THE PREPARATION

OF

α-ALKOXIMINO ACIDS AND THEIR DERIVATIVES

By

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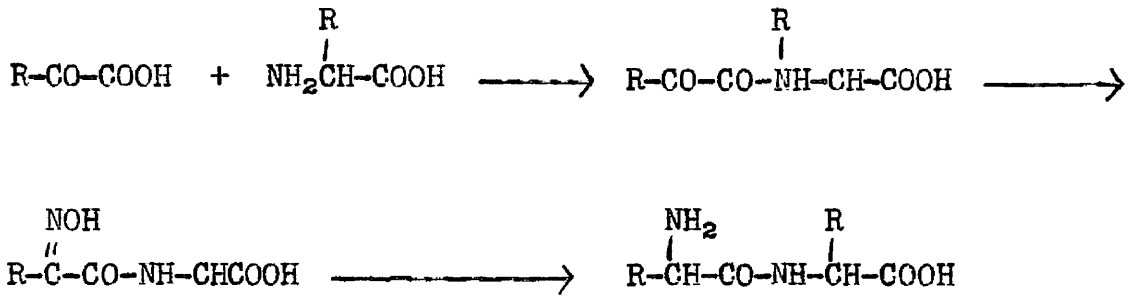
THE PROBLEM

The original aim of this investigation was to prepare intermediates which may be used in peptide synthesis. At first it was thought that it should be relatively simple to prepare α-ketoacids from the α-oximinoacids. By utilization of the α-ketoacids it was hoped to obtain dipeptides through the following steps:



Although it is possible and practical to prepare the α-oximino acids, no suitable procedure has been found thus far for converting them into α-ketoacids. Therefore, the next step was to try to protect the α-oximino group through alkylation, followed by preparation of the acid chloride, subsequent coupling with an amino-grouping to form an intermediate of the general structure $R-C(=NOR)-CO-NH-CHR-COOH$, and finally reducing the alkoximino group. Since this promises to be a more fruitful approach to the solution of the problem of peptide synthesis the work was directed toward this end.

Recently it has been stated (2) that the information relating to α-ketoacids in the literature is either missing, misleading, or erroneous; it was decided, therefore, that a literature-survey of this class of compounds would be desirable. This material is presented as a portion of the introduction, following a discussion of the dipeptide linkage.

INTRODUCTION

THE PEPTIDE LINKAGE

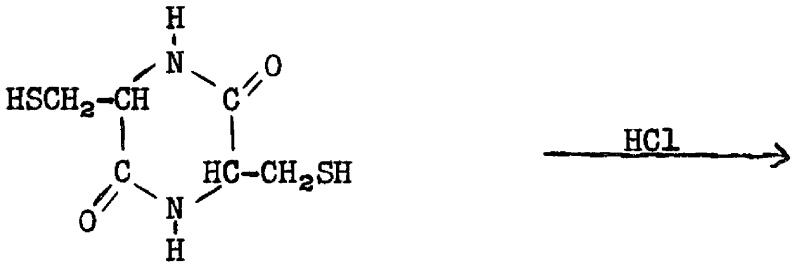
When a solution of acid, alkali, or proteolytic enzyme acts upon a protein, a decrease in the total mass of water in the solution occurs and the final product of the reaction is a mixture of α-amino acids. The manner of linkage of these amino acids in the protein has been the subject of a considerable amount of investigation. Several investigators (16, 123, 130) have shown that the action of pepsin and trypsin on various proteins always results in the simultaneous liberation of amino groups and carboxyl groups in the ratio of 1:1. The only type of linkage which, as far as is known, can yield, by hydrolytic splitting, an amino group and a carboxyl group is an amide, -CO-NH-. Therefore, the protein molecule may in general be conceived as consisting of amino acids bound to each other through condensation of the α-amino group of one acid with the carboxyl group of the adjacent acid. In proteins this amide type of union is usually referred to as the peptide linkage.

In view of the enormous complexity of the protein molecule, it is manifestly impossible to determine by hydrolytic splitting the sequence of the various groups of amino acids participating in the molecule. If we are to learn more of the proteins, we must make a study of known

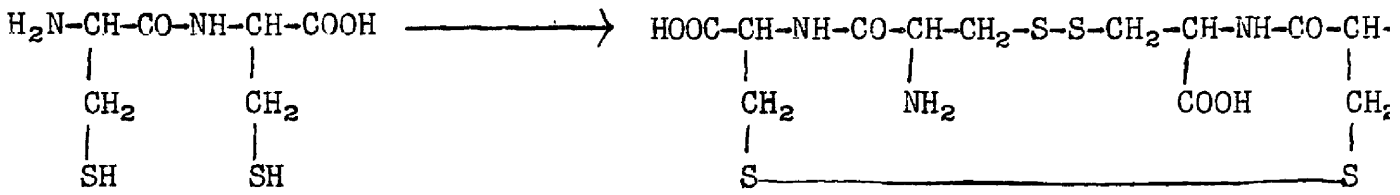
protein "models". These models can perhaps be best obtained through chemical synthesis. Such synthetic molecules may be used to study the physiochemical properties of the protein. When one considers, for example, the physiological properties of insulin, it is not inconceivable that the essential grouping of the molecule may be obtained synthetically.

It has been claimed that the first peptide linkage was prepared by Curtius (42) in 1882. However, since he was unable to remove the benzoyl group from his benzoylglycylglycine, the credit for preparing the first peptide goes to Fischer and Fourneau (60). This was accomplished by heating the diketopiperazine glycine anhydride, with concentrated hydrochloric acid thus, obtaining glycylglycine. Since then a number of procedures for the formation of the peptide linkage have been advanced. The procedures which have been most successfully used may be briefly summarized, as follows:

1. Hydrolysis of diketopiperazines



l-cysteine diketopiperazine

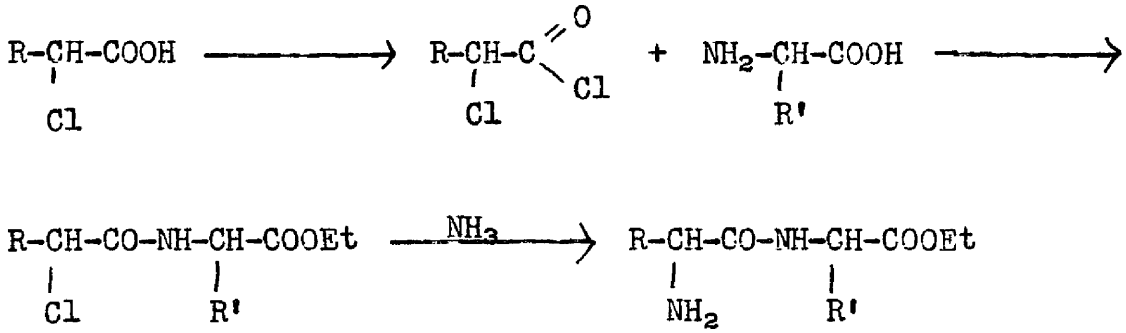


l-cysteinyl-l-cysteine

l-cystinyl-l-cystine

The most complex representative of this method of synthesis is l-cystinyl-l-cystine, accomplished by Greenstein (71), as shown in the equations above. The procedure is obviously limited, as only available diketopiperazines can be converted into dipeptides. In a heterogeneous anhydride molecule containing two amino acids it would, of course, be possible to obtain two isomeric dipeptides and one would be faced with the subsequent difficulty of separating them.

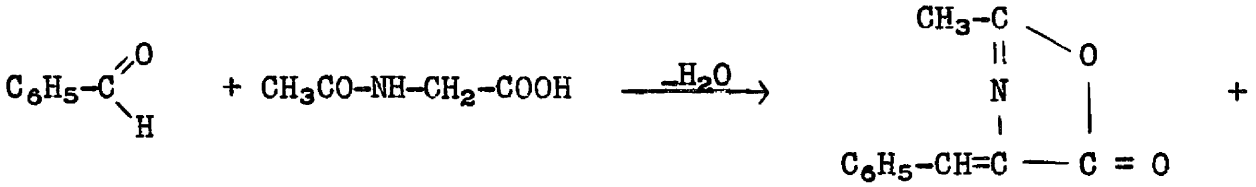
2. Use of α-Halogen Acids

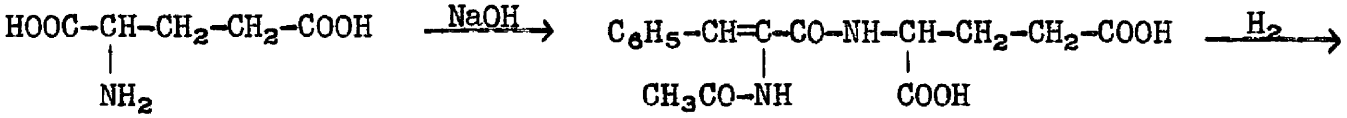


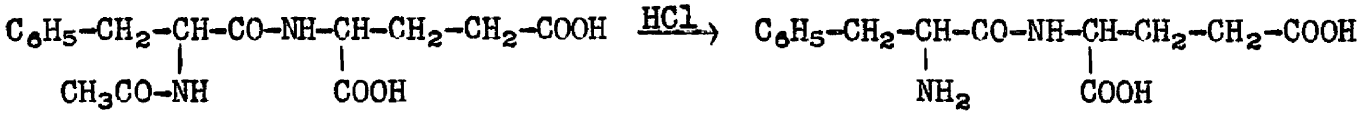
Fischer (61) was the first to use this procedure. From the α-halogen acid he prepared the acid chloride which was easily coupled

with an amino acid; the halogenated amide was allowed to react with ammonia and the resulting dipeptide separated. Using this procedure Fischer prepared a great number of peptides. Bertho and Maier (18) have introduced a modification for the substitution of halogen by NH_2; they treat the halogenated peptide ester with sodium azide; subsequent catalytic hydrogenation and saponification yields the dipeptide.

