.65^o/_o$; found N, $9.50^o/_o$.

When the melting point of Bouveault and Locquin could not be duplicated, a mixed melting point with the isomeric α-oximino-n-caproic acid was taken. This showed a decided depression. Since the analysis was correct and the reduction product (described later) was satisfactory, the compound was assumed to be α-oximino-β-methyl-n-valeric acid.

Preparation of ethyl oximinosuccinate. Using 21.6 gm. (0.1 mole) of ethyl acetylsuccinate, nitrosation was carried out as usual. The ester obtained was a reddish-brown oil. Preliminary attempts to hydrolyze the ester failed. Distillation of the ester resulted in complete decomposition, a phenomenon also reported by Schmidt and Dieterle (55). No further attempts were made to purify the ester. The yield of nitrosation was $85^o/_o$ based on the hydrogen absorbed in the reduction, described in a later paragraph.

Preparation of ethyl α-oximinoglutarate. By nitrosating 23.0 gm. (0.1 mole) of ethyl α-acetylglutarate in the manner described above, a $91^o/_o$ (19.7 gm.) yield of ethyl α-oximino-glutarate was obtained. The pure compound, recrystallized from benzin, melted at $62-63^o$ (56). Analysis of the ester gave the following results:

$C_9H_{15}O_5N$. Calculated N, $6.45^o/_o$; found N, $6.45^o/_o$.

Preparation of α-oximino-β-phenylpropionic acid. Nitrosation of ethyl benzylacetoacetate, 22 gm. (0.1 mole), was carried out. While adding the butyl nitrite, the reaction mixture turned a deep red. This color disappeared as nitrosation proceeded. After hydrolysis, the -oximino- -phenyl-propionic acid was recrystallized from dilute alcohol. The yield of pure compound was 89% (15.9 gm.). The melting point recorded was 168° (cor.) with decomposition. Wolff (100) reported 167° with decomposition.

Preparation of α-oximino-β-(p-methoxyphenyl)-propionic acid. Using 12.5 gm. (0.05 mole) of ethyl p-methoxybenzyl-acetoacetate, the general procedure for nitrosation was followed. During the addition of the butyl nitrite, the color changed to a reddish-purple which faded to a light brown, after nitrosation was completed. On hydrolysis, an 87% yield (9.1 gm.) yield of α-oximino-β-(p-methoxyphenyl)-propionic acid resulted. The compound, after recrystalliza-tion from dilute alcohol, melted at 156-157° (cor.) with decomposition. The melting point reported in the literature is 159° (101). An analysis of the material was made.

$C_{10}H_{11}O_4N$. Calculated N, 6.70%; found N, 6.77%.

Table V lists those oximino acids (or esters) synthesized with their respective melting points, analyses and yields from nitrosation.

TABLE V

α-Oximino Acids (or Esters) Synthesized

Oxime	Melting Point (cor.)	% Nitrogen		% Yield
		Calc.	Found	
Ethyl α-oximino- propionate	96°	10.69	10.91	88
α-Oximinopropionic acid	182° dec.			78
α-Oximinobutyric acid	155° dec.	11.96	11.72	80
α-Oximino- n-valeric acid	145.5° dec.	10.69	10.45	85
α-Oximino- n-caproic acid	137° dec.	9.65	9.56	89
α-Oximino-β-methyl- n-valeric acid	145° dec.	9.65	9.50	70
Ethyl oximinosuccinate	Oil			85
Ethyl α-oximino- glutarate	62°	6.45	6.45	91
α-Oximino-β-phenyl- propionic acid	168° dec.	7.76	7.76	89
α-Oximino-β- (p-methoxyphenyl)- propionic acid	156-157° dec.	6.70	6.77	87

Hydrogenation of Oximino Acids and Oximino Esters

Preparation of palladium-charcoal catalyst. The procedure of Hartung (77) was followed. To 3 gm. of norit was added 0.3 gm. of palladium chloride crystals. This mixture was placed in a 250 cc. flask and 100 cc. of distilled water added. On shaking in an atmosphere of hydrogen by means of a mechanical shaking device, the contents of the flask were saturated.
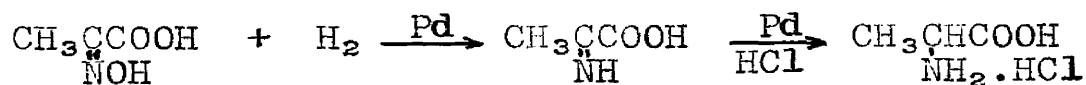
$$PdCl_2 \ + \ H_2 \ \rightarrow \ Pd \ + \ 2 \ HCl$$

