LONG-CHAIN α-AMINO ACIDS

By

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INTRODUCTION

General

It has been known for quite some time that proteins when hydrolysed by acids or alkalies or by the ferments yield proteoses, peptones, and finally α-amino acids. Of the numerous acids obtained in this manner, the constitution of the majority is now known and many have been prepared synthetically.

There are eighteen amino acids of general occurence; glycine, leucine, tyrosine, serine, glutamic acid, aspartic acid, phenylalanine, alanine, lysine, arginine, histidine, valine, proline, tryptophan, hydroxyproline, isoleucine, methionine, threonine. To these must be added seven acids of narrow distribution (thyroxine, diiodotyrosine, dibromotyrosine, norleucine, cystine, cysteine and hydroxyglutamic acid) and five of very limited occurence but which may be present in proteins (thiolhistidine, dihydroxyphenylalanine, citrulline, canavanine, djenkolic acid), to which must probably be added lanthionine. There are finally about twenty acids which have not been completely identified with rather vaguely defined amino acids such as those believed to be present in liver antianaemia factor. (1)

Two of the simplest α-amino aliphatic acids, namely glycine or α-aminoacetic acid, and alanine or α-amino propionic acid, are among the commonest α-amino acids found in proteins. On the other hand, α-aminocaproic acid or norleucine is known to occur in only a few proteins. It is often seen that the naturally occuring aliphatic α-amino acids contain one or more side chains or groups such as

hydroxy, methyl or imino in addition to the amino group. For instance, isoleucine or α-amino-β-methyl-n-valeric acid, and leucine or α-aminoisocaproic acid, which are isomeric with norleucine, contain a methyl group at the β and γ carbon atoms, respectively. The important naturally occuring aliphatic α-amino acids are listed in Table I. All of the other essential α-amino acids contain either a benzene, phenolic, or heterocyclic ring in their molecules.

TABLE I

Aliphatic Amino Acids Found in Proteins

Amino Acid	Chemical Name of the Acid	Formula
Alanine	α-aminopropionic	$CH_3-\underset{NH_2}{CH}-COOH$
Arginine	α-amino-δ-guanidino-n-valeric	$H_2N-\overset{NH}{C}-NH-(CH_2)_3-\underset{NH_2}{CH}-COOH$
Aspartic Acid	α-aminosuccinic	$HOOC-CH_2-\underset{NH_2}{CH}-COOH$
Cystine	di-(α-amino β-thiopropionic)	$(S-CH_2-\underset{NH_2}{CH}-COOH)_2$
Glutamic Acid	α-aminoglutaric	$HOOC-CH_2-CH_2-\underset{NH_2}{CH}-COOH$
Glycine	α-aminoacetic	$H_2\underset{NH_2}{C}-COOH$
-hydroxyglutamic acid	α-amino-β-hydroxy-glutaric	$HOOC-CH_2-\underset{OH}{CH}-\underset{NH_2}{CH}-COOH$

TABLE I (cont'd.)

Aliphatic Amino Acids Found in Proteins

Amino Acid	Chemical Name of the Acid	Formula
Leucine	α-amino-isocaproic	$(CH_3)_2-CH-CH_2-\underset{NH_2}{CH}-COOH$
Isoleucine	α-amino-β-methyl-n-valeric	$H_3C-CH_2-\underset{CH_3}{\underset{NH_2}{CH}}-CH-COOH$
Lysine	α-amino-ϵ-amino-n-caproic	$H_2\underset{NH_2}{C}-(CH_2)_3-\underset{NH_2}{CH}-COOH$
Methionine	α-amino-γ-methyl-thiol-n-butyric	$H_3C-S-(CH_2)_2-\underset{NH_2}{CH}-COOH$
Norleucine	α-amino-n-caproic	$H_3C-(CH_2)_3-\underset{NH_2}{CH}-COOH$
Serine	α-amino-β-hydroxy-propionic	$H_2\underset{OH}{C}-\underset{NH_2}{CH}-COOH$
Threonine	α-amino-β-hydroxy-butyric	$H_3C-\underset{OH}{CH}-\underset{NH_2}{CH}-COOH$
Valine	α-amino-isovaleric	$\begin{matrix} H_3C \\ H_3C \end{matrix}\!\!>CH-\underset{NH_2}{CH}-COOH$

HYDROLYSIS OF PROTEINS

Hydrolytic methods have contributed most to our present understanding of the chemical structure and nature of the proteins and their components. Hydrolysis of proteins can be accomplished mainly by three ways: (1) by acids, (2) by alkalies and (3) by enzymes; the most convenient being the treatment with hot aqueous mineral acids. The alkaline hydrolysis is limited in its scope, despite the fact that proteins are completely hydrolyzed to their constituents, for during the process a notable proportion of the amino acids which preexist in pure optically active form become racemized. Alkalies have one advantage over acids in that they do not destroy tryptophane. Hence they are often used when this acid is derived or estimated. Enzyme hydrolysis of proteins has the practical disadvantage of slow and often incomplete action, however it does not cause racemization or decomposition of the more sensitive acids.

All the simple proteins yield ammonia and mixtures of amino acids on hydrolysis by acids, alkalies, or enzymes. Purines, pyrimidines, carbohydrates, and lipoidal substances are also liberated during the hydrolysis of the "conjugated" proteins; however, the main products consist of amino acids.

Among the acids that have been used to hydrolyze proteins, sulfuric acid certainly holds first place, both from the historical and the practical standpoints. Braconnot (2) in 1820 isolated the amino acid, glycine from sulfuric acid hydrolysates of gelatin and

and also of meat. Since then sulfuric acid has been used almost by every investigator of protein chemistry. The concentration of sulfuric acid usually used is approximately 35 per cent. In 1873 Hlasiwetz and Habermann (3) introduced hydrochloric acid as an agent for hydrolyzing proteins. Hydrochloric acid is preferred to sulfuric acid when the monoamino acids are to be determined. Hydrolysis is generally carried out with 20 per cent acid. Other acids have also been used. Bernstein (4) hydrolyzed proteins with 57 per cent hydriodic acid in the presence of dihydrogen phosphate. A mixture of hydrochloric acid and formic acid was employed by Miller and du Vigneaud (5) for the hydrolysis of insulin. Use of titanous chloride in the presence of hydrochloric acid was reported by Hess and Sullivan. (6) This mixture hydrolyzes proteins with the formation of little humin and with a shortening of the time of hydrolysis.

Sodium hydroxide, potassium hydroxide, barium hydroxide are among the group of agents used for alkaline hydrolysis of proteins. The alkalies have the advantage over acids only in that they do not destroy tryptophane. Hence they are often used when this amino acid is to be isolated. Ammonium hydroxide or ammonium carbonate have also been used for the hydrolysis of proteins but offer no advantages.

The third group of hydrolytic agents consists of enzymes. Enzymes that are capable of catalyzing the hydrolysis of proteins are termed proteolytic enzymes. They occur widely in nature. Schulze and co-workers were the first to study the action of vegetable enzymes on proteins. Kühne, Chittenden, Kossel, Kutscher, Drechsel, Willstater, Leitz, Northrop, Sumner, have studied the action of animal enzymes

on many different proteins. Pepsin, trypsin, erepsin, papain are among the group of enzymes commonly used for the hydrolysis of proteins. The hydrolytic action of the proteolytic enzymes on protein is probably never complete, but in some instances may be nearly so as is alkaline or acid hydrolysis. (7) Hydrolysis of proteins by enzymes must be carried out under specific conditions. An intimate knowledge must be acquired of the potency of the enzyme, its activation, and inactivation, its specificity, the acidity of its optimum catalytic effect and the temperature.

REVIEW OF METHODS OF SYNTHESIS OF α-AMINO ACIDS

The majority of the methods of the synthesis of α-amino acids have been surveyed by Hamlin, (8) Barry, (9) and Mattocks, (10) but in most of them modifications have been introduced since then and hence their reconsideration here will not be out of place.

Amino acids may be synthesized by the following type reactions.

I. Amination of α-halogen acids, unsaturated dicarbonylic acid esters and anhydrides.

II. Hydrolysis of aminonitriles prepared by reaction of ammonia and hydrocyanic acid with aldehydes. (Strecker reaction)

III. Hydrolysis of the acyl, aryl or quaternary ammonium derivative of malonic ester, or cyanoacetic ester and of the pthalimido, benzamido, or α-oximino derivatives of these esters.

IV. Hydrolysis of the products prepared by the reaction of aldehydes with hippuric acid, hydantoins or diketopiperazines. (Erlenmeyer's synthesis)

V. Beckmann rearrangement and hydrolysis of cycloketoximes followed by bromination and amination of the resulting α-amino monocarboxylic acids.

VI. Oxidation followed by hydrolysis of benzoylamino alcohols.

VII. Reactions of hydrazoic acid and α-amino acids (Schmidt synthesis of diamino acids).

VIII. Addition of sodium enol malonates to α,β-unsaturated acid esters (Michael condensation).

IX. Degradation of substituted malonic acid hydrazides (Curtius reaction).

X. Catalytic reduction and amination of α-keto acids.

XI. Catalytic reduction of oximes and phenylhydrazones of α-keto acids.

I. One of the oldest and most general methods of preparing amino acids consists of treating an α-halogen acid with ammonia, (11,12,13,13a) α-bromo acids are readily prepared and are more reactive than α-chloro acids.

$$R - \underset{X}{CH} - COOH \xrightarrow{NH_3} R - \underset{NH_2}{CH} - COOH + NH_4X$$

The amonia is usually supplied in the form of concentrated ammonium hydroxide. Cheronis and Spitzmeuller (14) reported that the addition of ammonium carbonate to the ammonium hydroxide generally increased the yield. This method of preparing amino acids is useful only when the necessary α-halogen acids are readily available.

Aliphatic α-bromo acids are made by direct bromination of the corresponding fatty acid. In other instances the corresponding malonic acid is employed. Because of the much greater ease of bromination of this type of compound, it is possible to prepare bromo acids containing groups which would themselves be brominated under more drastic conditions.

Special methods of preparation of α-bromo acids have been developed in the synthesis of more complex amino acids. Thus serine is synthesized in the following manner. (15)

$$H_2C{=}CH{-}COOMe \xrightarrow{HOBr} \underset{HO\ \ Br}{H_2C{-}CH{-}COOMe}$$

$$\underset{HO\ \ NH{-}CO{-}\phi}{H_2C{-}CH{-}COOMe} \xrightarrow{HOH} \underset{HO\ \ NH_2}{H_2C{-}CH{-}COOMe}$$

serine ester

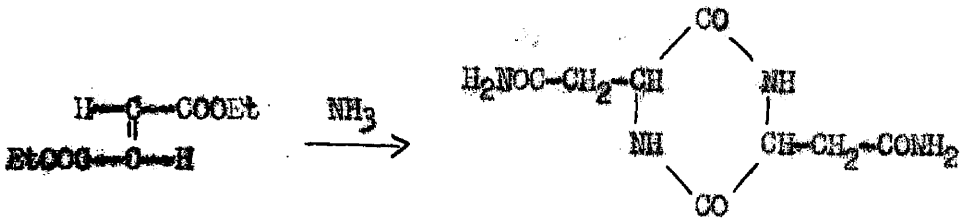
Schrauth and Geller (16) found that when mercuric salts in alcohol were allowed to react with α,β unsaturated carboxylic acid, HgBr was introduced in the α position and the alkyl group of the alcohol in the β position.