3. Azlactone procedure.





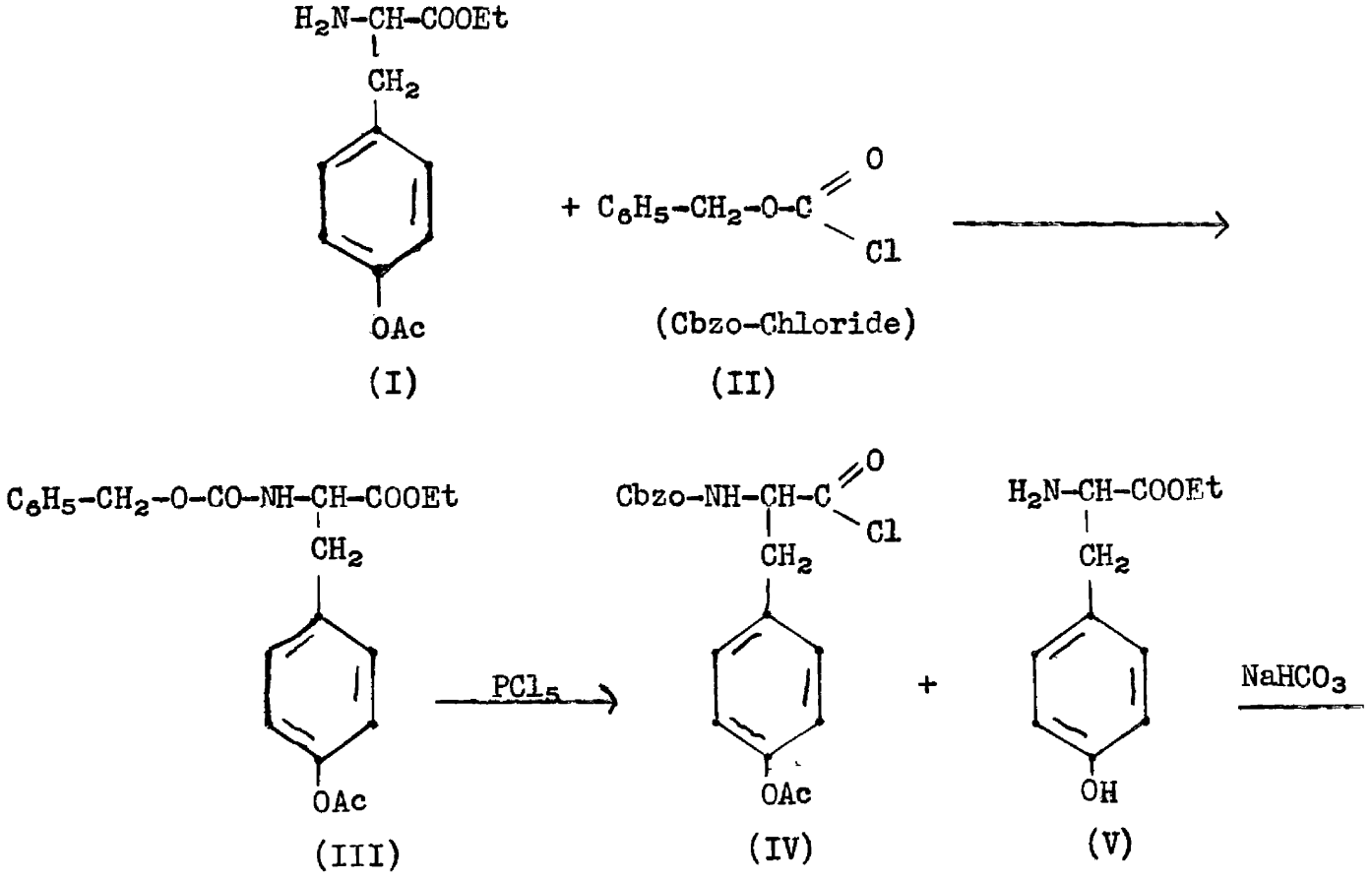


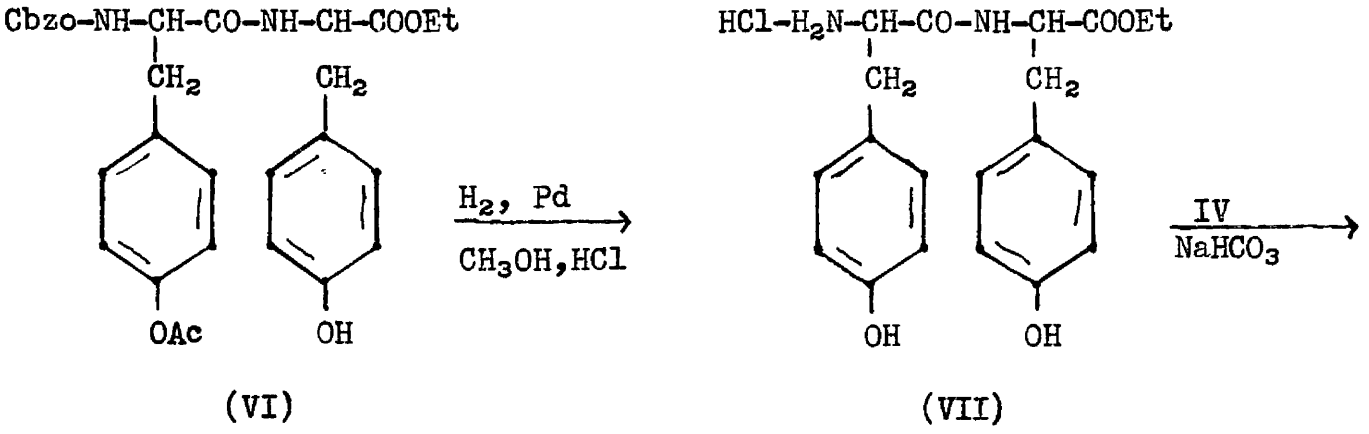
dl-Phenylalanyl-d-glutamic acid

This procedure is based on a reaction first reported by Erlenmeyer and Frŭstŭck (54) who observed that, if an aromatic aldehyde was

allowed to react with N-acylglycine in the presence of a dehydrating agent, an azlactone was formed. Bergmann, Stern and Witte (15) noted that the azlactone reacted energetically with amino acids in an alkaline solution forming N-acyl unsaturated dipeptides; the dipeptides were then obtained after catalytic hydrogenation and removal of the N-acyl group with hot hydrochloric acid. This method was an improvement over other peptide procedures and has led to the synthesis of a number of different peptides. One of the disadvantages of the procedure is that there is some breakdown of the molecule in the removal of the N-acyl group by the hot acid.

4. The "carbobenzoxy" synthesis.





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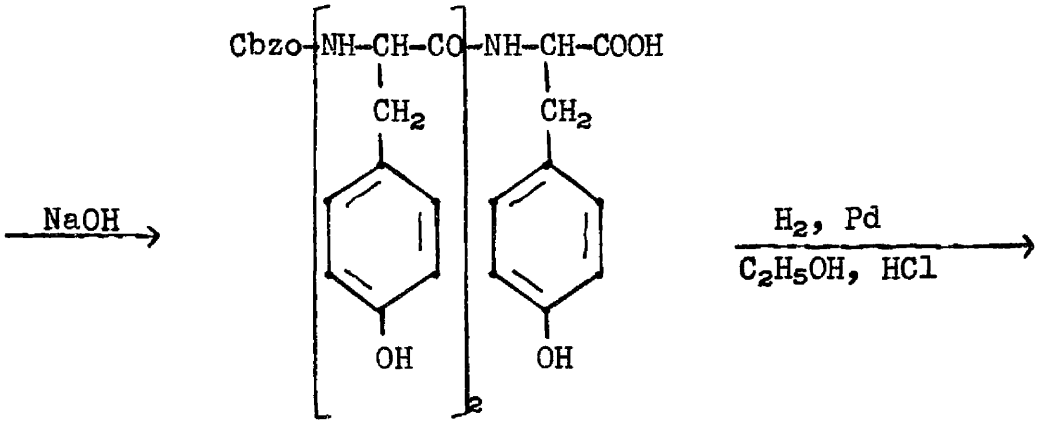

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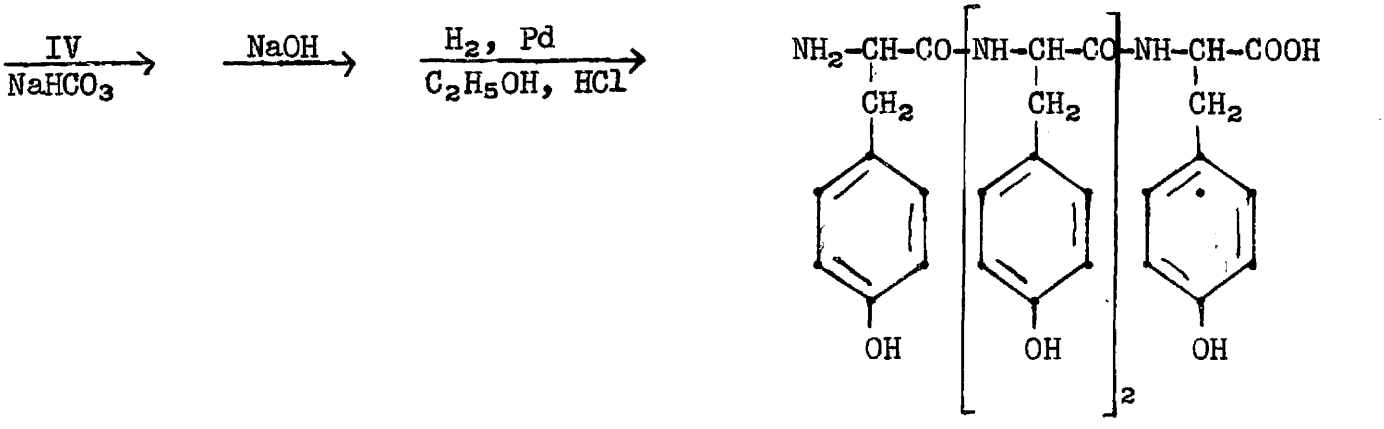
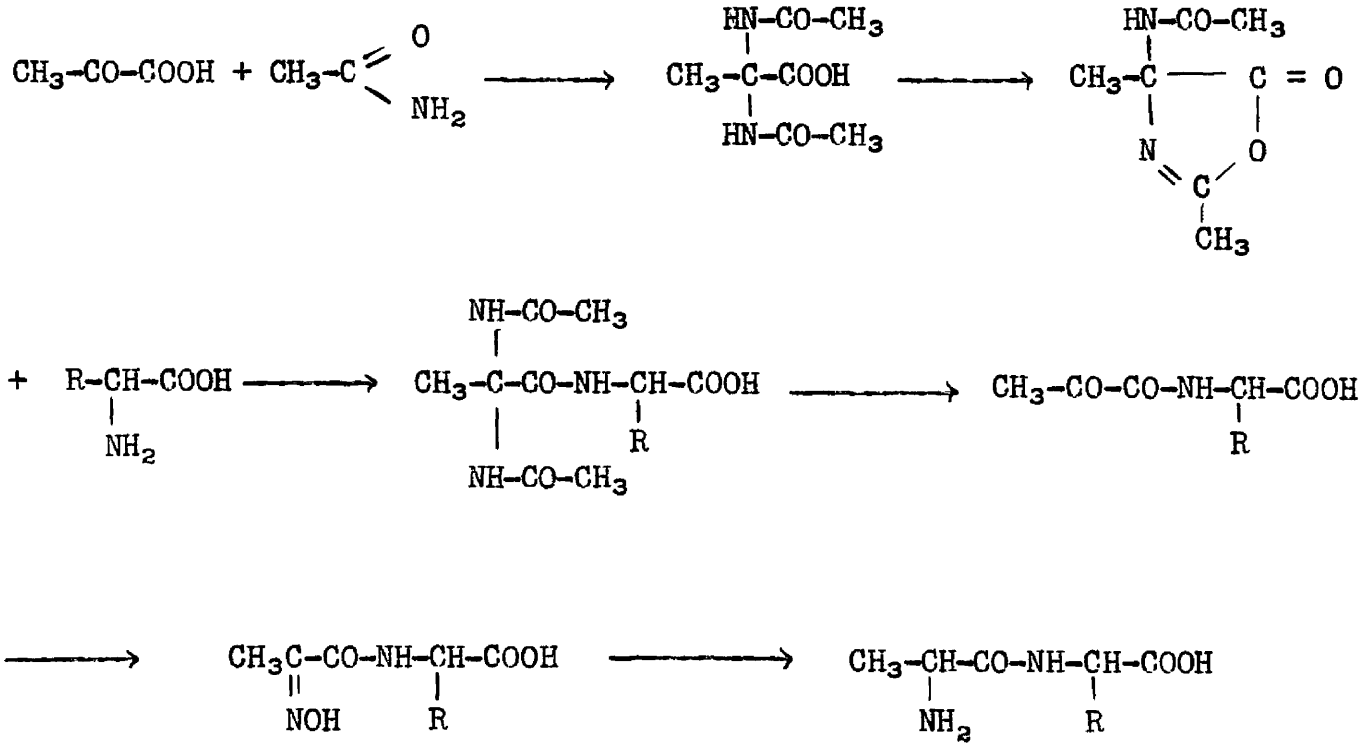


tyrosyl-tyrosyl-tyrosyl-tyrosine

This procedure was first proposed by Bergmann and Zervas (17) in 1932. The most recent synthesis reported in this manner is that of tyrosyl-tyrosyl-tyrosyl-tyrosine (Equations above) by Barkdall and Ross(7). The masking of the amino group with the carbobenzoxy group is unique in that this group can be removed quantitatively by the catalytic hydrogenation at room temperature yielding the regenerated amino group, carbon dioxide and toluene. A recent review by Bergmann and Fruton(13) gives extensive references to papers describing the preparation of peptides by this method.

5. Use of α-keto acids



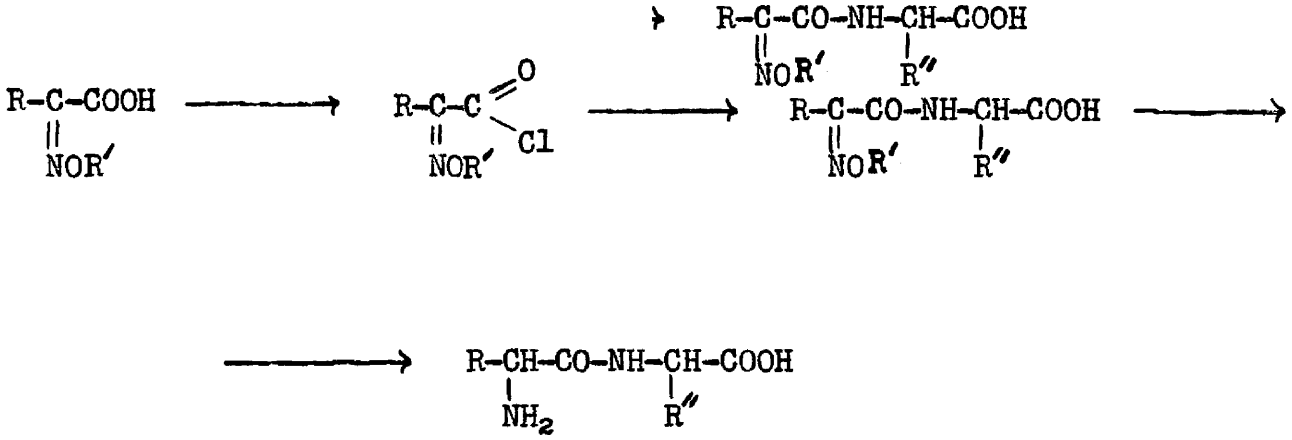
Schemin and Herbst (121) used the procedure represented by the equations above for the preparation of dipeptides. These investigators employed a reaction originally employed by Bergmann and Grafe(14) and were able to capitalize on Hartung's (79) reduction procedures.

Each of the above procedures has merit, although none may be considered ideal. The ideal procedure should employ intermediates of such a nature that there can be no doubt regarding the manner of linkage or the sequence of the components. The use of the halogen acids (Method 2 above) meets this requirement, but is limited in reaction since the conversion of R-CHX-CO-NH-CHR-COOH to the amino compound gives poor yields and since R-CHX-COX cannot be prepared for all desired compounds. The ideal intermediate might be prepared from R-C(=N-)-CO-NH-CHR-COOH, where (R-C(=N-)-CO-) could be first joined to an amino acid and then converted into the $R-CH(NH_2)-CO-NH-$ group with minimal or no effect or reaction on the rest of the reactive points in the molecule.

In view of Hamlin's and Barry's success in preparing the various α-oximinoacids, it was thought that, if proper experimental conditions could be developed, these substances might be converted into the corresponding α-ketoacids; these might, in turn, be joined (through amide linkage) to an amino acid to form intermediates of the general structure R-CO-CO-NH-CHR-COOH. It was anticipated that the keto group could be converted, by established procedures (92), into a carbinamine. Unfortunately as described in the experimental portion of this thesis

the α-ketoacids are not easily prepared from their oximino acids.