When the intake of hydrogen was completed, the flask was removed, and the contents filtered on a suction funnel. The palladinized charcoal was washed thoroughly with distilled water and then twice with 95% alcohol. Care must be taken to avoid spontaneous combustion of the material. The catalyst when nearly dried was placed in a vacuum desiccator over concentrated sulfuric acid. After standing overnight, it was ready for use.

Preparation of alanine (α-aminopropionic acid). After attempting reductions of the α-oximino acids and esters under various conditions of temperature, pressure and solvents, the following procedure was adopted as giving the best results:

To 5.15 gm. (0.05 moles) of α-oximinopropionic acid was added the palladium catalyst described above. An additional 0.5 gm. of palladium chloride crystals was added and 100 cc.

of 95% ethyl alcohol. About 10 cc. of 35% hydrochloric
acid (0.11 moles) was added and the entire mixture was
placed in a glass tube, fitted for use in a pressure hydro-
genator bomb, purchased from American Instrument Company.
After insertion of the glass liner, the bomb was subjected
to a pressure of 10 atmospheres of hydrogen and the mixture
agitated by means of a mechanical shaker. The rate of hydro-
gen absorption was followed by observing the fall in pressure
on the gauge. Reduction to the half-way point was rapid,
about thirty minutes being necessary. It is presumed, on the
basis of reports in the literature on analagous compounds,
that the imine was first obtained. The second step went much
more slowly, about three hours being required for the theo-
retical quantity of hydrogen to be absorbed. At this point,

$$CH_3\underset{NOH}{C}COOH \; + \; H_2 \xrightarrow{Pd} CH_3\underset{NH}{C}COOH \xrightarrow[HCl]{Pd} CH_3\underset{NH_2 \cdot HCl}{CH}COOH$$

no more hydrogen was taken up although shaking was continued
for an additional hour.

The glass container was then removed from the bomb. The
mixture was filtered by suction and the charcoal catalyst
washed on the filter with two 20 cc. portions of 95% alcohol.
The filtrate and alcohol washings were subjected to distilla-
tion under the pressure of the water pump, care being taken
to avoid bumping. After removal of the solvents, a white
crystalline material remained. This was dissolved in a mini-
mum of distilled water and filtered. The clear filtrate was
heated to boiling on a hot-plate and 28% ammonium hydroxide

was added until a drop of the solution was just yellow (pH 6.1) with a methyl red indicator. If too much base was added, it was neutralized with acetic acid. Usually, at this point, white crystals of alanine began to precipitate. If not, the solution was concentrated until crystals appeared. Then, three volumes of 95% alcohol were added to the hot solution. After standing in the refrigerator for twelve hours, the white crystalline precipitate was filtered and washed once with about 10 cc. of cold 95% alcohol. The mother liquor, after concentration, yielded a second crop of alanine. The amino acid was conveniently recrystallized from boiling distilled water to which three volumes of alcohol was added. The yield for the reduction step was 75% (3.3 gm.) of the theoretical. The nitrogen content was determined.

$C_3H_7O_2N$. Calculated N, 15.72%; found N, 15.52%.

It should be mentioned at this point that although the reductions all went in a manner to indicate a _quantitative_ yield, various factors combined to lower the quantities of amino acid obtained. Mechanical losses, solubility of the lower amino acids and the small quantities reduced were such factors. When larger runs were made and with the higher homologues, the amounts that could be isolated progressively increased.