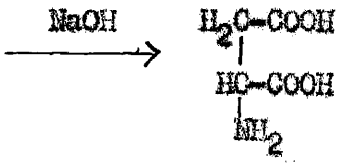
$$R_2C{=}C{-}COOH + Hg(OAc)_2 \xrightarrow[KBr]{CH_3OH} \underset{MeO\ \ HgBr}{R_2C{-}CH{-}COOH} \xrightarrow{Br_2}$$

$$\underset{MeO\ \ Br}{R_2{-}C{-}CH{-}COOH} \xrightarrow{NH_4OH} \underset{MeO\ \ NH_2}{R_2{-}C{-}CH{-}COOH} \xrightarrow{HBr} \underset{HO\ \ NH_2}{R_2{-}C{-}CH{-}COOH}$$

Threonine, serine, and hydroxynorvaline have been prepared by this method.

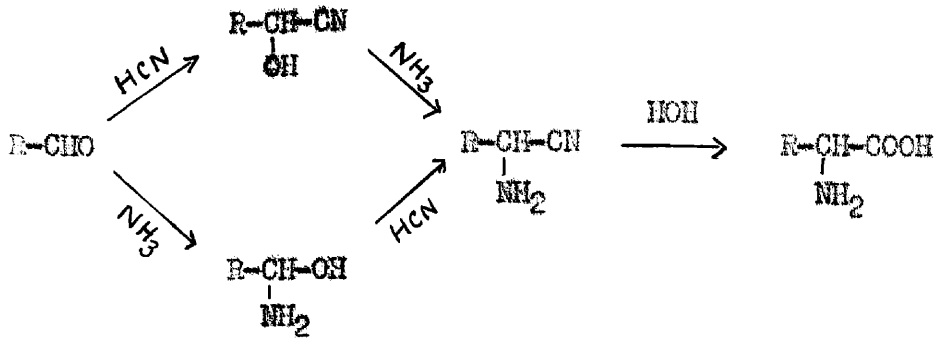
In some cases direct amination of unsaturated dicarboxylic acid ester is employed to prepare the amino acid. Thus aspartic acid is made in about 60 per cent of the theoretical yield by autoclaving a mixture of dry ammonia and diethyl fumarate. (17)





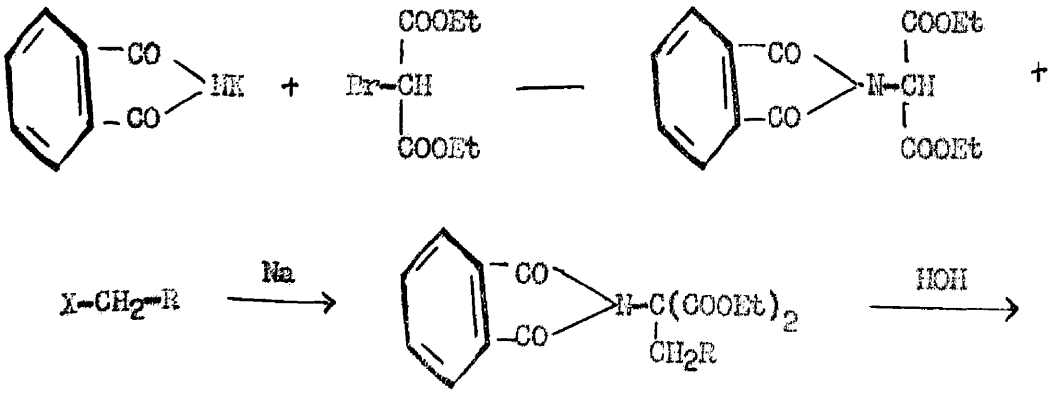
Aspartic acid is also obtained when maleic anhydride is heated with aqueous ammonia in an autoclave, followed by hydrolysis of the intermediate solids with concentrated hydrochloric acid.

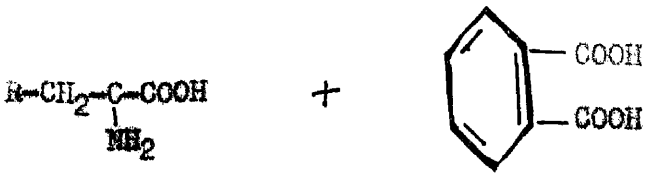
II. The conversion of an aldehyde or a ketone to an amino acid with one more carbon atom was accomplished by Strecker in 1850. (18) The reaction may be carried out in a variety of ways; the water, alcohol or ether solution of the aldehyde or the ketone may be treated with ammonia or ammonium salts followed by hydrogen cyanide or sodium cyanide, or the cyanohydrin may be formed first and then treated with ammonia.



In any case an aminonitrile results, which on hydrolysis with strong acids or bases is converted to the amino acid. The inavailability of many aldehydes and their tendency to undergo side reactions limit the application of this reaction. Despite the many improvements and modifications made in the Strecker synthesis, (19,20,21) it is rarely used except in the synthesis of glycine and alanine.

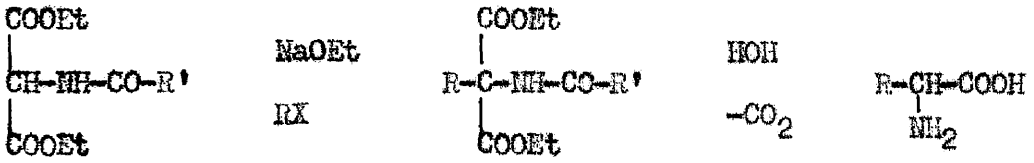
III. Another method for the preparation of -amino acid is the Sorensen's extension (22) of the Gabriel Phthalimide procedure. Phthalimidomalonic ester is easily obtained from potassium phthalimide and bromomalonic ester. (23) It contains a hydrogen atom which can be



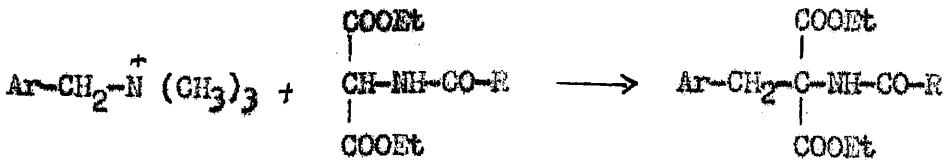


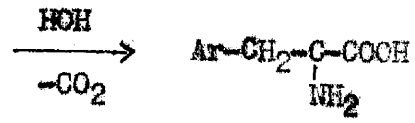
replaced by an alkyl group by treatment with sodium and alkyl halide. Lysine, (24) methionine, (25) cystine, (26) and tyrosine (27) have been prepared by this method.

With the advent of catalytic reduction procedures, aminomalonic ester has become readily available. It is not suitable for use in the synthesis of α-amino acids since the free amino group would undergo alkylation. However, N-acylated derivatives of this ester have proved very useful intermediates. (13,28,29,30,31,32)



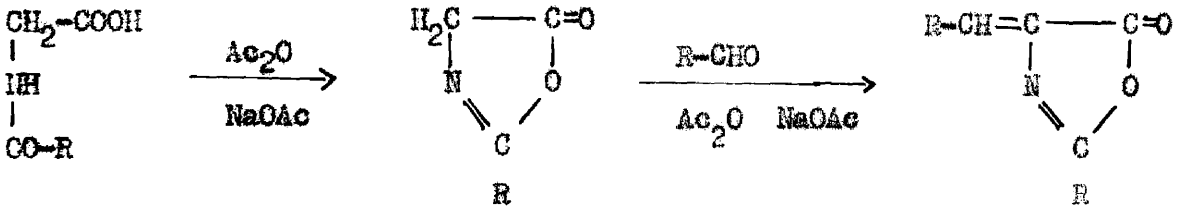
The alkylation proceeds smoothly, although the acylaminomalonic esters are not as easily alkylated as is malonic ester itself. The subsequent steps are effected in excellent yields. Ethyl acetamidocyanoacetate has been substituted in place of ethyl acetamidomalonate and has proved more advantageous. (33,34) An interesting variation of this method has been developed by Snyder and Smith. (35) They discovered that quaternary ammonium compounds of the type $Ar-CH_2-\overset{+}{N}(CH_3)_3$ will alkylate malonic esters.





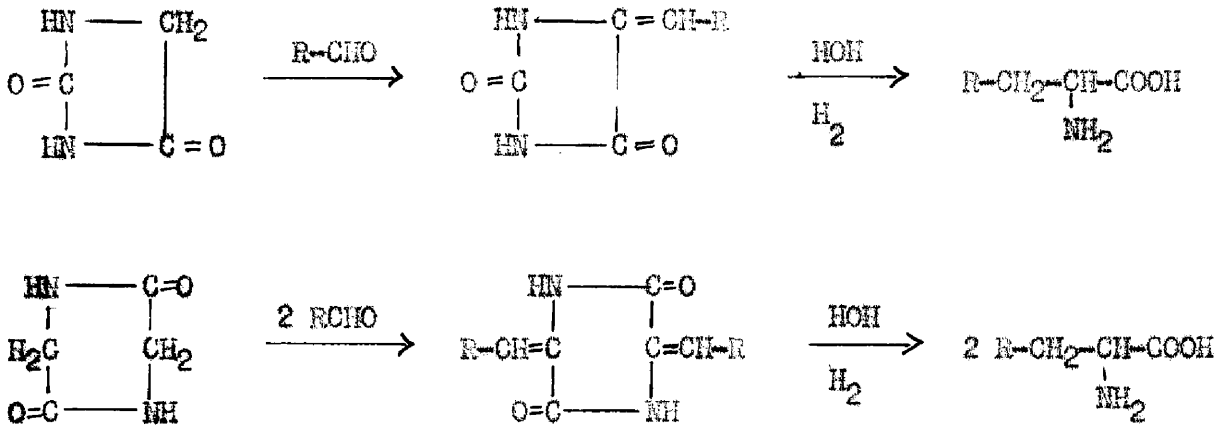
This discovery led to an excellent preparative method for tryptophane.

IV. α-Amino acids containing aromatic residues can be prepared by the so-called azalactone method of Erlenmeyer. (36) In this reaction the acyl derivative of glycine is treated with the aromatic aldehyde, sodium acetate and acetic anhydride. The acylglycine is converted into an oxazolone (azalactone) which possesses an active methylene group and condenses readily with the aldehyde to give an unsaturated azalactone. This can be hydrolyzed to N-acyl unsaturated acid, or



the reduction and hydrolysis can be performed all in one stage by heating with hydriodic acid and red phosphorus. Reduction of the unsaturated acid (acylaminoacrylic acid) may be effected with sodium amalgam (37) mixture of glacial acetic acid and acetic anhydride (38) or catalytically using platinum as the catalyst. (39,40) Other glycine derivatives such as hydantoin and diketopiperizine also readily condense

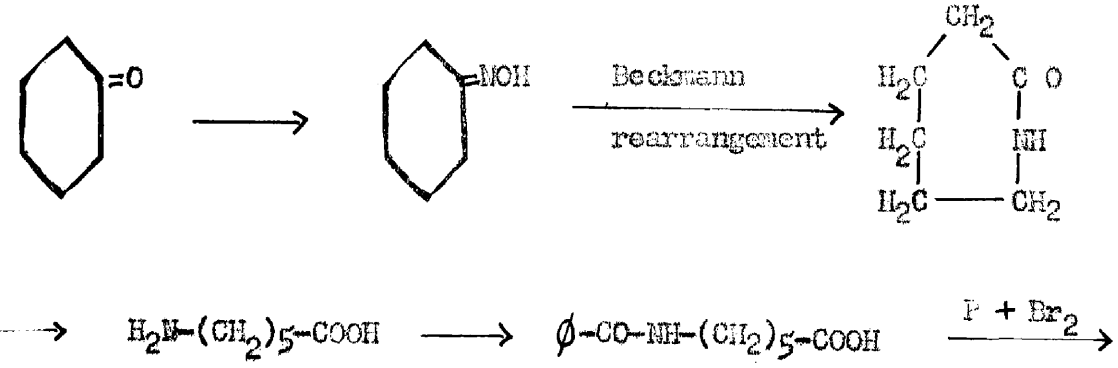
with aromatic aldehydes to give unsaturated derivatives which can be hydrolyzed and reduced to the free amino acid.