It was also found impossible to prepare the chloride of α-oximinoacids by means of either thionyl chloride or the phosphorus chlorides. Attention was next turned to the possibility of protecting the oximino group, e.g., by alkylation or acylation, during formation of the acid chloride. It has been found that alkyloximino acids may be conveniently synthesized; and since these form a new type of compound, a study of their properties was undertaken before investigating their value in the peptide synthesis, which should proceed according to the equation:



The alkyloximino acids may be converted to acid chlorides which react characteristically. At room temperatures, the ethoximino group is reduced to the amino group with difficulty under the conditions tried. Using glacial acetic acid as solvent the benzyloximino group may be reduced with hydrogen and palladinized charcoal at room temperature and about two atmospheres pressure. These studies are described in the experimental portion of this thesis.

THE α-KETOACIDS

Ever since Berzelius (19) described the properties of pyruvic acid in 1835, biochemists have shown considerable interest in α-ketoacids. Few issues of biochemical journals appear without some reference to them. Westerkamp (132) isolated a number of such acids from the blood serum of horses and hogs. Neuberger and Sanger (107) indicated that the metabolism of lysine passes through its keto analog to glutaric acid, suggesting that a knowledge of ketoacids is desirable to an understanding of amino acid metabolism. Although important, this class of compounds receives meager treatment in organic treatises. Elementary textbooks usually dismiss the subject with a brief discussion of pyruvic acid, and some of the advanced texts do but little better. Richter (119) gives a description of five α-ketoacids and outlines two general methods for their preparation. In view of the scarcity of such information a literature survey was undertaken. It was planned to check all the original references in Beilstein, then check all the references in Chemical Abstracts and finally present a condensation of this literature survey. However, it appears that since this would be the subject for a monograph, it would prove too extensive for presentation at this time. Some evidence of the magnitude of the literature on the α-ketoacids is indicated

by the fourteen and a half pages which Beilstein devotes to pyruvic acid alone and the more than one hundred references given for oxaloacetic acid. The completed survey of phenylpyruvic acid yielded about 120 references, including thirteen different procedures for its preparation. In view of this extensive material in the literature, it was decided to confine this review to:

1. A discussion of general properties of the α-ketoacids;
2. A discussion of the methods which have been found most general for their preparation; and
3. The presentation of a table showing some of their history and giving references to their preparation.

1. Properties of α-Ketoacids

The branched-chained α-ketoacids and the phenyl-substituted α-ketoacids vary in physical characteristics from liquids to high melting solids; 4-hydroxyphenylpyruvic acids melts at 220°. The normal straight-chain α-ketoacids are either liquids or low melting solids.

Adickes and Andresen (2) pointed out that if the straight chain α-ketoacids are arranged in two series, one of odd carbon atoms and one of even carbon atoms, there is a definite increase in the melting point as we go up the series. For example, going from 5 to 7 carbon atoms increases the melting point 23° and going from 6 to 8 carbon atoms

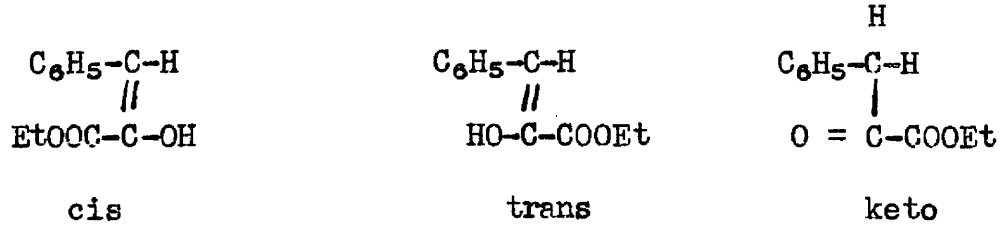
increases the melting point 25°; from 7 to 9 gives an increase of 14° and from 8 to 10 gives an increase of 14°. It may also be observed that the introduction of keto group in the α-position of the fatty acids causes an increase in the melting point. Table I serves to illustrate these observations:

TABLE I

MELTING POINTS OF STRAIGHT CHAIN α-KETOACIDS AND OF FATTY ACIDS

ODD			EVEN		
Number Carbon Atoms	α-Keto Acid m.p. °C.	Fatty Acid m.p. °C.	Number Carbon Atoms	α-Keto Acid m.p. °C.	Fatty Acid m.p. °C.
3	14	-24	4	31	-5
5	6	-34	6	7	-1
7	29	-10	8	33	16
9	43	12	10	47	31
11	55	29	12	57	43
13	62	44	14	64	54
15	68	53	16	69	63

Gault and Weick (68) have pointed out that the ester of phenylpyruvic acid exists in three forms, the cis-trans enol forms and the keto form.



No doubt the other α-ketoacids capable of enolization will show this tendency.

Many of the α-ketoacids are unstable and on standing lose carbon dioxide and form the lower fatty acid or corresponding aldehyde. Their decomposition by micro-organisms and by enzymes has been studied; generally CO_2 is eliminated and the lower alcohol or aldehyde is formed. Lead tetra-acetate effects a quantitative evolution of CO_2. Pyrolysis yields carbon dioxide, carbon monoxide, lower aldehyde or acid, and other dehydration products. Although the α-ketoacids appear to be somewhat unstable, they show remarkable stability when heated with acids and bases. This is evidenced by the fact that it is not unusual to find that a particular ketoacid has been prepared by heating an intermediate with acid or alkali, thus splitting out the α-ketoacid. A typical example of this is the preparation, described in Organic Synthesis (82), of phenylpyruvic acid by boiling α-acetaminocinnamic acid with normal hydrochloric acid for three hours; Erlenmeyer's (52) hydrolysis of phenylcyanopyruvic acid ester by heating for 12 hours with 66% sulfuric acid may also be mentioned. However, Karrer (89) states that all known α-ketoacids yield carbon monoxide when warmed with concentrated sulfuric acid.

The α-ketoacids generally form well-defined ketone derivatives. The oximes are usually stable compounds and are not easily reconverted to the original acids; they are stable to alkali and with

dilute acids lose carbon dioxide and form a nitrile with one less carbon atom (33). With ferric chloride many ketoacids develop a characteristic color. The acids may be titrated with bases, forming well defined calcium, barium and silver salts. The sodium bisulfite-addition compound of phenylpyruvic acid has been described. As a rule they reduce ammonical silver nitrate. They are reduced by hydrogen to the corresponding hydroxyacids, and hydrogenation in the presence of ammonia forms amino acids. Knoop and Osterlein (92), employing catalytic hdrogenation in the presence of mono- and of di-methylamine, respectively, were able to obtain several methylaminoacids.

Ketoacids form amino acids from ammonium salts in the presence of dog's blood. Transamination occurs when α-ketoacids are allowed to react with α-amino acids, the amino acid being reduced to the lower aldehyde (81). The condensation of the α-ketoacids with acetamide has been investigated by Shemin and Herbst (121) who assume that the first reaction is the formation of an α-hydroxy-α-acetamino derivative; this then reacts with another molecule of acetamide to form the disubstituted compound. With C_6H_5-CO-COOH, the disubstituted derivative was isolated, with $C_6H_5CH_2$-CO-COOH, a molecule of water is apparently lost at the first step with the formation of α-acetaminocinnamic acid; with α-keto-glutaric acid, decarboxylation takes place with the formation of γ,γ-diacetamino-butyric acid. A number of investigators have condensed amines with

α-ketoacids; Hahn and coworkers (73, 74, 75) have shown that α-ketoacids capable of enolizing react with the β-m-hydroxyphenethylamine derivatives to form the corresponding tetrahydroisoquinoline-1-carboxylic acids.

In the presence of strong acids or alkalies the α-ketoacids show a tendency to condense with themselves with the formation of lactones. The condensation with aldehydes follows the aldol condensation pattern.

It has been shown (115) that dimethylpyruvic acid reacts with benzene in the presence of concentrated sulfuric acid to form an addition compound which is then dehydrated to β,β-dimethyl-α-phenylacrylic acid. Toluene reacts with phenylpyruvic acid in the presence of cold concentrated sulfuric acid to form α,α-ditoluyl-β-phenylpropionic acid (20); ethylbenzene and xylene react similarly.

2. Preparation of α-Ketoacids

Probably more α-ketoacids have been prepared as oxidative degradation products in the course of proving the structure of a more complex compound than by any other method. Frequently special methods for preparing individual α-ketoacids are better than many of the so-called "general" methods. Notable among these are: the preparation of phenylpyruvic acid (82) and of benzoylformic acid (39)

as described in Organic Synthesis; the preparation of pyruvic acid by the distillation of tartaric acid (85); and the preparation of hydroxypyruvic acid (103) by the breakdown of nitrocellulose. Krebs (97) developed a biological technique for the deamination of the α-amino acids by means of kidney slices; this procedure has been used by a number of biological chemists for the preparation of α-ketoacids.

Four chemical methods seem to be rather general:

A. Hydrolysis of the Acyl Cyanide

$$R - CO - Cl + MCN \longrightarrow R\text{-}CO\text{-}CN \longrightarrow R\text{-}CO\text{-}COOH$$

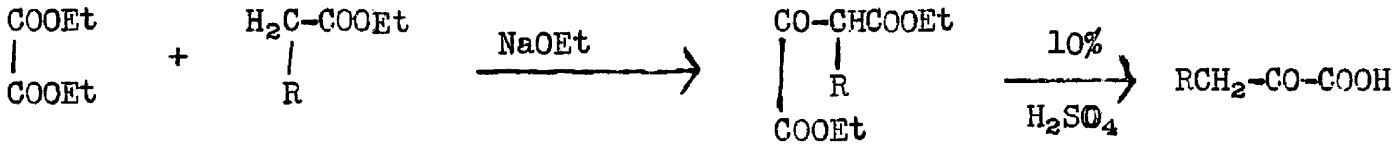
This method was first proposed by Claisen and Moritz (41) in 1880; they employed Hubner's (86) procedure, using silver cyanide to prepare the acyl cyanide. In 1929 Tschelinzeff and Schmidt (127) improved the procedure by substituting cuprous cyanide for silver cyanide. In some cases the hydrolysis was accomplished with cold concentrated hydrochloric acid and in others with a dilute, warm solution. The method was not successful for α-ketoacids containing more than five carbon atoms (127).

B. The Hydrolysis of the Oxime Ester

$$R\text{-}C(:NOH)\text{-}COOR_1 \xrightarrow[H_2SO_4.NO]{\text{formic acid}} R\text{-}CO\text{-}COOR_1 \longrightarrow R\text{-}CO\text{-}COOH$$

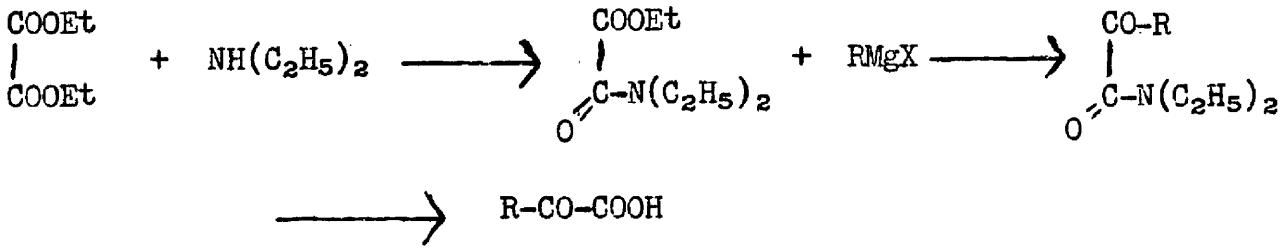
This method as originally used by Bouveault and Locquin (31) in 1902 calls for 85% formic acid and lead chamber crystals. Many variations have been tried, some with success. Attempts to prepare phenylpyruvic acid by this procedure were unsuccessful (77). The method has been quite popular and a great number of α-ketoacids have been prepared in this manner.

C. Hydrolysis Ethyl Esters of Oxaloacids



Adickes and Andresen (2) advanced this as a new method in 1943, and prepared twelve α-ketoacids in yields varying from 8 to 94 per cent, however, Wislicenus (136) had suggested an analogous reaction, using oxaloacetic acid in place of diethyl oxalate. The procedure is probably the most general yet developed.

D. Hydrolysis of the Addition Product of Grignard Reagents and Diethyloxamates



Barre (8) proposed this method in 1927 and showed its applicability in the preparation of four compounds. It would seem that the method should be very general; however, the necessity of performing the condensation at -15° and the tediously long refluxing time requisite for the preparation of diethyl oxamate have discouraged other investigators from extending the method. The author claimed 60 per cent yields and said that, when one considers the difficulties of the other methods for the preparation of α-ketoacids, this method could be used to advantage.

3. Additional Information on α-Ketoacids

In Table II is summarized part of the information which has been accumulated in the course of a literature survey. The data cover only the α-ketoacids having the general formula $C_nH_{2n-2}O_3$ and those acids which can be considered as precursors of the naturally occurring α-amino acids that are described in Beilstein and listed in the indices of Chemical Abstracts. In the majority of cases a complete literature survey has been made; the Beilstein reference is given for the four acids on which the survey is incomplete.