Preparation of the ethyl ester of alanine. When reduction of the α-oximino ester was carried out, the hydrochloride of the amino acid ester resulted. The procedure followed was identical with that outlined above. The isolation of the

amino acid ester was carried out following the method of Adkins and McElvain (102). After filtering the reduction mixture and distilling under reduced pressure, a semi-crystalline mass remained. This was dissolved in a minimum of water and placed in a 250 cc. round-bottomed flask (directions are for a 0.1 mole run). After adding 100 cc. of ether, the mixture was cooled in an ice-bath. Then 33% sodium hydroxide was added, while shaking well, until the aqueous layer was neutral to litmus. Next, powdered sodium carbonate was added with vigorous shaking to convert the watery layer into a paste. The ether layer was filtered into a flask and the residue was washed with two successive 50 cc. portions of ether, the washings being filtered into the main solution. The ether was then dried by shaking with anhydrous potassium carbonate for ten minutes and with anhydrous sodium sulfate for thirty minutes. The solution was filtered and distilled under the pressure of an aspirator. In the case of the low boiling esters, particular care had to be taken to cool the receiving flask. When the ether had been removed, the ester was distilled. The ethyl ester of alanine distilling from $39-45°/5$ mm., was a water-white liquid and had a strong ammoniacal odor. A yield of 78% (9.1 gm.) was obtained. The picrate of ethyl ester of alanine was prepared. The yellow crystals melted at $170.5-171°$ (cor.), the melting point previously being reported at $171°$ (103).

Hydrolysis of the ester was carried out by placing the ester in a round-bottomed flask and adding ten times its vol-

ume of distilled water. The flask was heated by means of an oil-bath and the liquid refluxed until the alkaline reaction had disappeared. The solution was then concentrated on a hot-plate until crystallization began and the alanine isolated as before. The yield from the hydrolysis step was 85%.

Preparation of α-aminobutyric acid. Following the procedure outlined above for the reduction of the α-oximino acids and the isolation of the amino acid, 5.85 gm. (0.05 moles) of α-oximinobutyric acid was hydrogenated to α-aminobutyric acid. The yield of the pure amino acid was 78% (4.0 gm.). An analysis by the Kjeldahl-Gunning method was made.

$C_4H_9O_2N$. Calculated N, 13.59%; found N, 13.73%.

The benzoyl derivative was prepared by the Schotten-Baumann method. The white crystalline derivative melted at 144° (cor.). The reported melting point is 147° (104).

Preparation of norvaline (α-amino-n-valeric acid). Norvaline was obtained in an 83% yield (4.9 gm.) by hydrogenating 6.55 gm. (0.05 moles) of α-oximino-n-valeric acid. Analysis for nitrogen was:

$C_5H_{11}O_2N$. Calculated N, 11.96%; found N, 12.22%.

The benzoyl derivative, prepared by the Schotten-Baumann procedure, melted at 153.5° (cor.). Slimmer (105) reported a melting point of 152.5°C.

Preparation of norleucine (α-amino-n-caproic acid). In the usual manner, 7.25 gm. (0.05 moles) of α-oximino-n-caproic acid was reduced to norleucine. The material was obtained in 85% yields (5.6 gm.) and gave the following analysis for nitrogen:

$C_6H_{13}O_2N$. Calculated N, 10.68%; found N, 10.73%.

The p-toluenesulfonamide was prepared by the method of Mc-Chesney and Swann (106). It melted at 122-124° (cor.). The reported melting point is 124° (104).

Preparation of isoleucine (α-amino-β-methyl-n-valeric acid). Using 7.25 gm. (0.05 moles) of α-oximino-β-methyl-n-valeric acid, isoleucine was obtained by reduction in the manner described above. The hydrogenation went smoothly to the theoretical endpoint and an 80% yield (5.2 gm.) of pure isoleucine was isolated. On analysis by the Kjeldahl-Gunning method, the following results were obtained:

$C_6H_{13}O_2N$. Calculated N, 10.68%; found N, 10.91%.

The p-toluenesulfonamide was prepared. The tan crystals melted at 137-138° (cor.), the reported melting point being 140° (104).