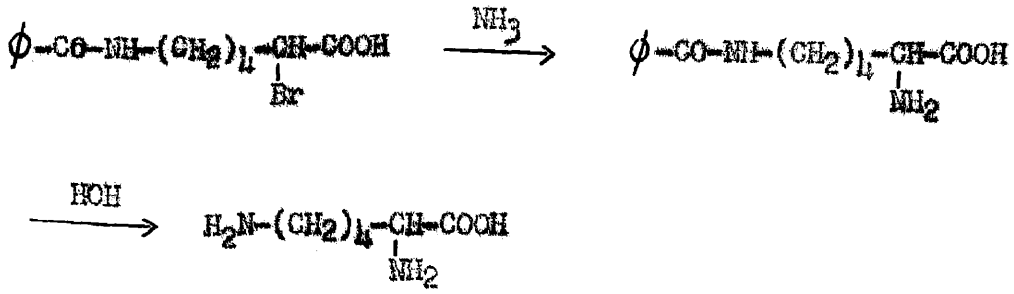


2:5 Diketopiperazine

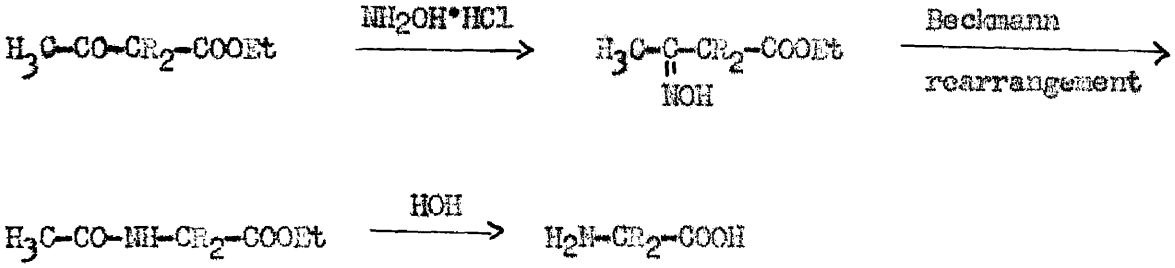
Phenylalanine, tyrosine, tryptophane and other aromatic amino acids have been prepared by this method, but not with any advantages over the azalactone method.

V. The oximes of cyclic ketones such as cyclopentanone, cyclohexanone undergo the Beckmann rearrangement when treated with sulfuric acid. Taking advantage of this fact Nek and Marvel (41) prepared lysine, starting with cyclohexanone.





Although several steps are involved, each proceeds smoothly and the overall yield is satisfactory. Ornithine has been prepared by applying the same series of reactions to cyclopentanone. (42) The formation of α-amino acids can also be accomplished by the Beckmann rearrangement of β-isonitroso esters. (43)

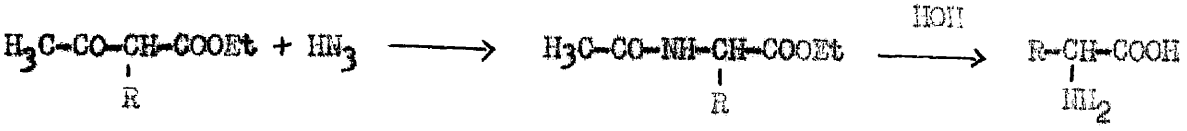


Yields of α-amino acids varying from 25-40 per cent are obtained by this procedure.

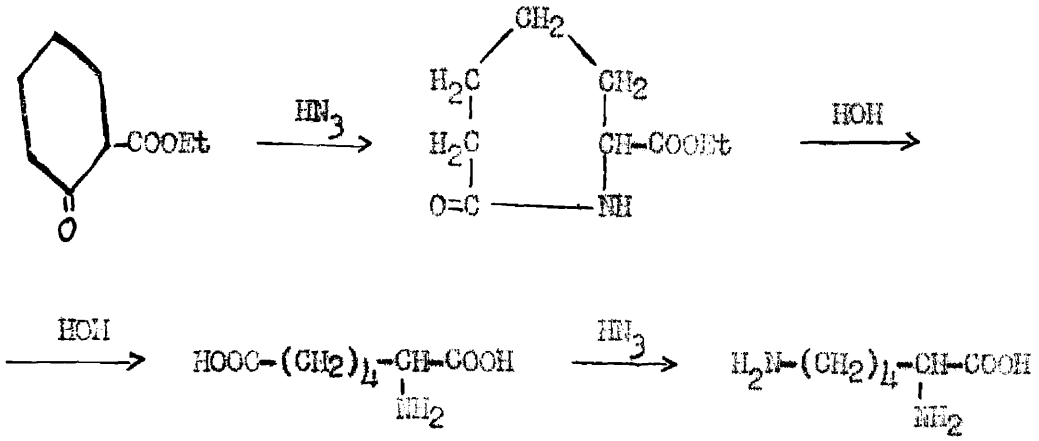
VI. In recent years α-amino alcohols have become available commercially. These alcohols can be smoothly converted into α-amino acids in good yields. (44)

Glycine, alanine, and many other simpler amino acids have been prepared by this procedure.

VII. Another interesting way of preparing α-amino acids is by Schmidt hydrazoic acid reaction. Schmidt prepared glycine, aspartic acid, phenylalanine in from 80-98 per cent yields by the action of hydrazoic acid on acetoacetic ester.

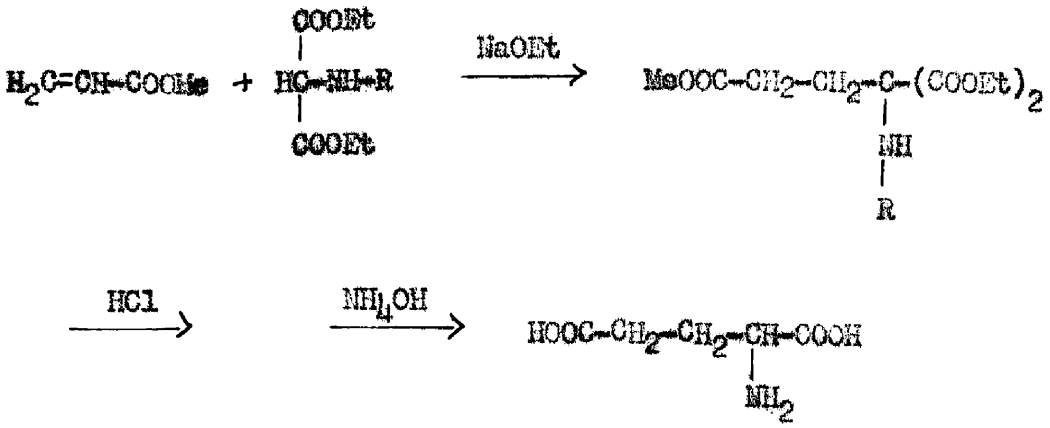


α-Amino acids do not undergo this reaction. Taking advantage of this fact, lysine was prepared from α-aminopimelic acid. (45)



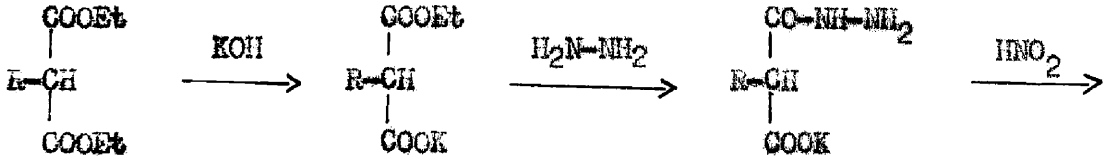
A serious drawback to this procedure is that hydrazoic acid is extremely toxic and explosive if not properly handled.

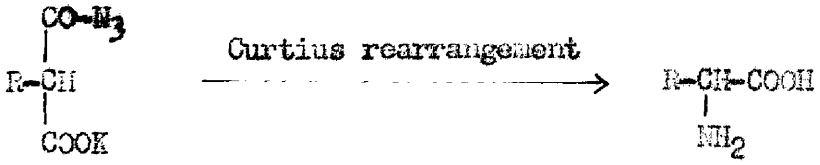
VIII. The Michael condensation has been applied in a very satisfactory manner to the preparation of glutamic acid from methyl acrylate and phthalimido or acetamidomalonic ester. (46,47)



Reactions of this type have been studied extensively in recent years by Connor, et. al.. (48,49,50,51,52,53)

IX. Substituted malonic esters may be converted into α-amino acids by the following reactions.





The use of substituted cyanoacetic esters has been employed by Darapsky (54,55) in the preparation of glycine, valine, leucine, and by Kuo-Hao Lin and co-workers (56) in the preparation of α-amino-isobutyric acid.

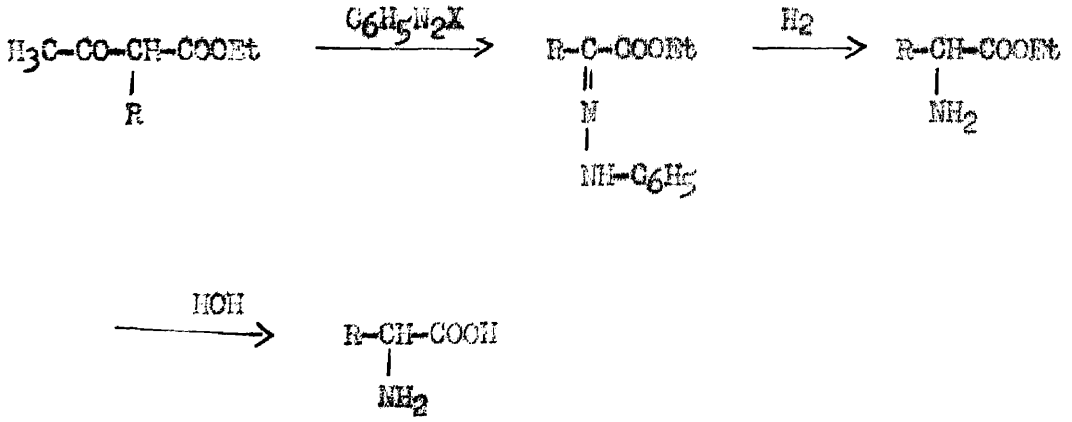
The Curtius reaction may be of special use when bromination

of substituted malonic acids cannot be carried out because of the reactivity of the substituent group. (57)

X. α-Keto acids when reduced with the palladium in the presence of ammonia yield α-amino acids. Knoop and Oesterlin (58) first studied this reaction. It was further developed by Shoenheimer and Ratner. (59) The method gives good yields of amino acids but is limited by the lack of available α-keto acids. (59a)

XI. Various derivatives of α-keto acids eg. oximes, phenylhydrazones can also be conveniently reduced to α-amino acids. This method of obtaining the amino acids has been used extensively in recent years and will be discussed here in greater detail.