The acids are classified as derivatives of pyruvic. The recorded melting points are given in a separate column. Under "First Prepared By" is given the author and date, along with the appropriate reference. In the fourth column, under "General Method", the letters

A, B, C, and D refer to the four general methods, respectively, discussed above on pages 16 to 18. In the last column are given literature references to other methods of synthesis. References to the chemical and biological reactions of the α-ketoacids have not been included.

TABLE II

INFORMATION ON α-KETOACIDS

$$R'-\overset{\overset{R}{|}}{\underset{\underset{R''}{|}}{C}}-CO-COOH$$

R	R'	R''	Melting Point °C.	First Prepared By	General Method (as described in text)	References to Other Methods of Preparation
H	H	H	13.6	Berzelius 1835 (19)		Beil. III. p. 608
H	H	CH_3	31	Claisen and Moritz 1880 (41)	A (41,127) B (31,102) C (135) D (8)	(137,65,43,5,128) (57,53,49,116,40)
H	H	C_2H_5	6	Moritz 1881 (105)	A (105) B (102) C (2)	(21, 58, 62)
H	CH_3	CH_3	31	Moritz 1881 (105) Brunner 1894 (35)	A (105,127) C (117)	(34,1,45,126) (98,70,93,35) (94,83,110,26)
H	H	nC_3H_7	7	Kondo 1912 (95)	B (95) C (2) D (8)	
H	H	iso C_3H_7	-1.5	Locquin (1904 (102)	B (102) C (2)	(111,70,1)
CH_3	CH_3	CH_3	125	Glucksman 1889 (69)		(4,118,23,24,38) (64a)
H	CH_3	C_2H_5	35	Mebus 1905 (104) Bouveault and Locquin 1905 (32)	B (101, 32)	

TABLE II (continued)

$$R'-\overset{R}{\underset{R''}{C}}-CO-COOH$$

R	R'	R''	Melting Point °C.	First Prepared By	General Method (as described in text)	Reference to Other Methods of Preparation
			52	Prezwalsky 1913 (114)		
H	H	n-C_4H_9		- - - - - - -		
			29	Adickes and Andresen 1943 (2)	(2)	
H	H	iso C_4H_9	22	Fittig and Kachlbrandt 1899 (63)	B (102)	
CH_3	CH_3	C_2H_5	liq.	Anschutz Rauff 1903 (4)		
			118	Smedley-MacLean and Pearce 1934 (122)	B (122)	
H	H	n-C_5H_{11}		- - - - - - - -		
			33	Adickes and Andresen 1943 (2)	C (2)	
			106	Smedley-MacLean and Pearce 1934 (122)	B (122)	
H	H	n-C_6H_{13}		- - - - - - - -		
			43	Adickes and Andresen 1943 (2)	C (2)	
			60	Smedley-MacLean and Pearce 1934 (122)	B (122)	
H	H	n-C_7H_{15}		- - - - - - - -		
			47	Adickes and Andresen	C (2)	

TABLE II (continued)

R'-C(R)(R")-CO-COOH			Melting Point	First Prepared By	General Method as described in text)	References to Other Methods of Preparation
R	R'	R"	°C.			
H	CH_3	n-C_6H_{13}	liq.	Locquin 1904 (102)	B (102)	
H	H	n-C_8H_{17}	55	Adickes and Andresen 1943 (2)	C (2)	
H	iso C_4H_9	iso C_4H_9	liq.	Freylon 1910 (67)		
H	H	n-C_9H_{19}	57	Adickes and Andresen 1943 (2)	C (2)	
H	H	n-$C_{10}H_{21}$	62	Adickes and Andresen 1943 (2)	C (2)	
H	H	n-$C_{12}H_{25}$	68	Adickes and Andresen 1943 (2)	C (2)	
H	H	n-$C_{13}H_{27}$	65	Kuwata 1938 (99)	(6)	
H	H	n-$C_{15}H_{29}$	83	Windaus and Van Schaar 1926 (134)		
H	H	OH	liq.	Wichelhaus 1867 (133)		Beil. III p. 869
H	H	SH		Parrod 1942 (109)		
H	OH	CH_3		Hoff-Jorgensen 1940 (84)		
H	H	CH_3SCH_2		Waelsch and Borek 1939 (129)		(36)
H	H	COOH	176	Fenton and Jones 1900 (56)		Beil. III p. 777

TABLE II (continued)

R'-C(R)(R")-CO-COOH			Melting Point	First Prepared	General Method (as described	References to Other Methods
R	R'	R"	°C.	By	in text)	of Preparation
H	H	CH_2-COOH	113	Blaise and Gault 1908 (22)		Beil. III. p. 789
H	H	C_6H_5	153	Plochl 1883 (112)	C (136) D (8)	(82,29,27,30,46) (47,120,49,50,52,12)
H	H	2-OH C_6H_4		Plochl and Woffrum 1885 (113)		(55,64)
H	H	3-OH C_6H_4	165	Flatow 1910 (64)		(76)
H	H	4-OH C_6H_4	220	Neubauer 1909 (106)		(12)
H	H	NH_2 $(CH_2)_3$	103	Wollfenstein 1893 (138)		(x)(Author in doubt as to exact formula)
H	H	$NH_2(CH_2)_2$		Krebs 1939 (96)		
H	H	-Imidazolyl		Novello Harrow Sherwin 1926 (108)		
H	H	-Indolyl	212	Ellinger and Matsuoka 1920 (48)		(72,11,87,10,25)
Thyroxine analog			173	Canzanelli Guild and Harrington 1935 (37)		

EXPERIMENTAL

SOURCE OF MATERIALS USED AS INTERMEDIATES

1. Commercially Available Chemicals

The following chemicals used in the preparations which are described later are commercially available: butyl alcohol, thionyl chloride, benzyl chloride, diethyl sulfate, diethylmalonate, acetoacetic ester, butyl bromide, and the common reagents. These chemicals were used without further purification.

2. n-Butyl Nitrite

n-Butyl nitrite was prepared in yields of 80 to 85 per cent according to the directions of Hamlin (77a). The product was stored in the refrigerator and redistilled at three month intervals.

3. Acetoacetic Ester Derivatives

Ethyl n-butylacetoacetate and ethyl benzylacetoacetate were prepared according to general directions of Marvel and Hager as described in Organic Synthesis (103a); yields were 72 per cent and 74 per cent, respectively. A small quantity of ethyl ethylacetoacetate was obtained from the graduate laboratory as a student preparation and was redistilled.

4. Malonic Ester Derivatives

Approximately 50 g. of benzylmalonic acid and approximately 10 g. of ethylmalonic acid were prepared by Dr. Richard Barry and have been used in this work. Ethyl n-butylmalonate was prepared in 88 per cent yield by the method of Adams and Kamm as described in Organic Synthesis (1a). The ester was converted into the acid in yields of 92 per cent by use of 50% . potassium hydroxide solution according to the directions of Barry (9).

5. α-Ketoximino Acids

The ketoximino acids used were prepared according to the methods described by Hamlin (78) and by Barry (9). Hamlin's procedure consist of nitrosation of substituted acetoacetic esters in the presence of 85 per cent sulfuric acid; it is somewhat more cumbersome and the product is not as pure as that obtained by Barry's method. Barry (9) nitrosated malonic acid derivatives in an ether solution.

During the nitrosation of the malonic acid derivatives certain observations were made which led to a modification of the procedure described by Barry. He employed absolute ether, giving the impression that anhydrous conditions were preferable for successful yields. During the course of the present investigation, it was observed that in the nitrosation of rather large quantities (30 g.) of α-oximinohexanoic acid yields were very low if anhydrous conditions

were maintained. Barry worked with smaller quantities and in an open beaker, which no doubt permitted the adsorption of water. The advantages of a modification of the Barry procedure may be seen in the following nitrosation studies.

A. Nitrosation under Anhydrous Conditions

To a 250 cc. three-necked flask equipped with stirrer, gas delivery tube, $CaCl_2$ tube and surrounded by an ice-salt bath, was added 16 g. of $C_4H_9CH(COOH)_2$ and 100 cc. of absolute ether. To this mixture was added 25 cc. of butyl nitrite. The stirrer was started and a stream of dry HCl gas passed into the reaction flask at a rate of 2 to 3 bubbles per second. After stirring for one hour, the mixture was transferred to a separatory funnel and extracted with 10% sodium hydroxide. The alkaline solution was then carefully neutralized with hydrochloric acid using congo red as the indicator; an oil separated which was first thought to be an ester of α-oximinohexanoic acid, however, when isolated and hydrolyzed with 10 per cent NaOH the original substituted malonic acid was obtained. In a repeat of this experiment, it was possible to isolate 8 g. of the original $C_4H_9CH(COOH)_2$ and 3 g. of the desired $C_4H_9C(NOH)COOH$.

B. Nitrosation with Moisture Present

Since this procedure was successfully employed in the preparation of three α-oximino acids (α-oximinobutyric, α-oximino-

hexanoic, and β-phenyl-α-oximinopropionic acid), it will be described as a general method.

In a 250 cc. beaker, equipped with a mechanical stirrer and surrounded by an ice salt bath, was placed 0.05 to 0.10 mole of substituted malonic acid, 100 cc. of ether, 1 cc. of water, 1 cc. of hydrochloric acid, and two equivalents of butyl nitrite. HCl gas was passed in at a rate of about 2 to 3 bubbles per second and the mixture stirred vigorously for about an hour; evaporated ether was replaced. The mixture was then transferred to a separatory funnel and the α-oximino acid extracted with 10 per cent sodium hydroxide solution. The alkaline extract was washed with several small portions of ether and made acid with concentrated hydrochloric acid, using congo red as the indicator. After standing for about an hour in the cold, the precipitated α-oximino acid was filtered and washed with cold water. In the case of α-oximinobutyric acid, which was somewhat soluble in water, the acidified solution was extracted with ether. The ether layer was washed with a saturated sodium chloride solution, then dried over anhydrous sodium sulfate, filtered, and the solvent evaporated in a stream of dry air. In general, purification was effected according to the recommendations of Barry and Hamlin.

Yields obtained using this procedure were from 70 per cent to 80 per cent of the theoretical.

C. Nitrosation with Aqueous Hydrochloric Acid as Catalyst

In a 250 cc. beaker was placed 16 g. of $C_4H_9CH(COOH)_2$ dissolved in 100 cc. of ether. To this was added 25 cc. of butyl nitrite and 1 cc. of concentrated hydrochloric acid. The mixture was stirred vigorously with a mechanical stirrer and after ten minutes an additional 1 cc. of hydrochloric acid was added. The stirring and the adding of hydrochloric acid was continued until 10 cc. of concentrated hydrochloric acid had been added. After adding 25 cc. of water the mixture was extracted with ether.

The ether solution was then extracted with 10 per cent sodium hydroxide solution. By acidification of the alkaline layer and filtration with subsequent purification 5 g. of $C_4H_9C(NOH)COOH$ was obtained. This would indicate that anhydrous conditions are not necessary for the nitrosation.

D. Nitrosation under Mild Conditions

The nitrosation of benzylmalonic acid under mild conditions which Barry reported (9) has been repeated. To 4 g. of benzylmalonic acid dissolved in 25 cc. of absolute ether was added 5 cc. of butyl nitrite and 1 cc. of absolute ethanol containing 0.1 g. of HCl per cc. This mixture was placed in the refrigerator for one week. A yield of 3 grams or 85 per cent of the theoretical amount of β-phenyl-α-oximinopropionic acid was obtained. This equals the yield reported by Barry (9).

ATTEMPTS TO HYDROLYZE α-KETOXIMINO ACIDS

The hydrolysis of aldoximes and ketoximes, by acids or by alkalies, to yield the corresponding aldehydes or ketones, is a reaction described in general organic texts. Unfortunately these reactions do not apply to the synthesis of α-ketoacids.

Bouveault and Locquin (33) found that if oximes of the α-ketoacids are heated with dilute acids, they lose carbon dioxide and water, forming a nitrile containing one less carbon atom:

$$R\text{-}C(:NOH)COOH \longrightarrow RCN + CO_2 + H_2O$$

This observation was confirmed during this investigation; the oxime of phenylpyruvic acid boiled with dilute acid, even with 0.1 N hydrochloric, formed phenylacetonitrile.

Organic text books also indicate that nitrous acid is capable of removing the oximino groups from aldoximes and from ketoximes. Keagle (91) was able to obtain succindialdehyde in yields of 90 per cent by treating succindialdoxime with nitrous acid. Accordingly, several procedures employing nitrous acid were tried, all unsuccessful, many forming phenylacetonitrile.

Phenylpyruvic acid cannot be prepared from its oxime by employing formic acid and nitrosyl sulfate (a method previously discussed) as reported by Hall, Hynes and Lapworth (77).

Sumerford and Dalton (124) have proposed the use of a mixture of cuprous oxide, hydrochloric acid and acetone for the hydrolysis of quinone oximes; this procedure does not seem to be applicable to the hydrolysis of α-oximino acids.

Karrer and Hoffmann (90) used hydrogen peroxide to effect the hydrolysis of a nitrosophenol and Wallach (131) employed aqueous oxalic acid to the same end; neither of these reagents showed promise for effective removal of the oxime group from the oxime of phenylpyruvic acid. Transoximination with acetone or with benzaldehyde did not appear to take place.

Since "unusual" reactions sometimes occur in liquid ammonia the oxime of three α-ketoacids, e.g. phenylpyruvic, α-keto-n-hexanoic, and α-ketobutyric acids were allowed to stand during two hours in an excess of liquid ammonia. The ammonium salts were formed and the original acid could be quantitatively recovered. A second series employing ammonium chloride and liquid ammonia gave similar results.