Preparation of Aspartic Acid (aminosuccinic acid). Using the oily ethyl oximinosuccinate from the nitrosation of 0.1 mole of ethyl acetylsuccinate, hydrogenation was carried out as usual. Contrary to the report of Cocker (78), who was unable to use palladium as a catalyst for the hydrogenation

of ethyl oximinosuccinate, the reduction proceeded very smoothly. Only 85% of the theoretical quantity of hydrogen (based on a 0.1 mole run) was taken up, indicating an 85% yield for the nitrosation step. Ethyl aspartate was isolated and distilled at 103-105°/2 mm. Cocker (78) reported 97-98° at 1 mm. as the boiling point. The yield of ester was 78% (11.2 gm.) based on the amount of hydrogen taken up.

Hydrolysis was carried out as described for the ethyl ester of alanine. A 88% yield (7.7 gm.) of pure aspartic acid was obtained in this step. The analysis gave these results:

$C_4H_7O_4N$. Calculated N, 10.53%; found N, 10.57%.

Preparation of glutamic acid (α-aminoglutaric acid).
Ethyl α-oximinoglutarate, 10.8 gm. (0.05 moles), was reduced catalytically by the general procedure. The theoretical quantity of hydrogen was taken up and the product isolated as ethyl glutamate. The ester distilled at 125-130°/3 mm. The yield of ester was 82% (8.3 gm.). Hydrolysis yielded glutamic acid in a yield of 90% (5.4 gm.).
Analysis of the pure amino acid gave:

$C_5H_4O_4N$. Calculated N, 9.52%; found N, 9.70%.

Preparation of phenylalanine (α-amino-β-phenylpropionic acid). Using 9.0 gm. (0.05 moles) of α-oximino-β-phenylpropionic acid, hydrogenation went to completion smoothly. Isolation in the usual manner gave an 89% yield (7.3 gm.)

of pure phenylalanine. An analysis of the material was made.

$C_9H_{11}O_2N$. Calculated N, 8.49%; found N, 8.27%.

The benzoyl derivative was prepared by the Schotten-Baumann procedure. The white crystalline product melted at 185° (cor.). The reported melting point is 188° (107).

Preparation of O-methyltyrosine [α-amino-β-(p-methoxy-phenyl)-propionic acid]. Reduction of 5.2 gm. (0.025 moles) of α-oximino-β-p-methoxyphenylpropionic acid was success-fully carried out to give a 90% yield (4.5 gm.) of O-methyl-tyrosine. The pure product gave the following analysis for nitrogen:

$C_{10}H_{13}O_3N$. Calculated N, 7.18%; found N, 7.15%.

Preparation of tyrosine (α-amino-β-(p-hydroxyphenyl)-propionic acid). Demethylation of 2 gm. (0.0086 moles) of O-methyltyrosine was carried out. The material was placed in a Pyrex glass bomb, 25 cc. of concentrated hydrochloric acid was added, and the glass was sealed. The bomb was placed in an oven and heated to 180° for a period of three hours. After cooling, the tube was carefully opened and the hydrochloric acid removed by distillation under reduced pressure. The res-idue was dissolved in a minimum of water, brought to the iso-electric point (pH 5.7) and worked up as described for alanine The yield for the demethylation step was 1.3 gm., 85% of the theoretical. Analysis of the recrystallized tyrosine gave these results:

$C_9H_{11}O_3N$. Calculated N, 7.73%; found N, 7.43%.

Table VI lists the amino acids synthesized, the yields from hydrogenation, the analyses and the derivatives prepared.

TABLE VI

Amino Acids Synthesized

Amino Acid	% Yield	% Nitrogen		Derivative
		Calc.	Found	
Alanine	75	15.72	15.52	Picrate of ethyl ester mp. 171° (cor.)
α-Aminobutyric Acid	78	13.59	13.73	Benzoyl mp. 144° (cor.)
Norvaline	83	11.96	12.22	Benzoyl mp. 153.5° (cor.)
Norleucine	85	10.68	10.73	p-Toluenesulfonamide mp. 122-124° (cor.)
Isoleucine	80	10.68	10.91	p-Toluenesulfonamide mp. 137-138° (cor.)
Aspartic Acid	69[1]	10.53	10.57	
Glutamic Acid	74[1]	9.52	9.70	
Phenylalanine	89	8.49	8.27	Benzoyl mp. 185° (cor.)
O-Methyltyrosine	90	7.18	7.15	
Tyrosine	85[2]	7.73	7.43	

[1] Yield based on combined hydrogenation and hydrolysis steps.