Preparation and Reduction of Phenylhydrazones: Substituted acetoacetic esters are converted into the phenylhydrazones of α-keto acids by the action of benzenediazonium chloride. The reduction of the phenylhydrazone yields α-amino acid. Fischer and Groh (60)



obtained a small amount of alanine by reduction of pyruvic acid phenylhydrazone with aluminum amalgam. Feofilaktov and collaborators (61,62,63,64,65) have modified the Fischer-Groh method into a general

procedure for the synthesis of α-amino acids. The oxime and phenylhydrazone of α-aceto-γ-butyrolactone, a valuable intermediate in the synthesis of methionine (66,67) were also prepared (68) by the action of nitrous acid and benzenediazonium chloride respectively on the γ-lactone. When this phenylhydrazone was reduced with tin and hydrochloric acid, the hydrochloride of α-amino-γ-butyrolactone was

γ-lactone. When this phenylhydrazone was reduced with tin and hydro-

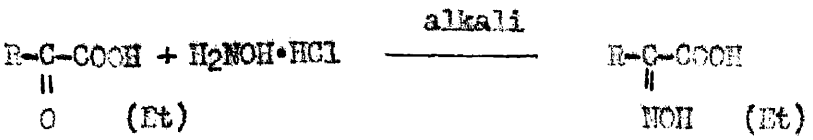
obtained. In the study of the catalytic reduction of the phenylhydrazone by Synder et. al., it was revealed that the reduction in alcohol at 100°-150° with Raney nickel yielded the diketopiperizine instead of the α-amino-γ-butyrolactone. However reduction in ethyl acetate containing acetic anhydride gave a mixture of acetanilide and α-acetamino-γ-butyrolactone.

Preparation and Reduction of Oximes: Oximes of α-keto acids can be smoothly reduced catalytically or chemically to the α-amino acids. The reduction probably proceeds through the intermediate imine stage. (8,9,69). However Waters and Hartung (69a) found that in the hydrogenation of the oxime of phenylpyruvic acid, if the reduction was interrupted before completion, only phenylalanine and unchanged oximino acid could be isolated.

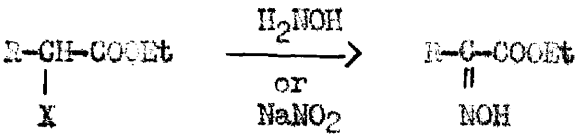
$$\text{R-C(=NOH)-COOH} \xrightarrow[\text{catalyst}]{H_2} \text{R-C(=NH)-COOH} \xrightarrow[\text{catalyst}]{H_2} \text{R-CH(NH}_2\text{)-COOH}$$

The α-oximino acids (or esters) may be prepared in several ways. These methods may be briefly classified as follows:

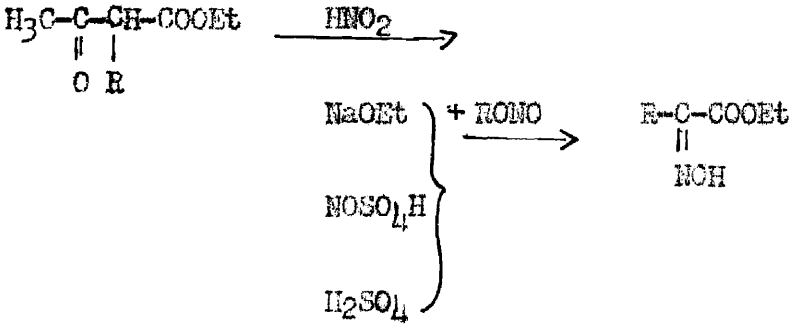
1. Reaction of α-keto acids and hydroxylamine.



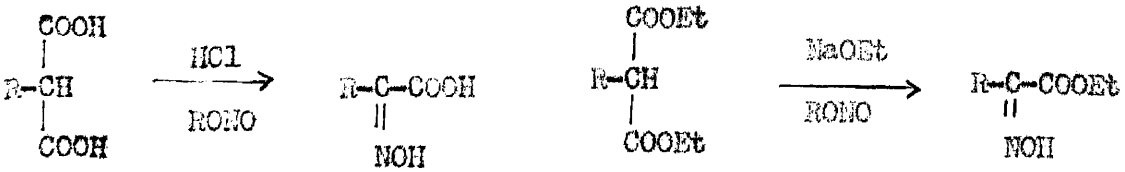
2. Reaction of esters of α-halogen acid with hydroxyl amine or inorganic nitrites.



3. Nitrosation of substituted aceto acetic esters.



4. Nitrosation of substituted malonic acids (or esters).



When an α-keto acid is treated with hydroxylamine hydrocloride in the presence of a base, the corresponding α-oximino acid is obtained. Many workers (70,71,72) have prepared the oximes of pyruvic acid, glyoxalic acid, α-ketoisovaleric acid by this procedure in good yields. The serious drawback of this procedure is the difficulty in obtaining the α-keto acids.

The esters of α-oximino acids are also obtained when esters of α-halogen acids react with inorganic nitrites such as sodium nitrite. (73) Sodium nitrite was replaced with hydroxylamine to prepare α-oximino-acetic,-propionic and -butyric acids in this manner. (74) Recently Hamlin (8) has prepared ethyl α-oximinocaproate in 65 per cent yield from ethyl α-bromocaproate and sodium nitrite. The required α-halogen esters can be easily obtained by the method of Zelinsky.

The oximes of the α-keto acids are more conveniently obtained directly from substituted acetoacetic esters than by the above described methods. Acetoacetic esters react readily with nitrous acid or nitrosylsulfuric acid or with alkylnitrite to yield the α-oximino esters. Ethyl α-oximinopropionate was prepared from methyl acetoacetic ester by Meyer, Zublin and by Dieckmann and Groeneveld (75,76) using nitrous acid and ethyl nitrite in presence of sodium ethoxide, respectively. A series of interesting papers were published by Bouveault and Locquin (77,78,79,80,81) on the use of nitrosylsulfuric acid with alkyl substituted acetoacetic esters. They obtained α-oximino acetic, -propionic,-butyric,-valeric,-isovaleric,-isocaproic,- β-methylvaleric and -isoheptanoic acids in yields ranging from 85-90 per cent.

Following the methods of Dieckmann and also of Bouveault and Locquin, Wislicenus and Grutsner (82) obtained diethyl α-oximinoglutarate and ethyl α-oximino phenylacetate. In 1915, Hall, Hynes, and Lapworth, (83) applying the method of Bouveault obtained the α-oximino acid corresponding to phenylalanine. Recently the method of Bouveault and Locquin has been modified and used extensively by several workers for obtaining α-oximino acids. (8,9,84,85,86,87)

α-Oximino acids or their esters are obtained also from substituted malonic esters. Baeyer (88) in 1864 prepared oximino malonic acid from oximinobarbituric acid. Following the underlined principle of the method of Baeyer, Dieckmann and Groeneveld (76) and later Fischer and Weigert (89) utilized the reaction for the preparation of ethyl α-oximinopropionate and ethyl α-oximinocyanovalerate from methylmalonic and cyanopropylmalonic ester, respectively. They nitrosated the malonic esters with ethyl nitrite in the presence of sodium ethoxide.

In 1903, Bouveault and Wahl (90) prepared oximinomalonic ester using sodium ethoxide and methyl nitrite which was improved lately by Redemann and Dunn (91) by use of n-butyl nitrite in place of methyl nitrite. Recently Shivers and Hauser (92,93) synthesized certain α-oximino esters from substituted malonic esters as well as from substituted acetoacetic ester and they have gotten better yields with substituted malonic esters.

The reduction of an α-oximino acid was first reported by Gutknecht (94) in 1880. He reduced the silver salt of α-oximino-propionic acid by means of tin and hydrochloric acid to alanine. Since that time many workers (80,81,95,96,97,98,99,100,101,102,103,104) have

applied this or similar chemical reduction procedures for the reduction of α-oximino acids to the corresponding α-amino acids. The catalytic reduction of these oximes have also been accomplished. Wassiljew (105) using nickel as a catalyst in the hydrogenation of oximes obtained both primary and secondary amines. A pyrasine derivative was obtained by the reduction of α-oximinoacetoacetic ester with Raney nickel by Adkins, (106) but he and Reeve (87) showed that good yields of primary amine can be obtained when the methyl ether of the oxime was used. In 1934 Bauguess and Berg (107) used the same catalyst for the reduction of the α-oximino acid corresponding to tryptophane. Levene and Schoemuller (108) and later Hedemann and Dunn (91) used it to reduce oximinomalonic ester to aminomalonic ester. Recently Shivers and Hauser (93) have reduced certain α-oximino esters with Raney nickel at high pressure and at temperatures 70°-75°C..

Platinum catalyst has been used in the reduction of oximes. Shemin and Herbst (40) used platinum oxide to reduce α-oximino acids to amino acids. Reductions of α-oximinoglutarate and α-oximino-δ-chloro-γ-valerolactone have been accomplished by the same catalyst. (84)

The use of palladium in the reduction of oximes was first made by Paal and Gerum (109) in 1908, when they reduced benzaldoxime. These two workers and Gulewitsch (110) found that the reduction leads to the formation of secondary amines and other side products. Rosenmund and Pfankuch (111) using palladium on barium sulfate were able to reduce benzaldoxime to benzyamine without the formation of secondary amine. The problem was further studied in greater detail by Hartung (112)

who was able to reduce oximes to primary amines in good yields with palladinized charcoal in alcoholic hydrochloric acid solution. This catalyst has been used by Harrington and Randall (86) to reduce ethyl α-oximino-β-ketoglutarate to hydroxyglutarate to hydroxyglutamic acid. Using two equivalents of hydrogen chloride in 95 per cent alcohol, Hamlin and Barry (7,8) were able to reduce several α-oximino acids or esters to the corresponding α-amino acids or esters in good yields. The reduction of the alkoximino acids at room temperatures and at low pressures has been studied by Waters (69) and it has been observed that hydrogenation of the alkoximino acid takes place slowly when alcohol is used as the solvent. However when alcohol was substituted by glacial acetic acid as solvent, a better yield of the corresponding α-amino acid was obtained.

METHODS OF PREPARING ALKYLMALONIC ESTERS

METHODS OF PREPARING ALKYLMALONIC ESTERS

The alkylated malonic esters can be synthesized in three ways:

(i) Alkylation of malonic ester.

(ii) Oxalate condensations.

(iii) Condensation of an aldehyde with malonic ester.

The alkylation of malonic ester is usually carried out by treating the sodiomalonic ester with an alkyl halide in absolute alcohol.

$$Na^{+}\ [CH(COOEt)]^{-} + RX \longrightarrow R\text{-}CH\begin{matrix}COOEt\\COOEt\end{matrix} + NaX$$

The reaction is a very general method for the preparation of alkyl derivatives of malonic ester, for there are very few limitations of the

type of alkyl halide that can be used with fully satisfactory results. Conrad and Bischoff (113) and later Michael (114) prepared ethylmalonic ester in this manner.

Ethyl n-butylmalonate was prepared by this procedure by Bischoff (115) and also Adams and Marvel (116). Directions for the preparation of this ester can be found in "Organic Synthesis". (117) Levene and co-workers (118) applied this reaction for the preparation of ethyl n-undecylmalonic ester, which indicates that higher alkyl halides can be employed for the alkylation of the malonic ester.