It was observed during the course of the investigation that small amounts of phenylpyruvic acid were formed if the oxime was allowed to stand at room temperature for a month in 85% sulfuric acid.

With ethanolic hydrogen chloride, Bouveault and Locquin (31) obtained small amounts of α-ketoacid from the oxime; a modification of their procedure substituting a reflux condenser for a sealed tube gave only traces of the α-ketoacid.

Hemmerle (80) has shown that phenylpyruvic acid forms a stable sodium bisulfite-addition compound. In attempts to utilize this property, it was noted that a small amount of the α-ketoacid was formed when a mixture of the oxime, sodium bisulfite and hydrochloric acid was allowed to stand at 35° for several days. Efforts to speed up this reaction through heating resulted in the formation of phenylacetonitrile.

In Table III are summarized, as briefly as possible, the procedures which have been employed in an effort to hydrolyze the oxime of phenylpyruvic acid. The general procedure was to treat the oxime with the reagent under given conditions, then acidify if not already acid, (or if strongly acid, dilute) and extract with ether. The solvent was then removed and an attempt was made to identify the resulting material. Small residues were checked by Erlenmeyer's (50) test, characteristic for phenylpyruvic acid, namely, the formation of a green ring at the interface between an ether solution containing the phenylpyruvic acid and a dilute solution of ferric chloride. The recovered oxime was identified by mixed melting points. Phenylacetonitrile was identified after isolating the oil resulting from a 10% acid hydrolysis; the nitrile was distilled under diminished pressure; the product distilling between 76° and 78° at 1 mm. was identical in boiling point and refractive index with a known sample of phenylacetonitrile as well as duplicating values reported in the literature.

Hydrolysis product: $[N]_D^{25} = 1.5212$;

Phenylacetonitrile (129a): $[N]_D^{25} = 1.52105$

TABLE III

SUMMARY OF ATTEMPTS TO HYDROLYZE β-PHENYL-α-OXIMINOPROPIONIC ACID

Reagent	Conditions of the Reaction	Reaction Time	Oxime Recovered Per Cent	Remarks
HCl, 3%	reflux	1 hr.	0	Phenylaceto-nitrile formed
HCl, 0.1 N	reflux	30 min.	0	Phenylaceto-nitrile formed
H_2SO_4, 10%	boiling	10 min.	30	Phenylaceto-nitrile formed
H_2SO_4, 10%	70°C.	1.5 hr.	30	Phenylaceto-nitrile formed
H_2SO_4, 85%	room temp.	1 week	75	Some ketoacid probably formed
H_2SO_4, conc.	room temp.	1 week	0	Carbonization
HOAc, 90%	room temp.	1 mo.	50	
$H_2C_2O_4$, 5%	steam bath	20 min.	40	Phenylaceto-nitrile formed
NaOH, 10%	room temp.	1 week	100	
NaOH, 10%	reflux	2 hr.	90	
NH_4OH, conc.	room temp.	1 week	100	
Alc. KOH, N/2	reflux	30 min.	90	
HNO_2, $NaNO_2$ dil H_2SO_4	25°, mech. stir.	30 min.	95	
HNO_2, $NaNO_2$ dil. H_2SO_4	50°C., mech.	20 min.	0	Phenylaceto-nitrile formed

TABLE III (continued)

Reagent	Conditions of the Reaction	Reaction Time	Oxime Recovered Per cent	Remarks
HNO_2, $NaNO_2$ H_2O	room temp.	18 hr.	0	Phenylaceto-nitrile formed
HNO_2, $BuNO_2$ 85% H_2SO_4	at 5°C.	1.5 hr.	95	
HNO_2, $BuNO_2$ 85% H_2SO_4	on ester, 5°C.	1 hr.	70	
$NaHSO_3$, dil. HCl	steam bath	10 min.	80	Phenylaceto-nitrile formed
$NaHSO_3$, dil. HCl	steam bath	30 min.	0	Phenylaceto-nitrile formed
$NaHSO_3$, dil. HCl	35°C.	36 hr.	70	Some ketoacid probably formed
Alc. HCl, Abs. alc. + 7% cond. HCl	reflux	1.5 hr.	0	Esterification and probably some ketoacid
H_2O_2, 0.1 N HCl	70°C.	10 min.	0	Phenylaceto-nitrile formed
H_2O_2, ROH HCl	boil	20 min.	90	No ketoacid identified
Cu_2O, HCl-acetone (124)	25°C.	45 min.	0	Test for ketoacid negative
Cu_2O, HOAc-acetone (124)	reflux	1 hr.	0	Test for ketoacid negative
Acetone, NaAC	reflux	1.5 hr.	80	Some ketoacid probably formed
C_6H_5CHO, neutral + alc.	shake-25°C.	3 hr.	70	Test for ketoacid negative
C_6H_5CHO, neutral + alc.	reflux	2 hr.	70	Test for ketoacid negative
NH_3, liquid		2 hr.	100	NH_4 salt formed
NH_3, liquid NH_4Cl		2 hr.	100	NH_4 salt formed

ATTEMPTS TO PREPARE AND HYDROLYZE α-KETIMINOACIDS

In view of the failure to obtain α-ketoacids by the methods described, it seemed that they might be prepared from their corresponding ketimines:

$$R\text{-}C(:NH)\text{-}COOH \xrightarrow{HOH} R\text{-}CO\text{-}COOH + NH_3$$

On the basis of previous work in these laboratories, it was believed that the desired ketimine might be formed as partial hydrogenation products of the oximino acids.

As stated earlier, Hamlin observed that during the hydrogenation of α-oximino acids approximately half of the theoretical amount of hydrogen needed for complete reduction was taken up rapidly; to complete the second half of the reduction usually required four to five times as long. Hamlin supposed that this slowing down was due to the complete formation of the imino intermediate which was then more slowly reduced to the amine. If Hamlin's supposition were correct, it should be comparatively easy to reduce to the half-way stage and hydrolyze the resulting imino intermediate to the α-ketoacid. Accordingly the behavior of oxime of phenylpyruvic acid on reduction was studied further.

The hydrogenation was carried out as described by Hamlin. The general procedure was to place a mixture of 3.3 g. of palladinized

charcoal (78), 96 cc. of 95 per cent ethanol, 4 cc. of concentrated hydrochloric acid and 2 g. of β-phenyl- α-oximinopropionic acid in a 250 cc. flask and connect to the hydrogenator. After exhausting the apparatus three times, the shaker was started and readings of the volume of hydrogen absorbed were made at intervals.

The results, given graphically in Figure 1, confirm Hamlin's observation that when approximately half the calculated hydrogen is taken up, the rate of reduction drops markedly, but if allowed to go to completion forms phenylalanine in substantially quantitative yields. In the next two experiments, reduction was interrupted at the half way point; the product consisted of a mixture of approximately equimolar amounts of unchanged oxime and phenylalanine. In a fourth experiment reduction was stopped when three quarters of the calculated amount of hydrogen had been taken up; the product consisted of a mixture of unchanged oxime and phenylalanine in a molar ratio of about 1 to 3.

These results suggest that the phenylalanine as it forms acts as an anti-catalyst. In order to test this hypothesis, several experiments were performed, in which 1 g. of phenylalanine and 2 g. of β-phenyl- α-oximinopropionic acid were mixed and subjected to catalytic hydrogenation. Figure II is a typical curve for this reduction. After two hours the reduction was only about 50 per cent completed. Obviously, the amino acid has some deterrent action on the speed of hydrogenation.

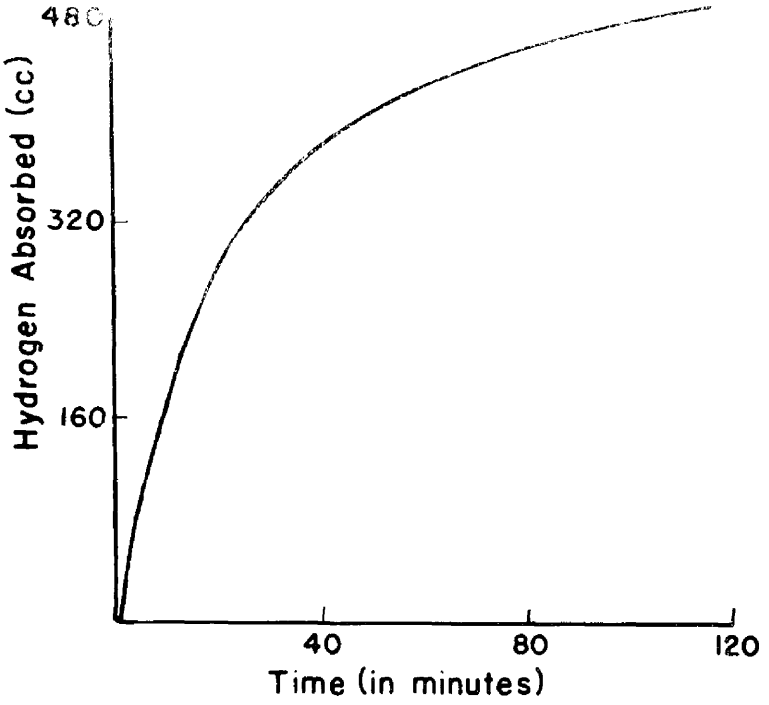


FIG. I

2 g. Oximino Acid

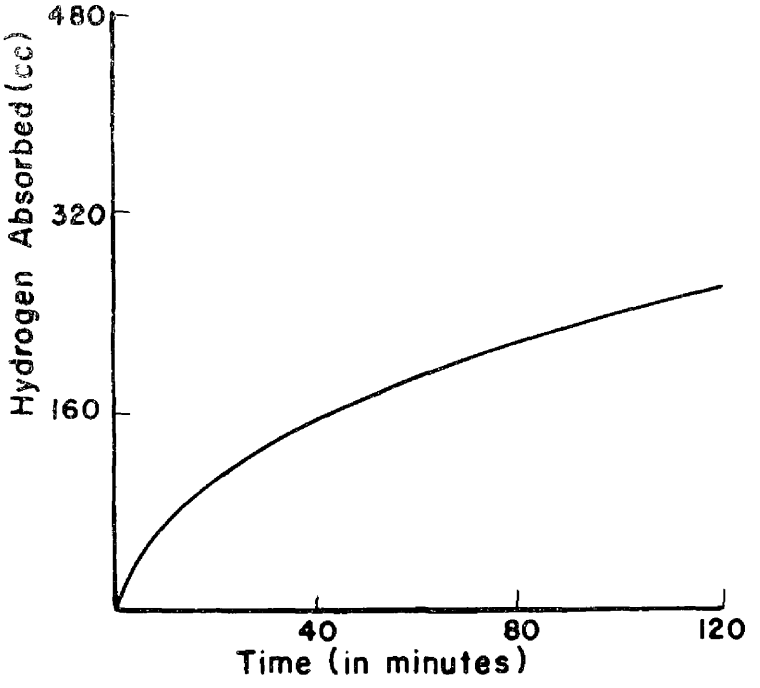


FIG. II

2g. Oximino Acid
Plus 1 g. Phenylalanine

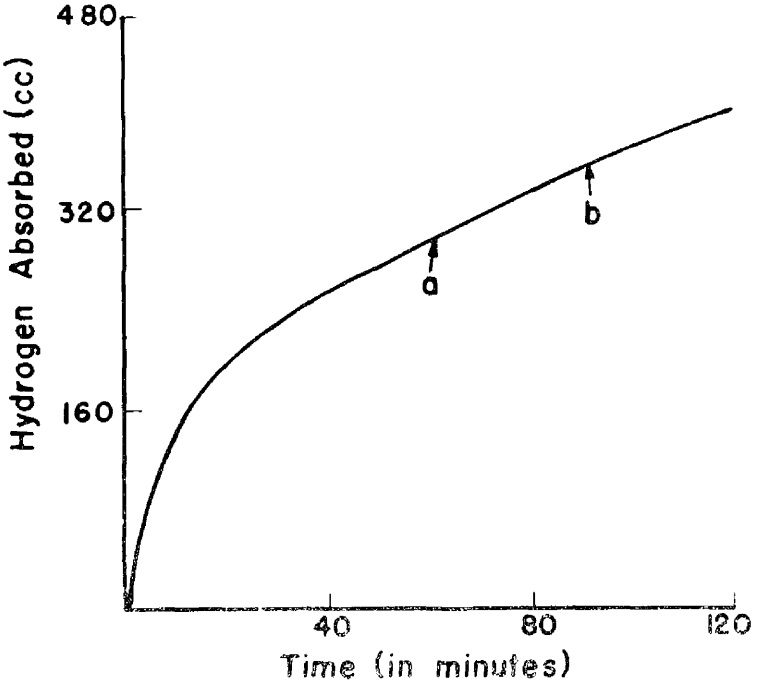


FIG. III

2 g. Oximino Acid Plus
Added Oxime At a And b

CATALYTIC HYDROGENATION OF β-PHENYL-α-OXIMINO PROPIONIC ACID.