[2] Yield based on demethylation of O-methyltyrosine.

Chemical Crystallography

An elementary study of the crystal habits of the oximino and amino acids was made. A Bausch and Lomb microscope, fitted with a mechanical stage, a rotating stage and two nicol prisms was used. The nicol prisms were mounted so as to act as polarizer and analyzer. Illumination was supplied by a "ribbon-filament" incandescent bulb, focused on a concave mirror. The heat generated was minimized by passing the light through a water-cell. Since maximum illumination was desired, no further control was needed.

The planes of vibration of the two nicol prisms were first determined by the method of Chamot and Mason (108). The nicol prisms were set to be exactly crossed (position of maximum darkness). Well-formed crystals of ammonium sulfate were placed in the field and the preparation turned by means of the rotating stage. The position of maximum extinction of any given crystal marked the planes of vibration of the two nicol prisms. Therefore, the cross-hairs of the eye-piece were set exactly parallel and perpendicular to the long edge of the crystal in order to represent the vibrational planes of the nicols.

Crystal preparations were made using a "hanging-drop" slide. A thin film of petrolatum was spread around the edges of a concavity in a microscope slide. Then a drop of the solvent to be used was placed in the center of a cover-glass. Small particles of the pure compound to be studied were added to this drop until a saturated solution resulted. Evaporation

caused crystallization to begin. When crystal growth was started, the microscope slide was inverted and placed over the drop, sealing it within the concavity. The film of petrolatum between the slide and cover-slide prevented further evaporation and permitted undisturbed growth of the crystals.

From their behavior between crossed nicols, the crystals can be classified as to their geometrical characteristics. This classification, with the geometrical characteristics and optical properties of each group, follows:

Cubic System - Three axes of equal length, all of which are perpendicular. Optically isotropic, since all orientations are alike.

Tetragonal System - Three perpendicular axes, two of which are equal in length, the third unequal. Parallel extinction.

Hexagonal System - Three axes of equal length in one plane, forming equal angles (60°) with each other; a fourth axis perpendicular to this plane, of unequal length. Parallel extinction exhibited by side views (rare) of the crystals, no extinction otherwise.

Orthorhombic System - Three axes of unequal length, all of which are perpendicular. Parallel extinction. Birefringence.

Monoclinic System - Three axes of unequal length, two of which are mutually perpendicular to the third but not to each other. Parallel extinction shown by one axis, oblique extinction by the others. Birefringence.

Triclinic System - Three axes of unequal length and forming

unequal angles with each other. Oblique extinction. Birefringence.

The results of the crystal studies accompany the photomicrographs which follow.

Photomicrography

By means of a Leica sliding-focusing attachment, the image from the microscope (described above) was focused upon the ground-glass. When the proper field was found and the image focused sharply, the Leica camera was slipped into position over the objective. The exposure was then made. The crystals photographed were prepared as described in the preceding section. Photomicrographs taken in this manner, with the appropriate descriptions, follow.

Ethyl **α**-oximinopropionate

From dioxane

Parallel extinction

No birefringence

Tetragonal

Mag. 50x   Enlarged 3x

Fig. 1.

α-Oximinopropionic acid

From dioxane

Parallel and oblique
  extinction

Birefringence

Monoclinic

Mag. 50x   Enlarged 3x

Fig. 2.