Condensations of the Claisen type between ethyl oxalate and esters of fatty acids producing α-ethoxalyl esters were first carried out by Wislicenus.(119) Applying this principle to the condensation of ethyl carbonate with other esters, Wallingford and others (120) were able to produce yields of 10 to 86 per cent of alkylmalonic esters. Replacing the ethyl carbonate with ethyl oxalate and under forcing conditions, it is possible to obtain good yields of α-ethoxalyl esters. (121)

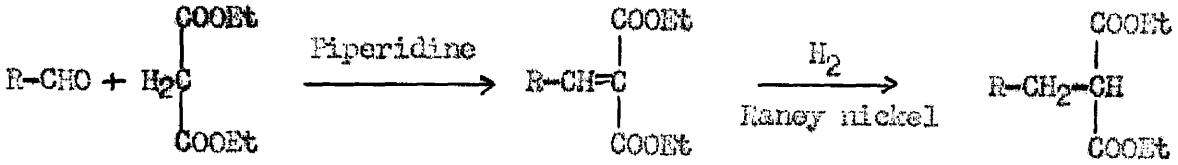
$$R\text{-}CH_2\text{-}COOEt + (COOEt)_2 \longrightarrow \underset{\underset{COOEt}{|}}{R\text{-}CH\text{-}CO\text{-}COOEt} \xrightarrow{-CO} R\text{-}CH\begin{matrix}COOEt\\COOEt\end{matrix}$$

α-ethoxalyl ester

The α-ethoxalyl esters may then be thermally decarbonylated to yield alkylmalonic esters.

A Knoevenagel type reaction between an aldehyde and malonic ester followed by catalytic hydrogenation of the resulting product over Raney nickel, (122) renders available another method for the alkylation

of malonic ester. (123) For this purpose an aldehyde is allowed to react with malonic ester in the presence of piperidine. The resulting unsaturated ester is then hydrogenated over Raney nickel at room temperature.



A number of alkylmalonic esters have been prepared by this procedure.

RESEARCH AIM

The usefulness of ∝-oximino acids as good potential intermediates for the synthesis of ∝-amino acids was nicely indicated by Hamlin and by Barry. (8,9) The latter described a general method of nitrosating substituted malonic esters by alkyl nitrite in the presence of sodium ethoxide, a reaction reported by Dieckmann and Groenveld. (76) Excellent yields of ∝-oximino acids were obtained by this procedure; besides, unlike Bouveault, Hamlin's method (nitrosation of substituted acetoacetic ester) this method has few limitations.

The primary aim of this investigation was to synthesise some long chain ∝-amino acids, study their properties and, time permitting, to link them in the form of a peptide chain by the method of Waters and Weaver. (69,136)

The long-chain ∝-amino acids, $RCH(NH_2)COOH$ where R varies from $n\text{-}C_6H_{13}$ to $n\text{-}C_{18}H_{37}$, may be looked upon as hybrids or crosses

between the components of proteins and those of fats. None has been identified from natural sources, yet their undiscovered biological properties excite one's imagination. It is not unlikely that they will be antagonists to some of the essential amino acids and perhaps, they may be antagonistic to the fatty acids also.

The peptide-like union of these long-chain amino acids should be of interest to the colloid chemist. For example, if R is $C_{16}H_{33}$,

$$\underset{\text{m.w. } 299}{RCH(NH_2)COOH} \longrightarrow \underset{\text{m.w. } 580}{RCH(NH_2)CO\text{-}NH\text{-}CHR\text{-}COOH} \longrightarrow$$

$$RCH(NH_2)CO\text{-}NH\text{-}CHR\text{-}CO\text{-}NH\text{-}CHR\text{-}COOH \longrightarrow \underset{\text{m.w. } 1704}{NH_2CHR\text{-}CO(NH\text{-}CHR)_4\text{-}NH\text{-}CHR\text{-}COOH}$$

m.w. 871

Thus appears the prospect of a transition from the molecular to macromolecular to colloid state, with perhaps many of the functional properties of the peptide linkage not too much masked.

To attain the purpose, the problem will be approached in the following way:

1. To find the optimum conditions for the alkylation of malonic ester with higher alkyl halides.
2. To extend the nitrosation method developed by Barry to these esters for the preparation of α-oximino acids.
3. To hydrogenate the α-oximino acids to the corresponding -amino acids.

EXPERIMENTAL

General

All temperatures recorded as the boiling points of the alkylmalonic esters synthesised are uncorrected.

The melting point determinations of all the products were carried out on the Fisher melting-point block built by Fisher Amend Company.

The "absolute alcohol" used in the various experiments was prepared from commercial absolute alcohol by the process of Lund and Bjerrum (124) given in the "Experiments in Organic Chemistry". (125) In this process, a mixture of 5 g. of magnesium turnings, 60 cc. of commercial absolute alcohol and 0.5 g. of iodine is refluxed in a large flask until a vigourous reaction starts and until nearly all the magnesium has reacted and is converted into magnesium ethoxide. Nine hundred cc. of commercial absolute alcohol is then added and the mixture is refluxed for one hour and the anhydrous alcohol is distilled into a receiver carrying an anhydrous calcium chloride tube. The product is stored in a well-stoppered bottle.

The various alkyl halides used in the alkylation of malonic ester and malonic ester itself are commercially available in fairly pure condition and were used without further purification.

SYNTHESIS

Ethyl n-butylmalonate:

This ester was synthesised by using a modification of

the method of Adams and Kamm. (117)

$$H_2C(CO_2Et)_2 + EtONa + n\text{-}C_4H_9Br \longrightarrow n\text{-}C_4H_9CH(CO_2Et)_2 + C_2H_5OH + NaBr$$

A dry one-litter three-neck flask equipped with a reflux condenser carrying a drying tube, mechanical stirrer and a separatory funnel was placed over a steam bath. In the flask was placed 250 cc. of absolute alcohol and then 11.5 g. of clean sodium (0.5 gram atom) was added slowly through the condenser. After all the sodium went in solution, the sodium alcoholate solution was cooled to about 50°C. and stirred after which 82.5 g. (78 cc., approx. 0.5 mole) of diethyl malonate was added dropwise through the separatory funnel. A half mole (68.5 g., 54.5 cc.) of butyl bromide was then added gradually. After the butyl bromide was added completely, the mixture was refluxed until neutral to moist litmus. The alcohol from the mixture was then distilled off over the steam bath, under slightly reduced pressure (water aspirator was used). The residue in the flask was dissolved in 200 cc. of water and the upper layer separated. The water layer was extracted twice with ether and the ether extracts were added to the upper layer of the first separation. This etherial solution was washed three times with water, twice with 50 cc. portions of dilute sulfuric acid and then washed completely free of acid with water. It was dried over anhydrous sodium sulfate and after removing the ether, distilled from a Claison flask under reduced pressure. Colorless ester, distilling at 128-132°/18 mm. was collected. The yield was 81 g. (75 per cent of the theoretical amount).

n-Butylmalonic Acid:

Following the method of Barry (9) for the saponification of ethyl benzylmalonate, 13.5 g. of ethyl n-butylmalonate was added dropwise through a separatory funnel into a solution of 13 cc. of H_2O and 13 g. of 85 per cent KOH in a three-neck flask, equipped with a mechanical stirrer. The flask was heated on a steam bath. Gentle suction was applied at the neck of the flask to remove ethyl alcohol formed during the hydrolysis of the ester. Heating and stirring were continued for two hours and water added to the mixture to prevent solidification. The mixture was poured in a beaker which was chilled by an ice-salt bath. It was made distinctly acidic to congo red paper with concentrated HCl. A white solid separated out which was filtered off and the filtrate was salted out and exhaustively extracted with ether. The ether extract was dried over anhydrous sodium sulfate and evaporated by means of a current of dry air. Very little solid was obtained from the ether extract which was added to the main product and the combined solids were crystallized from toluene. The yield of n-butylmalonic acid was 49 per cent; m.p. 101°, reported m.p. 102°. (126) The ester can be more conveniently saponified by the following procedure. In a 100 cc. beaker, 4.32 g. (.02 mole) of ethyl n-butylmalonate was saponified by heating with excess of 50 per cent NaOH solution. A white curdy mass formed floating on the top of the solution. Heating was continued for another five minutes and then water was added just sufficient to dissolve the soap (sodium salt of n-butylmalonic acid) in the hot solution. The solution was then cooled and extracted with ether and the water layer was acidified to congo red paper with concentrated HCl, keeping the container cool with an ice bath.

The yield of n-butylmalonic acid, m.p. 100°C., was 2.5 g., 78 per cent. The procedure was slightly modified when ethyl n-dodecylmalonate and higher alkylmalonates were saponified. After heating the esters with 50 per cent NaOH solution, the soap that was formed was washed twice with acetone and was decomposed with concentrated HCl to get the free acid.

Ethyl n-hexylmalonate:

By the procedure outlined for the preparation of ethyl n-butylmalonate, 41.25 g. (39 cc., 0.257 mole) of malonic ester was alkylated with 53 g. (0.25 mole) of n-hexyl iodide. The distillate fraction boiling at 155-158°/18 mm. was collected. It weighed 38 g. representing approximately a 64 per cent yield; the reported b.p. is 145-148°/15 mm.. (127) The melting point of acid corresponding to the ester was 104-105°; reported 105°. (127)

Ethyl n-heptylmalonate:

The alkylation of 41.25 g. (0.257 mole) of malonic ester with 56.5 g. (0.25 mole) of n-heptyl iodide was carried out in the usual manner. When the alkylated ester was distilled under reduced pressure, three fractions came over at different temperatures and a good amount of residue of dark brown color remained in the Claisen flask. When an attempt was made to distill this residue, there was considerable amount of decomposition and therefore this fraction was discarded. This variety in the distillation temperatures was probably due to some disubstitution of malonic ester. In order to overcome this discrepancy in stable distilling temperature, the amount of malonic ester was doubled with respect to the alkyl halide used in the second run. A half mole (80 g.) of malonic ester was alkylated with 56.5 g. (0.25 mole) of n-heptyl iodide. The yield of

ethyl n-heptylmalonate boiling at 142-144°/7 mm. was 46.5 g. (72 per cent of the theoretical amount). Reported b.p. in the literature is 136-138°/3 mm.. (123) The corresponding n-heptylmalonic acid melted at 94-95°; reported m.p. 95-96°. (123)

Ethyl n-octylmalonate:

Malonic ester weighing 80 g. (76 cc., 0.5 mole) was alkylated with 60 g. (0.25 mole) of n-octyl iodide in the usual manner. The weight of the alkylated ester boiling at 171-174°/13 mm. was 47.0 g. (68 per cent of the theoretical amount). The reported b.p. is 150-153°/7 mm.. (128) Saponification of the ester yielded n-octylmalonic acid, melting at 114-115°, reported m.p. being 116°. (129)

Ethyl n-decylmalonate:

The alkylation of 80 g. of malonic ester with 67 g. (0.25 mole) of n-decyl iodide resulted in the formation 54 g. of ethyl n-decylmalonate boiling at 178-181°/8 mm. (74 per cent of the theoretical amount). The alkyl ester was reported to boil at 155-158°/3 mm. (130) n-Decylmalonic acid which was obtained by saponification of the ester melted at 119-120°, when crystallized from toluene; reported m.p. 120°. (131)

Ethyl n-dodecylmalonate:

This ester was prepared in 53 per cent yield by alkylation of 80 g. of malonic ester and 75 g. (0.25 mole) of n-dodecyl iodide. The weight of the product boiling at 178-182°/5 mm. was 44 g.. Reported b.p. is 170-173°/3 mm.. (132) The corresponding malonic acid melted at 117-118°; reported m.p. in literature being 119°. (135)

Ethyl n-tetradecylmalonate:

By the usual procedure 80 g. (0.5 mole) of malonic ester was

alkylated with 81 g. (0.25 mole) of n-tetradecyl iodide. Ethyl n-tetra decylmalonate came over at 199-203°/10 mm., but unlike the product which should have a colorless appearance, it was dark yellow in color. This was due to the impurity in the crude alkyl ester which distilled just before the distillation temperature of the ester was reached. The product was redistilled to remove the impurity, but apparently there was very little change in color except for slight fading in it. Suspecting the impurity to be due to iodine resulting from the decomposition of sodium iodide adhering to the ester, it was thought that rewashing the ester with sodium thiosulfate solution and then with water might improve its appearance. When so treated and redistilled, the ester came over as colorless liquid. It weighed 22 g. representing 24 per cent yield.