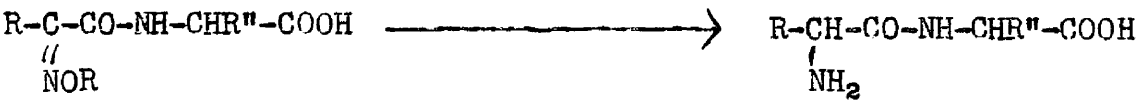
To prove further that the slowing down in the reduction of the oximino acid is an anti-catalytic phenomenon and not a formation of the intermediary imine as Hamlin postulated, which is subsequently reduced at a slower rate, the following experiment was performed: To the 250 cc. hydrogenation flask was attached a Claisen neck fitted with a burette so connected as to form a closed system which permitted no pressure change when additions were made to the reaction flask from the burette. In the reaction flask was placed the catalyst, 80 cc. of 95 per cent ethanol, and 1 cc. of concentrated hydrochloric acid. In the burette was placed a 10 per cent solution of β -phenyl- α - oximinopropionic acid (3.75 g. oxime, 33.5 cc. ethanol and 4 cc. of concentrated hydrochloric acid). Twenty cc. of the oxime solution (2 g.) was run into the reaction flask, the apparatus exhausted, and hydrogen introduced. The shaker was started and readings were taken at two minute intervals. After one hour, (that is, when approximately half of the theoretical amount of hydrogen had been taken up) another portion consisting of 10 cc. of the oxime solution (1 g. of oxime) was run into the reaction flask. It may be observed from Figure II, point a, that there was no increase in the rate at which the hydrogen was taken up. After the elapse of a further thirty minutes, the final 7.5 cc. of the oxime mixture (0.75 g. of oxime) was added; again there was no increase in the rate at which the hydrogen was taken up (Figure III, point b). This experiment shows that the addition of fresh β -

phenyl α -oximinopropionic acid to a mixture of the acid already in the process of hydrogenation does not cause an increase in the rate of hydrogen absorption. It must be concluded that the phenomenon of a slow-down in the rate of hydrogenation is an anti-catalytic phenomenon and is not due to the formation of a more difficult hydrogenated product. In passing it is interesting to note that in Figure I the curve after approximately one-half of the hydrogen has been taken up, is strikingly similar to the typical curve shown in Figure II.

α-ALKOXIMINOACIDS AND THEIR DERIVATIVES

Although the α-ketoacids are not easily prepared from the oximino acids, it is felt that the oximino acids may still be successfully employed in the preparation of the dipeptides. In 1938, Adkins and Reeve (3) made an interesting synthesis of threonine in which they prepared the O-ethyl ether of oximinoacetoacetate and later reduced the product thereby removing the O-ethyl to give the free amino group. It seemed logical that alkylation would protect the oximino group, permitting the synthesis of an acid chloride derivative which may be coupled with an amino acid; subsequent reduction should form the amino group, thus giving a dipeptide. The theoretical equations are:

I II III



IV V

During the course of this investigation three ethoximino (Type II, R' = Et) and two benzyloximino acids (Type II, R' = $CH_2C_6H_5$)

were prepared. Of these four were converted into their corresponding acid chloride (Type III, three where R' = Et and one where R' = $CH_2C_6H_5$). Time did not permit the condensation of these acid chlorides with amino acids; however, the fact that they will form amides is proved by the fact that the corresponding anilides have been synthesized. Reduction studies are incomplete.

Since these compounds are representative of a new class, Dr. E. J. Crane, Editor of Chemical Abstracts, was asked to give his opinion as to the proper nomenclature. Dr. Crane referred to the letter to Dr. Austin Patterson, who replied as follows:

> "Dr. Cappel and I rather like ethoxyimino better than ethylisonitroso and have no particular objection to ethoximino (We could use such names as ethoxyimino now in C.A. indexing since they are combinations of radical names already in our published list, but would have to regard ethoximino, methoximino, etc. as new radical names).
>
> I am sorry to have taken all this time to say that we see no objection to your proposed names, but think this was the best procedure.
>
> I might note that by the present C.A. practice the compound in your letter of December 30th would probably be indexed as pyruvic acid, phenyl, O-ethyloxime. Treating such compounds as derivatives of the corresponding Oxo compound accounts for our scant use of isonitroso".

A description of the experimental phase of this work is summarized under the following headings:

1. Preparation of α-alkoximino acids
2. Preparation of α-alkoximino acid chlorides
3. Preparation of ethyl esters of α-alkoximino acids
4. Preparation of the anilides of the α-alkoximino acids
5. Hydrogenolysis of the substituted α-oximino acids

1. Preparation of α-Alkoximino Acids

A. α-Ethoximino Acids

The preparation of the α-ethoximino acids was accomplished by ethylation with diethyl sulfate in a slightly alkaline medium in the presence of acetone. The method is adapted from a methylation procedure which Tipson and Levene (125) had found so successful in their sugar studies. The three acids which were prepared were found to be low melting solids and could be purified by distillation under diminished pressure.

(1) β-Phenyl- α -Ethoximinopropionic Acid

$$C_6H_5-CH_2-\underset{\underset{NOC_2H_5}{\|}}{C}-COOH$$

Since this synthesis may be used as a type procedure for the preparation of α-alkoximino acids, it will be described in detail. A tenth mole, 17.9 g. of β-phenyl- α -oximinopropionic acid was placed in a 500 cc. three-necked flask and dissolved in a 100 cc. of a 5 per cent aqueous solution of sodium hydroxide and 50 cc. of acetone.

The flask was equipped with a mechanical stirrer and two burettes so arranged that a solution of sodium hydroxide and diethyl sulfate could be added simultaneously. A total of 100 cc. of diethyl sulfate and 100 cc. of sodium hydroxide solution (30.5 g. per 100 cc. of solution) was added. The rate of addition after the initial addition of 10 cc. of the sodium hydroxide was such that 1 drop of alkali was added per drop of diethyl sulfate. Since the concentration of the alkali solution was such that 1 cc. of alkali would neutralize the acid formed during the hydrolysis of 1 cc. of diethyl sulfate, the mixture was slightly alkaline throughout the ethylation. The addition of the ethylating reagent required about thirty minutes and during this time the temperature of the water bath was raised to 70°. As a general rule 5 moles of diethyl sulfate were added per mole of oximino acid.

When the addition was completed, the burettes were replaced by a reflux condenser, and the temperature of the bath was raised to boiling. The mixture was refluxed half an hour, after which the reflux condenser was removed and the heating continued about an hour, or until most of the acetone had evaporated; the last traces being removed by suction. The reaction mixture was cooled, made definitely acid to congo red, using 25 cc. of concentrated hydrochloric acid, and extracted completely with ether. The combined ether extracts were washed with several small portions of water, filtered, and dried over

anhydrous sodium sulfate. The solvent was removed and the resulting material distilled at 1 to 2 mm. pressure. The fraction distilling at 115° to 120° was collected; a yield of 16.3 g. or 79 per cent of the theoretical β-phenyl- α-ethoximinopropionic acid was obtained. The colorless crystals melted at 58.5-59.0°.

Neutralization Equivalent (phenolphthalein)

Calc. for $C_{11}H_{13}NO_3$: 207.22 Found: 209.5
209.9

Nitrogen (Kjeldahl)

Calc. for $C_{11}H_{13}NO_3$: 6.76% Found: 6.59%
6.56%

(2) α-Ethoximinobutyric Acid

$$C_2H_5-C(=NOC_2H_5)-COOH$$

The ethylation was carried out according to the directions given for the preparation of β-phenyl- α-ethoximinopropionic acid. From 11.7 g. (0.1 mole) of α-oximinobutyric acid, 8.6 g.(60 per cent of theoretical) α-ethoximinobutyric acid was obtained. The fraction distilling at 89-95° under 12-14 mm. pressure was collected. When cooled the product crystallized in long colorless needles which melted at 61.0-61.5°.

Neutralization equivalent (phenolphthalein)

Calc. for $C_6H_{11}O_3N$: 145.2 Found: 146.5
146.8

Nitrogen (Kjeldahl)

Calc. for $C_6H_{11}O_3N$: 9.65% Found: 9.55%
9.58%

(3) α-Ethoximinohexanoic Acid

$$CH_3(CH_2)_3-C(=NOC_2H_5)-COOH$$

The ethylation of α-oximinohexanoic acid was carried out according to the directions given under the preparation of β-phenyl-α-ethoximinopropionic acid. From 29 g. (0.2 mole) of α-oximinohexanoic acid, 28.1 g. or 80 per cent of the theoretical α-ethoximinohexanoic acid was obtained. The fraction distilling at 83-88° under 1 to 3 mm. pressure was collected. The product was a clear liquid which when allowed to stand in the refrigerator solidified as long colorless needles. The crystals melted at 23°

Neutralization equivalent (phenolphthalein)

Calc. for $C_8H_{15}O_3N$: 173.2 Found: 174.0
174.6

Nitrogen (Kjeldahl)

Calc. for $C_8H_{15}O_3N$: 8.09% Found: 7.85%
7.88%

B. α-Benzyloximino Acids

Since various investigators have enjoyed considerable success with catalytic debenzylation, it was thought that it would be advantageous to prepare the benzyl substituted α-oximino acids and make a study of their reduction. As early as 1883, Janny (88) succeeded in preparing the benzyloximino grouping. He reacted benzyl chloride and acetoxime in the presence of sodium ethoxide to form benzyloximinoacetone. It was found that essentially the same procedure as described above for ethylation could be used for benzylation of the α-oximino acids. When five equivalents of benzyl chloride were used, the mode of isolation of the benzyl derivative was different from that used for the ethoximino acids. It was expedient to isolate the sodium salt of the α-benzyloximino acids since they were somewhat soluble in a mixture of ether, benzyl alcohol, and residual benzyl chloride. After isolation, the sodium salt was dissolved in water and the free α-benzyloximino acid precipitated by acidification. The yields obtained were 42 and 56 per cent, respectively, for the two acids prepared. It is believed that these yields may be greatly increased if the mode of isolation is improved.

(1) β-Phenyl- α-Benzyloximinopropionic Acid

$$C_6H_5CH_2C(=NOCH_2C_6H_5)-COOH$$

In a 500 cc. three-necked flask was placed 9 g. of β-phenyl-α-oximinopropionic acid dissolved in 25 cc. of water, containing 2.5 g. of sodium hydroxide, and 100 cc. of acetone. This mixture was heated to 40°, the mechanical stirrer started, and 5 cc. of a sodium hydroxide solution (34.7 g./100 cc.) was added from a burette. Benzyl chloride was then added drop by drop from a second burette and an equal volume of the alkali added drop by drop, simultaneously. This equal rate of addition was continued until 30 cc. of benzyl chloride (5 equivalents) and a total of 30 cc. of alkali had been added, the addition requiring about 30 minutes. During the addition the temperature of the water bath was slowly raised to 70°. The burettes were replaced by a condenser and the mixture was refluxed for an hour at 70° with continued stirring; the reflux condenser was then removed and the temperature of the bath raised to boiling, thus removing the acetone. The heating was continued for two hours; during the latter part of the heating, a current of air was drawn over the mixture and several portions of water were added to prevent the mixture from solidifying. The mixture was cooled and extracted with ether. The etherial extracts were allowed to stand overnight in a stream of dry air. The resulting semi-solid material was treated with small portions of absolute ether; the oily constituents dissolved leaving behind 10.3 g. of colorless solid which was soluble in water and gave a strong alkaline reaction after ignition. It was assumed to be the

sodium salt of β-phenyl- α-benzyloximinopropionic acid. The 10.3 g. of "sodium salt" was dissolved in water and the solution made acid to congo red. The precipitate which formed was filtered and recrystallized from ethyl alcohol. A yield of 7.5 g., or 56 per cent of the theoretical β-phenyl- α-benzyloximinopropionic acid, was obtained as colorless crystals melting at 79-80°.

Neutralization Equivalent (phenolphthalein)

Calc. for $C_{16}H_{15}O_3N$: 269.3 Found: 272.0, 271.7

Nitrogen (Kjeldahl)

Calc. for $C_{16}H_{15}O_3N$: 5.20% Found: 5.12%, 5.13%

(2) α-Benzyloximinohexanoic Acid

$$CH_3(CH_2)_3C(=NOCH_2C_6H_5)-COOH$$

Using the procedure described for the preparation of β-phenyl- α-benzyloximinopropionic acid, 7.2 g. of α-oximinohexanoic acid gave 4.8 g. (42 per cent theoretical) of α-benzyloximinohexanoic acid. The white crystals melted at 61.0-61.5°.

Neutralization Equivalent (phenolphthalein)

Calc. for $C_{13}H_{17}O_3N$: 235.3 Found: 236.3, 236.3

Nitrogen (Kjeldahl)

Calc. for $C_{13}H_{17}O_3N$: 5.95% Found: 5.87%
5.87%

2. Preparation of the α-Alkoximino Acid Chlorides

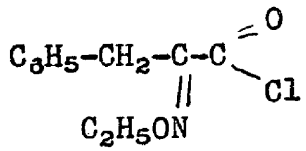
The chlorides of the α-alkoximino acids may be prepared in good yields by the action of thionyl chloride on the free acid. Two α-ethoximino acid chlorides were prepared and their structures confirmed by analysis, and by subsequent preparation of the corresponding ester and anilide. One α-benzyloximino acid chloride was prepared and its structure verified through analysis and subsequent preparation of the corresponding anilide. The preparation of another acid chloride is described, although the product was not completely characterized. Two of the acid chlorides were prepared by allowing the reaction to take place in a dry solvent. The other reaction was effected without the use of a solvent. There are indications that in the preparation of the acid chlorides of lower molecular weight, the solvent is necessary; it also seems that in the case of the lower molecular weight compounds, heat may cause a lowering of the yield. There was a marked darkening when thionyl chloride was refluxed with α-ethoximinohexanoic acid, resulting in the formation of tars during the distillation under diminished pressure. When this reaction was repeated using a solvent, permitting the reaction mixture

to stand overnight and then refluxing for a short time, the yields were good, although a small amount of unreacted acid was recovered; the mixture darkened after refluxing for 20 minutes. It may be that only a slight excess of thionyl chloride should be employed; however, in the preparation of β-phenyl- α-benzyloximinopropionyl chloride, three equivalents of thionyl chloride were added and the mixture refluxed in dry benzene for a couple of hours.