α-Oximinobutyric acid
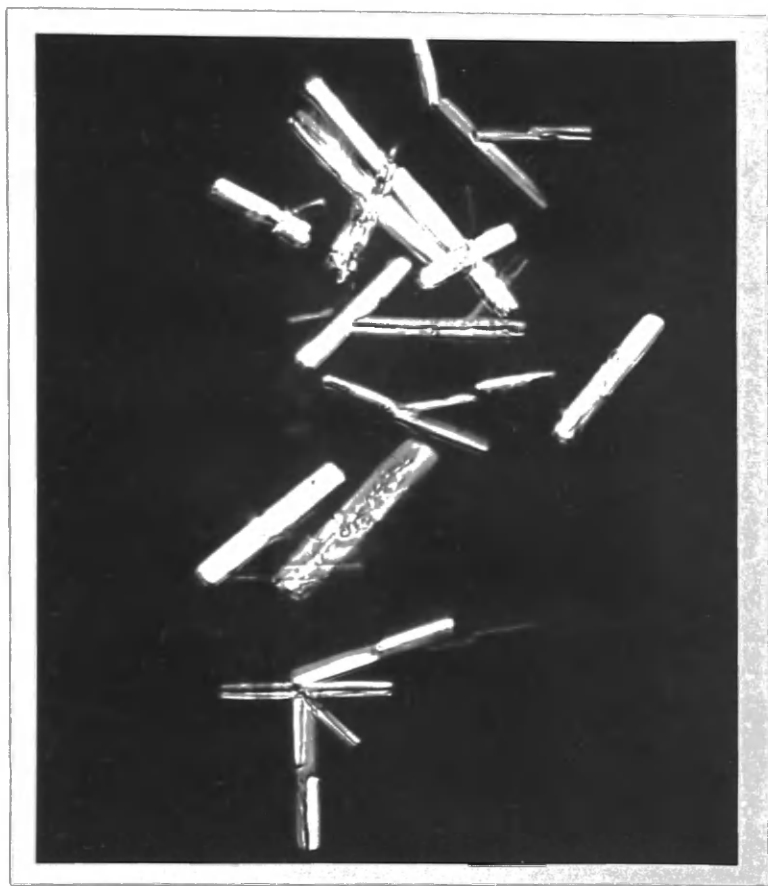
From dioxane

Oblique extinction

Birefringence

Triclinic

Mag. 50x   Enlarged 3x

Fig. 3.

α-Oximinovaleric acid

From dioxane

Parallel and oblique
    extinction

Birefringence

Monoclinic

Mag. 50x   Enlarged 3x

Fig. 4.

Ethyl α-oximinocaproate

From dioxane

Parallel and oblique
   extinction

Birefringence

Monoclinic

Mag. 50x   Enlarged 3x

Fig.5.

α-Oximinocaproic acid

From dioxane

Parallel extinction

No birefringence

Tetragonal

Mag. 50x   Enlarged 3x

Fig. 6.

Fig. 7.

α-Oximino-β-methyl-n-valeric acid

From dioxane

Parallel extinction

Birefringence

Orthorhombic

Mag. 50x   Enlarged 5x

α-Oximino-β-methyl-n-valeric acid

Note phenomenon on rotating crystals between polarized light.

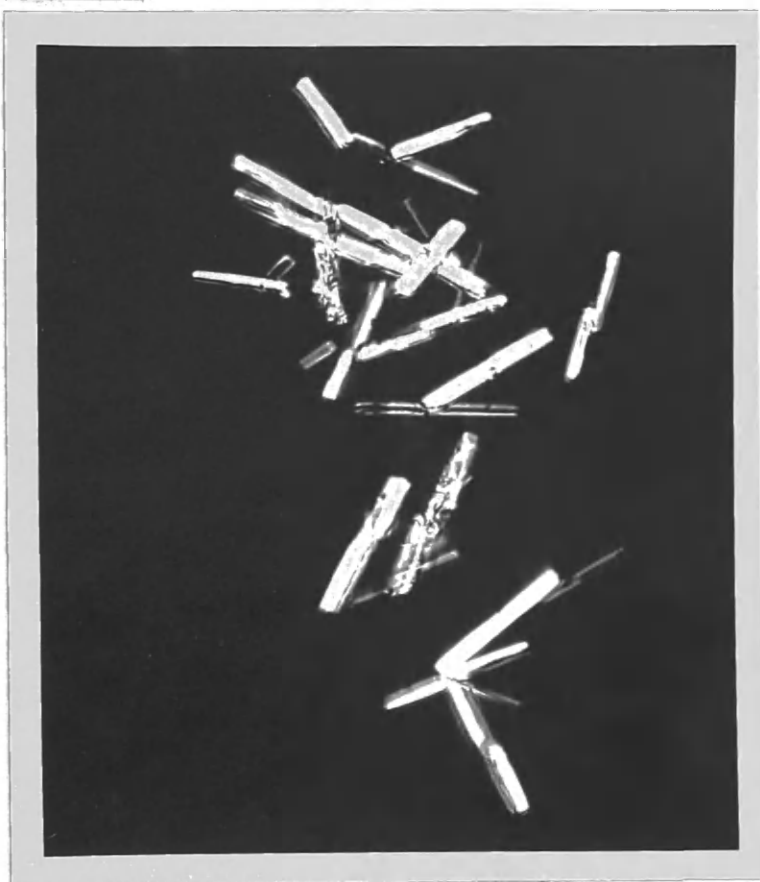Figs. 7,8,9 and 10 show a peculiar "breaking" of crystals on rotation.