In another run 80 g. of malonic ester was alkylated with 69.2 g. (0.25 mole) of n-tetradecyl bromide instead of the iodide. Condensation seemed to procede very smoothyl. The yield of ethyl n-tetradecylmalonate by this slight variation increased from 24 to 52 per cent. The weight of the alkylmalonic ester boiling at 198-201°/9 mm. was 46.3 g.; reported b.p. being 182-185°/2 mm. (130) The melting point of n-tetradecylmalonic acid was 120-121°. Hell and Jordanoff reported the same melting point for the acid. (133)

Ethyl n-hexadecylmalonate:

This ester was prepared in 49 per cent yield when 48 g. (0.3 mole) of malonic ester was alkylated with 55.5 g. (approx. 0.15 mole) of n-hexadecyl bromide. The boiling point of the alkyl ester was 212-215°/10 mm. and the weight was 28 g.. Reported b.p. is 196-199°/2 mm.. (134) The corresponding n-hexadecylmalonic acid melted at 117-119°, reported m.p. 119-120°. (135)

Ethyl n-octadecylmalonate:

This alkyl ester was prepared like the others by alkylating 80 g. of malonic ester with 83 g. (0.25 mole) of n-octadecyl bromide. n-Octadecyl bromide being a solid at room temperature was dissolved in 50 cc. of absolute alcohol and was then added to the sodio-malonic ester solution in the reaction flask through the separatory funnel. The weight of ethyl n-octadecylmalonate boiling at 202-205°/4 mm. was 45 g. (approximately 44 per cent). The n-octadecylmalonic acid melted at 122-123°.

TABLE II

Alkylmalonic Esters and Acids Synthesized

Ester	Boiling Point (uncorrected)	Per Cent Yield	M.P. of corresponding alkylmalonic Acids
Ethyl n-butylmalonate	128-132°/18 mm.	75	100-101°
Ethyl n-hexylmalonate	155-158°/18 mm.	64	104-105°
Ethyl n-heptylmalonate	142-144°/7 mm.	72	94-95°
Ethyl n-octylmalonate	171-174°/13 mm.	68	114-115°
Ethyl n-decylmalonate	178-181°/8 mm.	74	119-120°
Ethyl n-dodecylmalonate	178-182°/5 mm.	53	117-118°
Ethyl n-tetradecylmalonate	198-201°/9 mm.	52	120-121°
Ethyl n-hexadecylmalonate	212-215°/10 mm.	49	117-119°
Ethyl n-octadecylmalonate	202-205°/4 mm.	44	122-123°

α-OXIMINO ACIDS FROM ETHYL n-ALKYLMALONATES

α-Oximino acids were prepared by Barry, (9) Waters, (69) and Weaver (136) by nitrosation of alkylmalonic esters and alkylmalonic acids under alkaline and acid conditions respectively. In order to find out a suitable method for the preparation of α-oximino long-chain acids, both methods were employed and the results compared.

In all the nitrosation experiments n-butyl nitrite was used as a nitrosating agent. It was prepared by the following procedure.

n-Butyl nitrite:

In a three liter three-neck flask equipped with mechanical stirrer, a thermometer and a separatory funnel were placed 370 g. (5 moles) of n-butyl alcohol, 380 g. (5.5 moles) of finely powdered sodium nitrite and 500 g. of crushed ice. The flask was cooled with an ice-salt bath. Keeping the mixture in the flask well stirred, 500 cc. of cold concentrated HCl was added through the separatory funnel. The flow of the HCl was so adjusted that the temperature of the reacting mixture never rose above 5°. After all the HCl was added, the stirring was continued for another five minutes and then the mixture was allowed to separate in a three liter separatory funnel. The slightly greenish-yellow layer of butyl nitrite was separated and washed once with ice water, twice with saturated solution of sodium bicarbonate and then twice with ice water. The light-yellow liquid was dried over anhydrous sodium sulfate and distilled. The weight of the fraction boiling at 75-78° was 450 g. (87 per cent). The product was stored in the refrigerator in a well-stoppered flask. It keeps for a period of two months without appreciable decomposition.

α-Oximino-n-hexanoic Acid:

In a 500 cc. three-neck flask carrying a reflux condenser with drying tube, mechanical stirrer and a separatory funnel, was placed 50 cc. of absolute alcohol. Clean, freshly-cut sodium weighing 2.3 g. (0.1 gram atom) was added to it. After the reaction of the sodium, 21.6 g. (0.1 mole) of ethyl n-butylmalonate was added to the warm mixture, which was then cooled in an efficient ice-salt bath to 0°. Then 16.8 g. (0.15 mole) of butyl nitrite was slowly introduced beneath the surface of stirred solution. As the addition of the nitrite proceeded, the color of the mixture changed from light-yellow to deep red, but no appreciable rise in temperature was noted (below 5°). After all the butyl nitrite was added, the solution was stirred for 15 minutes longer. The ice-salt bath was removed and the solution was allowed to come to room temperature. The flask was connected to suction (condenser and dropping-funnel removed) and placed in a warm water bath at 65-70°. After the ethanol and butanol were nearly removed, (solution becomes pasty) 25 cc. of cold water was added to the pasty mass and the mixture was acidified with hydrochloric acid to Congo red. It was extracted throughly with ether and the combined ether extracts were shaken several times with 10 per cent sodium hydroxide solution to extract the oximino compound. The alkaline extracts were treated with 3 g. of norite and heated on a steam-bath for an hour. The solution was filtered through a fluted filter paper. The light brown solution was cooled in an ice bath and acidified with concentrated HCl. A light brown solid separated which was filtered off; the filtrate was salted out and exhaustively extracted with ether; evaporation of the ether gave more of the solid which was added to the main product and the combined solids (11.6 g., m.p. 122-125°, 80 per cent) were crystallized from a mixture of ether and n-heptane. The product crystallized in long white needles, and melted at 138-139° (dec.).

The reported melting point is 137°. (8)

In another experiment a solution of 8 g. (0.05 mole) n-butylmalonic acid in 30 cc. of ether was placed in a 200 cc. three-neck flask surrounded by an ice-salt bath. To this solution was added with stirring 10.3 g. (0.1 mole) of n-butyl nitrite, and a steady stream of dry HCl was slowly passed into the solution. After all the butyl nitrite was added, stirring was continued for another five minutes after which the solution was poured in an evaporating dish and ether blown off by means of a current of air. The brown residue when crystallized twice from a mixture of ether and n-heptane melted at 134° (dec.). The product weighed 4.3 g. (59 per cent).

α-Oximino-n-octanoic Acid:

By the procedure outlined for the preparation of α-oximino-n-hexanoic acid, 24.4 g. (0.1 mole) of ethyl n-hexylmalonate was nitrosated. In the extraction of the oximino compound from the ether extract with 10 per cent sodium hydroxide solution in this case, it was observed that mere shaking of the etherial solution with the alkali did not extract the oximino compound completely. Therefore the etherial solution after the first three or four extractions with the 10 per cent NaOH solution was shaken with additional 25 cc. of the alkali and left over night. After that period, it was seen that the alkali-layer was tinted dark red indicating the extraction of the oximino compound. Furthermore, it was noticed that when this layer was acidified with concentrated HCl after heating it on a steam bath as in the other case, more of the desired α-oximino acid was obtained. Crude α-oximinooctanoic acid after two recrystallizations from n-heptane weighed 12.2 g. (70 per cent) and

melted at 109-110°.

α-Oximino-n-nonanoic Acid:

When 12.9 g. (0.05 mole) of ethyl n-heptylmalonate was nitrosated by the general method outlined, the yield of α-oximino-n-nonanoic acid melting at 93-94° was 4.7 g. (50 per cent).

It has been reported (137) that excess of the alkyl nitrite causes removal of the α-oximino group. Attributing this sudden drop in the yield to this observation, it was decided to use lesser quantities of the alkyl nitrite.

In another preparation of α-oximino-n-nonanoic acid, 25.8 g. (0.1 mole) of ethyl n-heptylmalonate was nitrosated using 11 g. (0.1 mole and about 5 per cent excess) of n-butyl nitrite. The weight of the crude product, melting at 83-86° was 16.2 g.. When crystallized twice from n-heptane, 13.3 g. (71 per cent) of oximino acid was obtained. The compound melted at 99°.

α-Oximino-n-decanoic Acid:

By following the general nitrosation procedure outlined, 40.8 g. (0.15 mole) of ethyl n-octylmalonate was nitrosated with 16.3 g. of n-butyl nitrite. The crude yield of α-oximino-n-decanoic acid was 24.5 g. (81 per cent). Recrystallized from n-heptane, the melting point of the oximino acid was raised about 11 degrees, now melting at 95°. The recovery of the pure product from recrystallization was about 80 per cent.

α-Oximino-n-dodecanoic Acid:

Following the general method of nitrosation, 19 g. (83 per cent) of α-oximino-n-dodecanoic acid (m.p. 83-85°) was obtained from

30 g. (0.1 mole) of ethyl n-decylmalonate. Recrystallized from n-heptane, the oximino acid was obtained in white silky flakes melting at 93°.

α-Oximino-n-tetradecanoic Acid:

Nitrosation of 16.4 g. (0.05 mole) of ethyl n-dodecylmalonate was tried in the usual manner. On acidifying the residue obtained after removal of alcohol, a light-yellow solid was separated. When the mixture was extracted with ether, it went into solution in the solvent. When the ether extract was shaken with 10 per cent NaOH, there was no color change in the alkali-layer (usually alkali-layer turns brown). The separatory funnel containing the ether extract and NaOH solution was therefore left over night to extract the oximino acid. The next day it was observed that a light-yellow solid had settled down and clogged the separatory funnel. There was a small ether layer on the top. The solid was filtered off and washed several times with water. It was then suspended in about 50 cc. of 10 per cent sodium hydroxide solution and heated on a medium temperature hot plate and water was added until all the solid went into solution. The hot solution was treated with 5 g. of norite and filtered hot, applying suction. On acidification of the cooled filtrate, the oximino acid precipitated as a light yellow solid which was filtered off and dried in a vacuum desiccator. When crystallized from a mixture of n-heptane and ether, the compound melted at 82°.

In another run, the procedure was slightly changed. The light-yellow solid which was obtained on acidifying the residue from the nitrosation mixture was filtered off and washed thoroughly with water. This solid was suspended in 50 cc. of 10 per cent NaOH after which the same procedure as above, was applied. The oximino acid from 32.8 g. (0.1 mole)

of ethyl n-dodecylmalonate weighed 22.3 g. (83 per cent), m.p. 76°.