The acid chlorides are clear colorless liquids which may be distilled under diminished pressure. They do not react actively with cold water, but hydrolyze slowly to give a positive chloride test when shaken with water for several minutes. The lower molecular weight acid chlorides have a rather pungent odor; β-phenyl-α-benzyloximinopropionyl chloride, on the other hand, is practically odorless. It was observed that α-ethoximinohexanoyl chloride darkens when held in a sealed glass ampule during a period of two months.

A. α-Ethoximino Acid Chlorides

(1) β-Phenyl- α-Ethoximinopropionyl Chloride



To 6.6 g. of β-phenyl- α-ethoximinopropionic acid in a 125 cc. flask, was added 6.6 g. (4.0 cc.) of freshly distilled thionyl

chloride. The mixture was refluxed for half an hour, during this time the condenser was protected from moisture by a calcium chloride tube. To remove the excessive thionyl chloride and the liberated hydrogen chloride, two portions of 50 cc. each of dry benzene were added and removed under reduced pressure. After all the benzene had apparently been removed, the product was distilled under 1 to 2 mm. pressure and the fraction distilling at 95-97° was collected. A yield of 6.0 g. or 85 per cent of the theoretical β-phenyl- α-ethoximino-propionyl chloride was obtained.

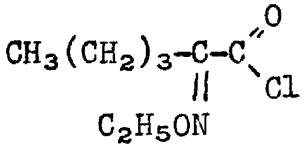
Nitrogen (Kjeldahl)

Calc. for $C_{11}H_{12}O_2NCl$: 6.21% Found: 6.04%
6.03%

Chlorine

Calc. for $C_{11}H_{12}O_2NCl$: 15.72% Found: 15.61%
15.57%

(2) α-Ethoximinohexanoyl Chloride



To 10.0 g. of α-ethoximinohexanoic acid dissolved in 25 cc. of dry benzene was added 9.8 g. (6.0 cc.) of thionyl chloride dissolved in 15 cc. of dry benzene. The mixture was allowed to stand overnight,

protected from atmospheric moisture, and then refluxed on the steam bath for about 20 minutes or until the mixture started to darken. The excess thionyl chloride and the liberated hydrogen chloride were removed by the addition of two portions of dry benzene with subsequent distillation under reduced pressure. After all the benzene had apparently been removed distillation of the acid chloride was effected at 1 to 3 mm. pressure. There was obtained 7.8 g. of product distilling between 58° and 63°. An additional 1.2 g. of material distilling within the range of the original α-ethoximinohexanoic acid (78-83°) was then collected. Allowing for the recovered acid, the yield of chloride was 81 per cent of the theoretical α-ethoximinohexanoyl chloride.

Nitrogen (Kjeldahl)

Calc. for $C_8H_{14}O_2NCl$: 7.31% Found: 7.30%
7.19%

Chlorine*

Calc. for $C_8H_{14}O_2NCl$: 18.51% Found: 15.6%
16.1%

(3) α-Ethoximinobutyryl Chloride

To 7.3 g. of α-ethoximinobutyric acid was added 13.2 g. (8.0 cc.) of thionyl chloride and the mixture refluxed on the steam

* The analysis of chlorine is admittedly unsatisfactory, but is reported here as a rapid rough quantitative analysis to indicate the high chlorine content of the distilled product. The identity of the compound is further substantiated by its ester and anilide, both of which are described later.

bath for ten minutes during this time the mixture became very dark, accordingly the heating was discontinued. The excess thionyl chloride and the liberated hydrogen chloride were removed by adding two portions of 50 cc. each of dry benzene and distilling under diminished pressure. After all the benzene had apparently been removed, the product was distilled under 15 to 18 mm. pressure, and the product distilling between 50° and 55° was collected. Only 3 g. or 36 per cent of the theoretical α-ethoximinobutyl chloride was obtained. The material remaining in the flask seemed to have polymerized and no unreacted original products could be distilled out of the mixture.

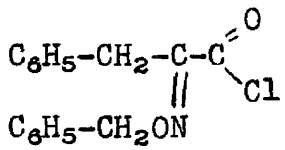
The analysis for chlorine was unsatisfactory, but it is reported here to indicate the high chlorine content of the distilled product. No nitrogen analysis was made; however, the structure was indicated by the fact that hydrolysis with 10 per cent sodium hydroxide gave the original α-ethoximinobutyric acid. It is believed that if technique worked out subsequent to this attempted preparation were employed, the product could be obtained in good yields.

Chlorine

Calc. for $C_6H_{10}O_2NCl$: 21.7% Found: 18.2%

B. α-Benzyloximino Acid Chlorides

(1) β-Phenyl- α-Benzyloximinopropionyl Chloride



To 5.0 g. of β-phenyl- α-benzyloximinopropionic acid dissolved in 25 cc. of dry benzene was added 6.6 g. (4.0 cc.) of thionyl chloride dissolved in 15 cc. of benzene. The mixture was refluxed for two hours, during this time the mixture was protected from atmospheric moisture by means of a calcium chloride tube. To remove the excess thionyl chloride and liberated hydrogen chloride, two portions of 50 cc. each of dry benzene were added and removed under diminished pressure. After all the benzene had apparently been removed, the product was distilled under 1 to 2 mm. pressure, and the product distilling at 170-175° was collected. A yield of 4.7 g. or 88 per cent of the theoretical β-phenyl- α-benzyloximinopropionyl chloride was obtained.

Nitrogen (Kjeldahl)

Calc. for $C_{16}H_{14}O_2NCl$: 4.87% Found: 4.82%
4.76%

Chlorine

Calc. for $C_{16}H_{14}O_2NCl$: 12.34% Found: 12.13%
12.09%

3. The Preparation of the Ethyl Esters of α-Alkoximino Acids

The ethyl esters of the α-ethoximino acids may be easily prepared by permitting the acid chloride to react with absolute ethyl alcohol. Two of the esters were prepared in order that the structure of the corresponding acid and acid chloride could be more conclusively established. The esters are clear colorless liquids which may be distilled under diminished pressure. They have shown no darkening in color over a three months period.

A. Ethyl Esters of α-Ethoximino Acids

(1) Ethyl β-Phenyl- α -ethoximinopropionate

$$C_6H_5CH_2-C(=NOC_2H_5)-COOC_2H_5$$

About 3 g. of β-phenyl- α -ethoximinopropionyl chloride was added to 10 cc. of absolute ethyl alcohol and the mixture was refluxed on the steam bath for half an hour. The excess alcohol was removed under diminished pressure, and the residue taken up with ether. The ethereal solution was washed with two small portions of saturated salt solution, then dried over anhydrous sulfate, and filtered into the distilling flask. After removal of the solvent, the residue was distilled at 1 to 3 mm. pressure. The fraction distilling at 118-120° was collected. The ester so prepared was a clear colorless liquid.

Nitrogen (Kjeldahl)

Calc. for $C_{13}H_{17}O_3N$: 5.95% Found: 5.79%
5.75%

Saponification Value

Calc. for $C_{13}H_{17}O_3N$: 235.3 Found: 231
232

(2) Ethyl ∝-ethoximinohexanoate

$$CH_3(CH_2)_3-C(=NOC_2H_5)-COOC_2H_5$$

To 4.0 g. of ∝-ethoximinohexanoyl chloride was added 25 cc. of absolute ethyl alcohol, and the mixture was refluxed on the steam both for one hour. The excess alcohol was removed under diminished pressure, the the residue taken up with ether. The ethereal solution was washed with two small portions of saturated salt solution, then dried over anhydrous sodium sulfate and filtered into a distilling flask. After removal of the solvent, the ester was distilled under 1 to 3 mm. pressure. The product distilling at 60-65° was collected. A yield of 3.7 g. or 88 per cent of the theoretical ethyl ∝-ethoximinohexanoate was obtained as a clear colorless liquid.

Nitrogen (Kjeldahl)

Calc. for $C_{10}H_{19}O_3N$: 6.96% Found: 6.82%
6.73%

Saponification Value

Calc. for $C_{10}H_{19}O_3N$: 201.3 Found: 201.1
199.0

4. Preparation of the Anilides of the α-Alkoximino Acids

The anilides of the α-alkoximino acids may be prepared by the reaction of aniline with the acid chloride. Two α-ethoximino anilides and one α-benzyloximino anilide have been prepared in order that the structure of the corresponding acids and acid chlorides could be more conclusively established. It was also desired to attempt the reduction of a compound of this class.

Two of the anilides are colorless crystalline products; their melting points are very close to those of the acid from which they may be considered as being derived. α-Ethoximinohexanilide is a liquid and may be distilled under diminished pressure; α-ethoximinohexanoic acid is also a liquid.

A. α-Ethoximino Anilides

(1) β-Phenyl- α-ethoximinopropionanilide

$$C_6H_5-CH_2-\underset{\underset{NOC_2H_5}{\|}}{C}-CO-NH-C_6H_5$$

To 9.2 g. of crude β-phenyl- α-ethoximinopropionyl chloride (product not distilled but excess thionyl chloride removed under diminished

pressure) dissolved in 25 cc. of dry benzene was added 9.2 g. (9.0 cc.) of aniline. The mixture was refluxed on the steam bath for half an hour, then cooled and the precipitated aniline hydrochloride filtered. The benzene was removed by distillation under diminished pressure, and the residue taken up with 20 cc. absolute ethyl ether. To this solution was added 80 cc. of petroleum ether; a light yellow precipitate was deposited which was at first thought to be the desired anilide. This product weighed 3.2 g. and on further purification (dissolving in absolute ether and precipitating with petroleum ether) had a constant melting point of 88-89°. Nitrogen analysis, neutralization equivalent and hydrolysis products indicate that this material is a salt of aniline and β-phenyl-α-ethoximinopropionic acid.

Nitrogen (Kjeldahl)

Calc. for $C_{11}H_{13}NO_3 \cdot C_6H_5NH_2$: 9.30% Found: 8.87%
8.77%

Neutralization Equivalent (0.1 N NaOH)

Calc. for $C_{11}H_{13}NO_3 \cdot C_6H_5NH_2$: 300.3 Found 304 to 318

(End point with phenolphthalein indefinite because of the inherent color of the solution; apparently liberated aniline)

Hydrolysis with 10 per cent Sodium Hydroxide

Aniline identified as a product.
β-Phenyl-α-ethoximinopropionic acid identified as a product.

β-Phenyl- α-ethoximinopropionanilide was isolated from the ethereal filtrates by evaporation to dryness and purification of the residue by dissolving in ethyl alcohol and precipitating by the addition of water. A total of 5.8 g. of anilide which melted at 59-60° was obtained.

Nitrogen (Kjeldahl)

Calc. for $C_{17}H_{18}O_2N_2$: 9.92% Found: 9.84%
9.79%

(2) α-Ethoximinohexanilide

$$CH_3(CH_2)_3-C(=NOC_2H_5)-CO-NH-C_6H_5$$

To 4.2 g. of α-ethoximinohexanoyl chloride, dissolved in 25 cc. of dry benzene, was added 4.2 g. (4.1 cc.) of aniline dissolved in 15 cc. of dry benzene. The aniline hydrochloride, which formed as a white precipitate, was filtered after the mixture had stood for half an hour. The solvent was removed under diminished pressure, and the residue distilled under 1 to 3 mm. pressure. The product distilling at 133-138° was collected. A yield of 4.3 g.,or 77 per cent of the theoretical α-ethoximinohexanilide, was obtained as a slightly yellow somewhat viscous liquid, which failed to crystallize on prolonged standing at room temperature.

Nitrogen (Kjeldahl)

Calc. for $C_{14}H_{19}O_2N_2$: 11.31% Found: 11.18%
11.14%

B. α-Benzyloximino Anilides

(1) β-Phenyl- α-benzyloximinopropionanilide

$$C_6H_5-CH_2-C(=N-O-CH_2-C_6H_5)-CO-NH-C_6H_5$$

To 1.1 g. of β-phenyl- α-benzyloximinopropionyl chloride dissolved in 25 cc. of absolute ethyl ether was added 0.8 g. of aniline dissolved in 10 cc. of absolute ethyl ether. The mixture was allowed to stand for one hour and the precipitated aniline hydrochloride filtered. The solvent was removed under diminished pressure; the residue was purified by dissolving in warm 95 per cent ethyl alcohol and precipitating by the addition of water. A yield of 1.2 g. or 90 per cent of the theoretical β-phenyl- α-benzyloximinopropionanilide was obtained as a colorless crystalline substance which melted at 73.5-74.0°.