Fig. 8.

α-Oximino-β-methyl-
n-valeric acid

Fig. 9.

α-Oximino-β-methyl-
n-valeric acid

Fig. 10.

Ethyl **α**-oximinoglutarate

From dioxane

Parallel and oblique
    extinction

Birefringence

Monoclinic

Mag. 50x   Enlarged 3x

Fig.11.

Ethyl **α**-oximinoglutarate

Fig.12.

α-Oximino- β-phenyl-
   propionic acid

From dioxane

Parallel extinction

Birefringence

Orthorhombic

Mag. 50x   Enlarged 3x

Fig.13.

α-Oximino-β - [p-methoxy-
   phenyl] -propionic acid

From dioxane

Parallel and oblique
   extinction

Birefringence

Monoclinic

Mag. 50x   Enlarged 3x

Fig.14.

Fig. 15.

Alanine

From water and alcohol

Parallel extinction

No birefringence

Tetragonal

Mag. 50x   Enlarged 3x

α-Aminobutyric acid

From water and alcohol

Parallel extinction

No birefringence

Tetragonal

Mag. 50x   Enlarged 3x

Fig. 16.

Norvaline

From water and alcohol

Parallel extinction

No birefringence
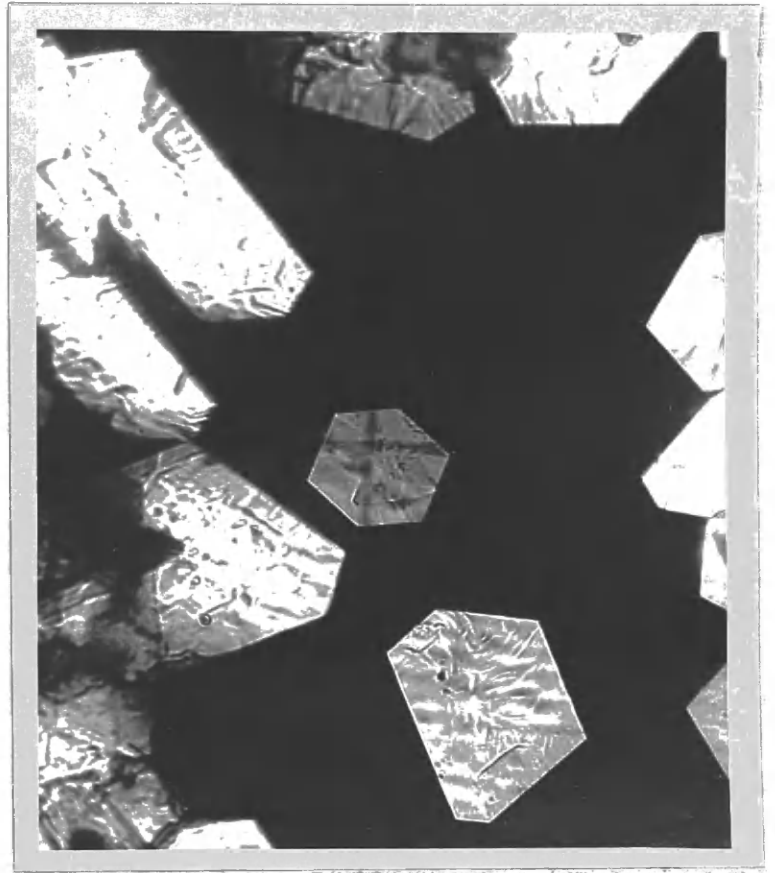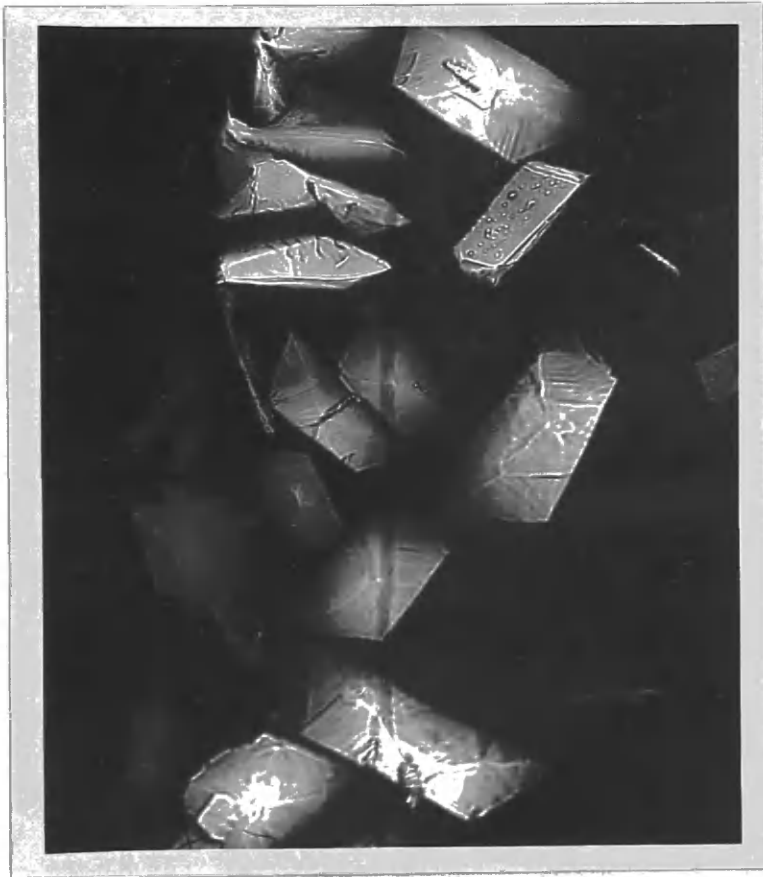
Tetragonal