α-Oximino-n-hexadecanoic Acid:

By following the general method with a few variations as in α-oximinotetradecanoic acid, 17.8 g. (0.05 mole) of ethyl n-tetradecylmalonate was nitrosated. The weight of the crude oximino acid melting at 73° was 13 g. (91 per cent). On recrystallization from a mixture of ether and n-heptane, the compound melted at 76°.

α-Oximino-n-octadecanoic Acid:

Nitrosation of 19.2 g. (0.05 mole) of ethyl n-hexadecylmalonate was carried out by following the modified method used in the preparation of α-oximinotetradecanoic acid. α-Oximinooctadecanoic acid ethyl ester obtained from acidifying the nitrosation residue when suspended in 10 per cent NaOH and heated, did not go into solution even after the addition of a large amount of water. However it was heated for an hour (no norite was added), cooled and acidified with concentrated HCl. The crude oximino acid when crystallized from a mixture of ether and n-heptane weighed 11.8 g. (73 per cent). The compound melted at 77°.

Nitrosation of Ethyl n-octadecylmalonate:

Nitrosation of 41.2 g. (0.1 mole) of ethyl n-octadecylmalonate was tried in the usual manner. When the residue from the nitrosation mixture was acidified, 20 g. of white low-melting solid was obtained. Ten grams of this solid was suspended in excess of 10 per cent NaOH and heated for an hour, the mixture cooled, and acidified with concentrated HCl. Many attempts were made to refine the product (m.p. 25-28°) but apparently with very little success. It was therefore set aside for future study.

Neutralisation Equivalents of α-Oximino Acids:

It was observed by Barry (9) that α-oximino acids behave like monobasic acids and can be titrated with standard alkali in hydro-ethanolic solution, using phenolphthalein indicator. Sharp end points are obtained in such titrations. The observation was varified by work of Waters (69) who was able to obtain good values of the neutralization equivalents of several α-alkoximino acids having the general structural formula $R-\underset{\underset{NOR}{\|}}{C}-COOH$. It was decided therefore to characterize the oximino acids synthesized, by this procedure. The results are summerized in the following table.

TABLE III

Neutralisation Equivalents of α-oximino Acids

Compound	Per Cent Alcohol Used in Titration	Molecular Weight	Neutralization Equivalent
α-Oximinohexanoic Acid	40	145	146
α-Oximinooctanoic Acid	40	173	173
α-Oximinononanoic Acid	40	187	183
α-Oximinodecanoic Acid	40	201	203
α-Oximinododecanoic Acid	60	229	231
α-Oximinotetradecanoic Acid	95	257	260
α-Oximinohexadecanoic Acid	95	285	286
α-Oximinooctadecanoic Acid	Insoluble in 95		

Characterization of α-oximinooctadecanoic Acid:

An α-oximino acid can be converted to a nitrile with one less carbon atom on treatment with acetic anhydride according to the following equation. (138) The resulting nitrile can be then hydrolyzed by one of

$$R-\underset{\underset{NOH}{\|}}{C}-COOH \xrightarrow{(Ac)_2O} R-C\equiv N + CO_2 + H_2O$$

the conventional methods to the corresponding carboxylic acid which can be identified by its melting point or boiling point. Thus the method affords another way of identifying an α-oximino acid.

In the titration studies on α-oximino acids, it was found that α-oximinooctadecanoic acid could not be titrated owing to its insolubility in 95 per cent alcohol which is used as a solvent for the oximino acid. It was characterized therefore by converting it to n-heptadecanoic or margaric acid, the melting point of which is known. The experimental procedure is given below.

One gram of α-oximinooctadecanoic acid was placed in a 50 cc. test tube, carrying a reflux condenser, with 10 cc. of acetic anhydride. The mixture was heated gently on a low flame. The oximino acid went in solution first in acetic anhydride and then there was a spontaneous evolution of a gas (CO_2). After boiling the solution for 10 minutes, it was evaporated to dryness under reduced pressure. To the cooled residue was added 25 cc. of 10 per cent NaOH solution and the resulting mixture was refluxed for an hour. It was cooled slightly, acidified with concentrated HCl, cooled again and extracted with ether. On evaporation

of the ether extract a brown, waxy solid was obtained which was dissolved in acetone, treated with 0.5 g. of norite, heated, and filtered hot. A dull-white solid melting at 58-59° was obtained on evaporation of acetone. The melting point reported for margaric acid is 61°.

HYDROGENATIONS

Hydrogenation of an α-oximino acid or its ester to the corresponding α-amino acid has been studied by many workers in these Laboratories and elsewhere. (8,9,10,93) The reduction proceeds to completion and the yields of α-amino acids are good. However Hamlin and Barry (8,9) observed that the reduction after reaching to the half-way point of the uptake of hydrogen, slowed down considerably which they attributed to the formation of intermediate imine state. This hypothetical conclusion was

$$R\text{-}\underset{\underset{NOH}{\|}}{C}\text{-}COOH \xrightarrow[H_2]{Pd.} R\text{-}\underset{\underset{NH}{\|}}{C}\text{-}COOH \xrightarrow[H_2]{Pd.} R\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}COOH$$

shown to be wrong by Waters and Hartung (69a) who were able to isolate only phenylalanine and unchanged oximino acid when the hydrogenation of β-phenyl-α-oximinopropionic acid was interrupted before completion. In trial runs Waters (69) reduced α-alkoximino acids to the corresponding α-amino acids. The yields of α-amino acids were not very satisfactory. Weaver (136) was faced with the difficulty of reducing amides, anilides of α-alkoximino acids, the resulting products being diketopiperazines instead of dipeptides. Anticipating these difficulties, the hydrogenations of the α-oximino acids were undertaken.

Preparation of Palladium Catalyst:

To 3 g. of norite were added 0.3 g. of palladium chloride and 13.6 g. of fused sodium acetate. The mixture was placed in 250 cc. bottle with 100 cc. of distilled water and shaken on the Parr shaker in an atmosphere of hydrogen for one-half hour. The bottle was removed from the hydrogenator and the contents filtered on a suction funnel. The palladinised norite was washed well with distilled water, then twice with 95 per cent alcohol. The catalyst was dried in a vacuum desiccator over concentrated sulfuric acid.

α-Amino-n-hexanoic Acid (Norleucine):

In a glass liner was placed a solution of 7.25 g. (0.05 moles) of α-oximino-n-hexanoic acid in 100 cc. of 95 per cent alcohol, 3 g. of palladium catalyst prepared as described above, and 10 cc. of concentrated HCl (0.11 mole). An additional 0.5 g. of palladium chloride was added to the mixture after which the liner was carefully fitted in a pressure bomb of the American Instrument Company, and subjected to a pressure of 10 atmospheres of hydrogen. The rate of absorption of hydrogen can be followed by observing the fall in pressure on the guage. Reduction to the halfway point was rapid (about 30 minutes) as noticed by the previous workers (8,9) but the theoretical amount of hydrogen was absorbed when the mixture was shaken about three hours longer.

The glass container was then removed from the bomb and the contents were filtered by suction and the catalyst was washed twice with 25 cc. portions of hot 95 per cent alcohol. The filtrate and washings were combined and the solvents removed under reduced pressure. The resulting residue was dissolved in a minimum of distilled water

and filtered. The clear filtrate was warmed and adjusted to pH between 6.5-7 by 28 per cent NH_4OH using a Beckmann pH meter. At this point a dull-white solid began to precipitate. After standing in the refrigerator overnight, the solid was filtered. The filtrate after concentration yielded additional solid. The combined solids were recrystallized from boiling distilled water to which three volumes of alcohol were added. The yield of the amino acid was 75 per cent. It melted at 322-326° (dec.).

α-Amino-n-octanoic Acid:

Following the procedure outlined above, the reduction of 3.5 g. (about 0.02 mole) of α-oximinooctanoic acid was tried. One-half of the theoretical amount of hydrogen absorbed in about an hour but after that the reduction slowed down considerably. The mixture after shaking for an additional four hours , was removed from the bomb and 0.1 g. of platinic oxide (Adam's catalyst) was added to it. The liner was again placed in the bomb and the mixture was agitated with hydrogen for three hours longer. Even then the reduction did not proceed to completion. The crude amino acid was isolated by procedure described for isolation of norleucine. The white solid was recrystallized by solution in 200 cc. of water containing 25 cc. of 28 per cent NH_4OH and adding excess glacial acetic acid to precipitate the amino acid, yield 1.6 g. (50 per cent). The compound melted at 257-259°, the reported melting points being 263-264°, (139,140) 270(30).

Nitrogen (Kjeldahl)

Calc. for $C_8H_{17}O_2N$: 8.80 % Found: 8.64%

α-Amino-n-nonanoic Acid:

Reduction of 3.75 g. (0.02 mole) of α-oximinononanoic acid

was tried. After four hours the reduction was interrupted and 0.1 g. of platinic oxide was added as in the reductions of α-oximinooctanoic acid. After an additional five hours of shaking, the mixture took the required amount of hydrogen. However on isolation and purification only 0.5 g. of the desired amino acid was obtained. No unreacted α-oximino acid was recovered. Apparently the misleading guage-reading was due to a small leak in the pressure bomb. The amino acid melted at 265-267°(dec.). Melting point reported in literature is 270-273°. (30)

Nitrogen(Kjeldahl)

Calc. for $C_9H_{19}O_2N$: 8.08% Found N: 7.89%

The reduction of α-oximinononanoic acid even after nine hours was not complete. It was decided therefore to attempt the reductions of the other oximino acids in different solvents and with different catalysts.

Reduction of α-oximinodecanoic Acid:

1. Using palladium-platinum catalyst in ethyl alcohol containing 20% acetic acid.

A solution of 4.02 g. (0.02 mole) of α-oximinodecanoic acid in 80 cc. of ethyl alcohol was placed in a bottle with 20 cc. of glacial acetic acid and 3 g. of palladium catalyst, described before. The mixture was hydrogenated on a Parr shaker at 4 atmospheres pressure of hydrogen. The mixture was shaken for six hours but there was no appreciable fall in the pressure-guage reading. The shaker was stopped and the contents of the bottle were filtered and the catalyst was washed twice with 10 cc. portions of hot glacial acetic acid. The combined filtrates were concentrated until almost dry at 50-60° under reduced pressure, after which 50 cc.

of ether were added to the residue and the flask kept in the refrigerator for an hour. White solid precipitated which was filtered and washed with 10 cc. of ether. The filtrates were saved. The solid when crystallized from 50 per cent acetic acid, weighed 0.2 g. and melted at 230°. The etherial filtrates when evaporated down to dryness gave the unchanged α-oximino acid characterized by its melting point. It weighed 3.1 g.

2. Using platinic oxide (Adam's catalyst) in glacial acetic acid.

As above 4.02 g. (0.02 mole) of α-oximinodecanoic acid was suspended in 100 cc. of glacial acetic acid, 0.1 g. of platinic oxide catalyst added to it and hydrogenated on the Parr shaker at four atmospheres pressure. After shaking the mixture for six hours about one-third of the calculated amount of hydrogen was absorbed. When the α-amino acid was isolated, as in the preceding experiment, it weighed 0.87 g. (23 per cent) and melted at 237-239°. Reported m.p. is 264° (30); unchanged α-oximino acid weighed 2.9 g.

Nitrogen (Kjeldahl)

Calc. for $C_{10}H_{21}O_2N$: 7.48% Found N: 7.12%

Reduction of α-Oximinododecanoic Acid:

Reduction of 4.58 g. (0.02 mole) of α-oximinododecanoic acid was carried out on Parr shaker. When the hydrogenation was interrupted after six hours, a white solid had precipitated and covered the catalyst. The mixture was filtered and the residue on the filter paper was treated with 10 cc. of boiling acetic acid and refiltered to remove the amino acid adhering to the catalyst. This filtrate was added to the main filtrate and from the combined filtrates, the amino acid was isolated as previously

described. Recrystallized from glacial acetic acid, the yield of α-amino-dodecanoic acid melting at 250-252°, was 0.7 g. (16 per cent). The recovered oximino acid weighed 3.15 g..

Nitrogen (Kjeldahl)

Calc. for $C_{12}H_{25}O_2N$: 6.51% Found N: 6.32%

Reduction of α-oximinotetradecanoic Acid:

When reduction of 5.14 g. (0.02 mole) of α-oximinotetradecanoic acid was carried out over a period of five hours in glacial acetic acid on the Parr shaker using 0.1 g. of platinic oxide catalyst, 1.12 g. (23 per cent) of α-aminotetradecanoic acid was obtained along with 3.7 g. of the original oximino acid. The amino acid melted at 232-234°.

Nitrogen (Kjeldahl)

Calc. for $C_{14}H_{29}O_2N$: 5.76% Found N: 5.60%

Reduction of α-oximinohexadecanoic Acid:

When 2.85 g. (0.01 mole) of α-oximinohexadecanoic acid was hydrogenated for six hours, in 100 cc. of glacial acetic acid with 0.1 g. of platinic oxide catalyst and isolated in the usual manner, 0.2 g. of α-aminohexadecanoic acid melting at 221-223° was obtained along with 2.1 g. of the unchanged oximino acid.

Nitrogen (Kjeldahl)

Calc. for $C_{16}H_{33}O_2N$: 5.16% Found N: 4.82%

Reduction of α-oximinooctadecanoic Acid:

α-Oximinooctadecanoic acid weighing 3.13 g. (0.01 mole) was suspended in 100 cc. of glacial acetic acid along with 0.1 g. of platinic

oxide. The mixture was hydrogenated on the Parr shaker for five hours but no noticable drop in the guage-reading occurred. When the mixture was subjected to the same treatment as outlined previously, 2.5 g. of the original oximino acid was recovered. No amino acid was obtained.

The following hydrogenations were carried out with the palladium catalyst that was recovered from a bottle marked "used palladium catalyst", used by the former workers in these laboratories. It is believed that this catalyst contained an appreciable amount of platinum in it, however, no elemental analyses were carried out. It will therefore be designated from now on as "recovered palladium catalyst". It proved to be more active than the former one in the reductions of α-oximino acids.

α-Aminodecanoic Acid Hydrochloride:

A 100 cc. alcoholic solution of 4.02 g. (0.02 mole) of α-oximino-decanoic acid containing 5 cc. of concentrated HCl was hydrogenated in the bomb at 10 atmospheres, using 3 g. of palladinized norite. The theoretical amount of hydrogen was absorbed in about five hours, after which the solution was filtered and the catalyst washed three times with hot 95 per cent alcohol. The filtrates were concentrated under reduced pressure. When 50 cc. of water was added to the residue, a white solid precipitated which was filtered on a suction funnel. Additional amount of solid was obtained on concentrating the filtrate. The combined solids were washed with ether to remove any unchanged α-oximino acid and the crude product was purified by recrystallizing from glacial acetic acid. The weight of the hydrochloride of α-aminodecanoic acid was 1.8 g. (40 per cent). It melted at 243-245° (dec.).

Nitrogen (Kjeldahl)

Calc. for $C_{10}H_{21}O_2N \cdot HCl$: 6.26% Found N: 6.16%

α-Aminododecanoic Acid Hydrochloride:

Reduction of 4.58 g. (0.02 mole) of α-oximinododecanoic acid was carried out in the bomb as described above. On isolation and recrystallization, the hydrochloride of the amino acid melting at 262-265° (dec.) weighed 1.9 g. (37 per cent). Recovered oximino acid weighed 1.5 g..

Nitrogen (Kjeldahl)

Calc. for $C_{12}H_{25}O_2N \cdot HCl$: 5.56% Found N: 5.60%

α-Aminotetradecanoic Acid Hydrochloride:

Following the method described in the preceding two experiments, 5.14 g. (0.02 mole) of α-oximinotetradecanoic acid was hydrogenated in the pressure bomb. The theoretical uptake of hydrogen was complete in about four and one-half hours, but hydrogenation was continued for an additional hour. It was observed that there was a small but gradual fall in the guage reading. It was probably due to a small leak in the apparatus. Weight of the purified amino acid hydrochloride melting at 247-249° (dec.) was 1.1 g. (23 per cent). Unchanged α-oximinotetradecanoic acid weighed 2.8 g..

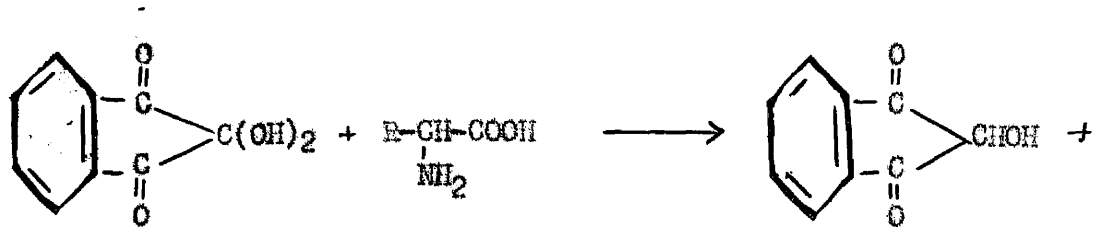
Nitrogen (Kjeldahl)

Calc. for $C_{14}H_{29}O_2N \cdot HCl$: 5.00% Found N: 4.90%

Ninhydrin Test:

A specific test for α-amino acids is the so called "Ninhydrin test" (tri-ketohydrindene hydrate). The reaction that takes place

between ninhydrin and α-amino acid may be represented by the equation shown below:



$$R\text{-}CHO + NH_3 + CO_2$$

a qualitative test was preformed therefore on all the amino acids synthesized.

In a clean 25 cc. test tube was placed about 25 mg. of α-amino acid. Four drops of 1 per cent ninhydrin solution and 5 cc. of distilled water were added to the tube and it was placed in a water-bath at 90°. In 5-10 minutes, the solution in the tube turned violet. The intensity of coloration decreased as the molecular weight of the amino acid increased.

COMMENTS ON RESULTS

The reaction of a substituted malonic ester with an alkyl nitrite in the presence of sodium ethoxide, first reported by Dieckmann and Groeneveld (76) was modified and developed by Barry (9) to obtain satisfactory yields of α-oximino acids. He and others (8,93) have also shown the usefulness of the α-oximino acids and their esters as good potential sources of amino acids.

The present investigation was an attempt to extend the nitro-

sation reaction to higher alkylmalonic esters. Suffice it to say that these esters have been nitrosoted in good yields to the corresponding α-oximino acids by varying the experimental conditions slightly. Some of the alkylmalonic acids were nitrosated by the method outlined by Barry (9), however, it was found increasingly difficult to isolate the resulting oximino acids in pure condition from the reaction mixtures. The nitrosation of substituted malonic ester in presence of sodium ethoxide was adopted therefore as a suitable procedure for the preparation of α-oximino long-chain acids.

Several alkylmalonic esters have been synthesized by alkylation of malonic ester and the optimum conditions for its alkylation found. There was a gradual drop in the yield of these esters as the alkyl chain lengthened, however, it was due to the fact that a considerable amount of pyrolysis took place during their distillations.

The behavior of α-oximino acids as monobasic acids was observed by Barry (9). This observation has been confirmed as can be seen from Table III.

The hydrogenation studies of α- oximino acids are by no means complete, although several attempts have been made to effect the reduction at room temperature and at moderate pressures using different catalysts . These reductions have not been crowned with complete success, however hydrogenations have taken place to give small amounts of the corresponding amino acids. As shown by Waters, (69a) this is probably due to the inhibitory effect of the α-amino acid as it is formed by reduction of the α-oximino acid, on the rate of reduction of unchanged oximino acid. Also it is believed that as the reduction of an oximino acid (especially the higher ones) procedes the α-amino acid formed precipitates from the solvents and covers the catalyst thus rendering it less active. Carrying

out these reductions at higher temperature, pressure, and in proper solvent may remedy this situation.

At this point we have at hand sufficient information regarding the melting points, solubility behavior of ∝-oximino, and ∝-amino acids. As these acids are nitrogen derivatives of the corresponding fatty acids, it will be interesting to compare their properties with the acids from which they are derived. (Refer to Table IV).

TABLE IV

Properties of Oximino Acids, Amino Acids and Corresponding Fatty Acids

		Solubility			Corresponding ∝-Oximino Acid	Solubility			Corresponding ∝-Amino Acid	Solubility		
Fatty Acid	M.P. °C.	W.	A.	E.	M.P. °C.	W.	A.	E.	M.P. °C.	W.	A.	E.
Hexanoic	-1.5	Sl	Sl	Sl	138	Sl	C	C	326	Sl	VSl	I
Octanoic	16.5	VSl	C	C	110	VSl	F	C	259	Sl	VSl	VSl
Nonanoic	12	VSl	C	C	99	Sl	F	C	266	Sl	Sl	I
Decanoic	31	VSl	C	C	95	VSl	F	C	238	VSl	VSl	I
Dodecanoic	48	VSl	F	C	93	VSl	F	C	252	I	VSl	I
Tetradecanoic	58	VSl	F	C	82	VSl	F	C	234	I	VSl	I
Hexadecanoic	64	VSl	Sl	C	76	VSl	F	C	223	I	VSl	I

A = Alcohol
C = Completely
E = Ether
F = Fairly
I = Insoluble
Sl = Slightly
VSl = Very Slightly
W = Water

The introduction of the oximino and amino groups into the aliphatic chain profoundly alters the character of the fatty acid as can be seen from the above table. The lower members are colorless, crystalline solids, but with increasing chain length, they tend to become waxy in nature. The melting points of both the oximino and amino acid are markedly higher than those of the fatty acids from which they are derived. However, in the oximino acids, they tend to approach to the corresponding fatty acid. The lower members of oximino and amino acid series are moderately soluble in water, but the solubility decreases as the series is ascended. Unlike the fatty and oximino acids, which are soluble in alcohol and ether, the corresponding amino acids are only slightly or completely insoluble in these media. Little may be said about the other properties of these long chain oximino and amino acids, however it may be surmised, that they display a variety of properties depending on the chain of fatty acid and the point of attachment of the oximino and amino groups.

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SUMMARY

I.A survey of synthetic methods for the preparation of α-amino acids has been made.

II. Nine long-chain alkylmalonic esters have been synthesized and the optimum conditions for the alkylation have been determined.

III. These esters have been converted to α-oximino acids by treating them with n-butyl nitrite in the presence of sodium ethoxide in good yields.

IV. Barry's observation that α-oximino acids behave as monobasic acids has been confirmed.

V. Hydrogenations of α-oximino acids have been studied; the acids have been converted to the corresponding α-amino acids. It appears that the reduction reaction is "anti-catalyzed" by the amino acid as it forms.