Mag. 50x   Enlarged 3x

Fig. 17.

Norleucine

From water and alcohol

Parallel extinction

No birefringence

Tetragonal

Mag. 50x   Enlarged 3x

Fig. 18.

Isoleucine

From water and alcohol

Parallel extinction

No birefringence

Tetragonal

Mag. 50x   Enlarged 3x

Fig. 19.

Aspartic acid

From water and alcohol

Oblique extinction

Birefringence

Triclinic

Mag. 50x   Enlarged 3x

Fig. 20.

Glutamic acid

From water and alcohol

Parallel extinction

No birefringence

Tetragonal

Mag. 50x   Enlarged 3x

Fig.21.

Phenylalanine

From water and alcohol

Parallel and oblique
    extinction

Birefringence

Monoclinic

Mag. 50x   Enlarged 3x

Fig.22.

O-Methyltyrosine

From water and alcohol

Oblique extinction

Birefringence

Triclinic

Mag. 50x   Enlarged 3x

Fig. 23.

Tyrosine

From water and alcohol

Parallel extinction

No birefringence

Tetragonal

Mag. 50x   Enlarged 3x

Fig. 24.

SUMMARY

The synthesis of alanine, α-aminobutyric acid, norvaline, norleucine, isoleucine, aspartic acid, glutamic acid, phenylalanine, tyrosine, and O-methyltyrosine has been described. After synthesis of the appropriately substituted acetoacetic esters, nitrosation was carried out through a modification of the procedure of Bouveault et al (49-53). The resulting intermediate oximino acids (or esters) were characterized and then hydrogenated using palladinized charcoal as a catalyst. The amino acids were isolated readily and in good yields. As far as the present investigation has disclosed, it appears likely that any mono-substituted acetoacetic ester may be converted into an α-amino acid by application of these reactions.

An elementary study of the crystal habits of the oximino and amino acids was made under the polarizing microscope. Photomicrographs, illustrating these habits, were also prepared.

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