Nitrogen (Kjeldahl)

Calc. for $C_{22}H_{20}O_2N_2$: 8.14% Found: 8.03%
8.01%

5. Hydrogenolysis of the Substituted α-Oximino Acids

Jones and Major (88a) made studies of the catalytic reduction

of aryl oximino groups in 1930. Later Adkins and Reeve (3) reduced an ethoximino group in their synthesis of threonine. These investigators employed what may be considered drastic conditions for the reduction of the alkoximino group; Adkins and Reeve used a pressure of 300 atmospheres and a temperature of 90°. It was hoped that it would be possible to reduce the alkoximino acids catalytically without resorting to high pressures and elevated temperatures. Attempts have been made to effect this reduction at room temperature and at a low pressure using several different catalysts. These experiments have not been crowned with complete success; however, reduction has taken place to give small amounts of the corresponding amino acid. It appears that benzyloximino acids are more easily reducible since a yield of 50% has been obtained. These hydrogenation experiments will be briefly described:

A. Reduction of β-Phenyl-α-ethoximinopropionic Acid

(1) Using Palladium as Catalyst

To 5.0 g. of β-phenyl-α-ethoximinopropionic acid 100 cc. of 95 per cent ethyl alcohol, 5 cc. of hydrochloric acid and 3 g. of prepared palladium on charcoal catalyst (78) were added. This mixture was shaken with hydrogen at a pressure of 2 atmospheres at room temperature for 3 hours. About 0.4 g. of phenylalanine was obtained along with 3.8 g. of the original acid.

(2) Using Raney's Nickel

To 5.0 g. of β-phenyl- α-ethoximinopropionic acid was added sodium hydroxide solution (the calculated amount to make the sodium salt), 0.2 g. of prepared Raney's nickel catalyst and 100 cc. of distilled water. This mixture was shaken with hydrogen at a pressure of 2 atmospheres at room temperature for three hours. About 0.6 g. of phenylalanine was obtained; no original acid was recovered.

B. Reduction of β-Phenyl- α -ethoximinopropionanilide

(1) Using Palladium at Two Atmospheres Pressure

To 5.0 g. of β-phenyl- α -ethoximinopropionanilide 95 cc. of 95 per cent ethyl alcohol, 5 cc. of hydrochloric acid, and 3.3 g. of prepared palladium charcoal catalyst (78) were added. This mixture was shaken with hydrogen at 2 atmospheres pressure at room temperature for four hours. There was no noticeable adsorption of hydrogen. Accordingly, the mixture was transferred to the high pressure hydrogenator.

(2) Using Palladium at Seventy-five Atmospheres Pressure

The mixture which had been subjected to hydrogenation at two atmospheres for four hours was transferred to the high pressure hydrogenator and shaken with hydrogen at a pressure of 75 atmospheres at room temperature for 16 hours. After this treatment, 3.6 g. of the original anilide was isolated.

C. Reduction of α-Ethoximinohexanoic Acid

(1) Using Adams' Catalyst in Methyl Alcohol

To 3.5 g. of α-ethoximinohexanoic acid, 100 cc. of methyl alcohol and 0.2 g. of Adams' platinic oxide catalyst were added. This mixture was shaken with hydrogen at a pressure of two stmospheres at room temperature for four hours. At the end of this time about 0.4 g. of isoleucine was obtained along with 2.3 g. of the original acid.

(2) Using Adams' Catalyst in Ethyl Alcohol

To 3.5 g. of α-ethoximinohexanoic acid, 100 cc. of ethyl alcohol and 0.2 g. of Adams' platinic oxide catalyst were added. This mixture was shaken with hydrogen at a pressure of two atmospheres at room temperature for four hours. At the end of this time about 0.2 g. of isoleucine was obtained along with 2.0 g. of the original acid.

D. Reduction of β-Phenyl- α-benzyloximinopropionic Acid

(1) Using Palladium Catalyst in Ethyl Alcohol

To 5.0 g. of β-phenyl- α-benzyloximinopripionic acid 95 cc. of 95 per cent ethyl alcohol, 5 cc. of hydrochloric acid and 3.3 g. of prepared palladium charcoal catalyst (78) were added. This mixture was shaken with hydrogen at two atmospheres at room temperature for five hours. About 0.6 g. of phenylalanine was obtained along with 2.5 g. of the original acid.

(2) Using Palladium Catalyst in Glacial Acetic Acid

To 4.0 g. of β-phenyl-α-benzyloximinopropionic acid 100 cc. of glacial acetic acid and 3.3 g. of prepared palladium charcoal catalyst (78) were added. This mixture was shaken with hydrogen at two atmospheres at room temperature for six hours. The glacial acetic acid was removed under diminished pressure; the last traces being removed in a vacuum desiccator containing soda lime. The residue which remained weighed 2.5 g.; from the portion which was insoluble in absolute ether 1.2 g. of phenylalanine was obtained, representing a yield of 50 per cent of the theoretical.

From the experiments described above, it appears that the hydrogenation of benzyl substituted oximino acids may be accomplished using glacial acetic acid as the solvent. Time has not permitted a study to ascertain the conditions which may prove more favorable for the reduction. The hydrogenations performed, which may justly be considered as preliminary experiments, have indicated that the benzyl substituted compound may be reduced in yields of at least 50 per cent.

SUMMARY

I. A comprehensive literature survey of the α-ketoacids has been made.

II. The catalytic hydrogenation of the oxime of phenylpyruvic acid has been further studied; the hypothetical intermediate imine is reduced more rapidly than the oxime. Phenylalanine acts anti-catalytically in the reduction of the oxime of phenylpyruvic acid.

III. A method for the alkylation of the α-oximino acids has been devised and employed successfully.

IV. It has been shown that if the oximino group of the α-oximino acid is protected by alkylation, the corresponding acid chlorides may be prepared in good yields.

V. The acid chlorides of the α-alkoximino acids have been shown to react as characteristic acid chlorides.

VI. In a study of the catalytic hydrogenation of the alkoximino acids at room temperatures and at low pressures, it has been observed that hydrogenation takes place very slowly when alcohol is used as the solvent. However, if glacial acetic acid is substituted for alcohol as solvent, a yield of at least 50 per cent of phenylalanine can be obtained from the corresponding α-benzyloximino acid.

VII. Table IV summarizes the new compounds prepared.

TABLE IV

NEW COMPOUNDS PREPARED

Name	Formula	Melting Point or Distillation Range	
α-Ethoximino-butyric Acid	$CH_3CH_2C(=NOC_2H_5)-COOH$	m. 61-61.5	b. 89-95° 12-14
α-Ethoximino-hexanoic acid	$CH_3(CH_2)_3C(=NOC_2H_5)-COOH$	m. 23°	b. 83-88° 1-3
β-Phenyl- α-ethoximino propionic acid	$C_6H_5CH_2C(=NOC_2H_5)-COOH$	m. 58.5-59	b. 115-120° 1-2
α-Benzyloximino-hexanoic acid	$CH_3(CH_2)_3C(=NOCH_2C_6H_5)-COOH$	m.61.0-61.5°	
β-Phenyl- α-benzyl-oximino propionic acid	$C_6H_5CH_2C(=NOCH_2C_6H_5)-COOH$	m. 79.0-80°	
α-Ethoximinobutyryl-chloride	$CH_3CH_2C(=NOC_2H_5)-CO-Cl$		b. 50-55° 15-18
α-Ethoximinohexanoyl-chloride	$CH_3(CH_2)_3C(=NOC_2H_5)-CO-Cl$		b. 58-63° 1-3
β-Phenyl- α-ethoximino propionylchloride	$C_6H_5CH_2C(=NOC_2H_5)-CO-Cl$		b. 95-97° 1-2

TABLE IV (continued)

Name	Formula	Melting Point or Distillation Range
β-Phenyl- α-benzyloximino-propionyl chloride	$C_6H_5CH_2-C(=NOCH_2C_6H_5)-CO-Cl$	b. 170-175° 1-2
α-Ethoximinohexanilide	$CH_3(CH_2)_3C(=NOC_2H_5)-CO-NHC_6H_5$	b. 133-138° 1-3
β-Phenyl- α-ethoximino-propionanilide	$C_6H_5-CH_2-C(=NOC_2H_5)-CO-NHC_6H_5$	
β-Phenyl- α-benzyloximino-propionanilide	$C_6H_5CH_2-C(=NOCH_2C_6H_5)-CO-NH-C_6H_5$	m. 73.5-74.0°
Ethyl α-ethoximino-hexanoate	$CH_3(CH_2)_3-C(=NOC_2H_5)-COOC_2H_5$	b. 60-65° 1-3
Ethyl β-phenyl- α-ethoximino propionate	$C_6H_5CH_2-C(=NOC_2H_5)-COOC_2H_5$	b. 118-120° 1-3

CONCLUSIONS

A method for the preparation of the α-alkoximino acids has been presented which is believed to be applicable to the preparation of any such acid. The method of isolation of the α-ethoximino acids has been well established; they may be conveniently separated by distillation. It should prove possible to increase the yields of the α-benzyloximino acids reported in the present work; distillation of these acids was not attempted. It is probable that the excess of alkylating agent, particularly of benzyl chloride, is a hindrance to obtaining high yields.

The preparation of the acid chlorides of the α-alkoximino acids appears to be a relatively simple matter; good yields of the α-ethoximino chlorides were isolated, and practically quantitative yields were obtained in the preparation of the α-benzyloximino chloride reported. It is thought that any α-alkoximino chloride may be prepared by using thionyl chloride in a low boiling, inert solvent.

The reaction of aniline with the chlorides of the α-alkoximino acids gives anilides in excellent yields. It is reasonable to assume that the reaction of the acid chloride with an amino acid will take place giving a dipdptide linkage.

Experiments on the hydrogenation of the α-alkoximino acids, which must be considered as trial runs rather than as a comprehensive investigation, have indicated that the acids may be reduced under mild conditions. To find the optimum conditions for the reduction of these acids, or of their derivatives, it will be necessary to conduct a routine investigation of the effect of varying the catalyst and the solvent.

The next phase of this problem should logically be the coupling of the acid chlorides with amino acids. If subsequent study of the reduction of these coupled compounds should prove fruitful, the possibility of preparing peptides in which there can be no doubt regarding the manner of linkage or sequence of the components would indeed appear encouraging.

BIBLIOGRAPHY

1. Abderhalden and Rossner, Z. physiol. Chem. 163, 149, 261 (1927).

1a. Adams and Kamm, "Org. Syntheses" Col. vol. I, John Wiley and Sons, New York (1941) p. 250.

2. Adickes and Andresen, Ann., 555, 41 (1943).

3. Adkins and Reeve, J.Am.Chem.Soc. 60, 1328 (1938).

4. Anschütz and Rauff, Ann. 327, 201 (1903).

5. Aristow and Demjamow, Ber. ref. 20, 697 (1887); J.Russ, Phys.Chem.Soc. 1, 257 (1887).

6. Asano and Yamaguti, J.Pharm.Soc. Japan 60, 585 (1940)

7. Barkdall and Ross, J.Am.Chem.Soc. 66, 951 (1944).

8. Barre, Compt. rend. 184, 825 (1927); Ann.Chim. (10) 9, 231,204 (1928).

9. Barry, U. of Md., Doctors Diss. (1943).

10. Bauguess and Berg, J. Biol. Chem. 104, 675 (1934).

11. Berg, Rose, and Marvel, J. Biol.Chem. 85, 219 (1929).

12. Bergmann and Delis, Ann. 458, 76 (1927).

13. Bergmann and Fruton, "Advances in Enzymol", Nord and Werkmann, 1, 63 (1941).

14. Bergmann and Grafe, Z. physiol.chem. 187, 187 (1930).

15. Bergmann, Stern, and Witte, Ann. 449, 277 (1926).

16. Bergmann, Zervas, and Schleich, Ber. 65, 1747 (1932).

17. Bergmann and Zervas, Ber. 65, 1192 (1932).

18. Bertho and Maier, Ann. 498, 50 (1932).

19. Berzelius, Ann. Physik. 36, 1 (1835).

20. Bistrzycki and Mauron, Ber. 43, 2883 (1910).

21. Blaise, Compt.rend. 157, 1440 (1913); Bull.soc.chim. 19, 10 (1916).

22. Blaise and Gault, Compt.rend. 147, 198 (1908).

23. Boeseken, Rec.Trav.Chim. 30, 142 (1911).

24. Boeseken and Van Tonningen, Rec.Trav.chim. 39, 187 (1920).

25. Borchers, Berg, and Whitmann, J.Biol.Chem. 145, 657 (1942).

26. Botvinnik, Prokofeu, and Zelinski, Compt.rend.acad.Sci. U.S.S.R., 30, 129 (1941).

27. Bougault, J.pharm.chim. (7) 10, 297 (1914).

28. Bougault, Bull.soc.chem. (4), 21, 246 (1917).

29. Bougault, Compt.rend. 158, 1424 (1914).

30. Bougault, Compt.rend. 163, 363 (1916).

31. Bouveault and Locquin, Compt.rend. 135, 179 (1902).

32. Bouveault and Locquin, Compt.rend. 141, 115 (1905).

33. Bouveault and Locquin, Bull.soc.chim. iii, 31, 1142 (1904).

34. Bouveault and Wahl, Compt.rend. 132, 416 (1901); Bull.soc.chim.iii, 25, 1031 (1901).

35. Brunner, Monats. 15, 751 (1894).

36. Cahill and Rudolph, J.Biol.Chem. 145, 201 (1942).

37. Canzanelli, Guild, and Harington, Biochem.J., 29, 1617 (1935).

38. Carlinfanti, Gazz.chim.Ital. 29 I, 271 (1899).

39. Carson, Dodge, Harris, and Hazen, "Org. Synthesis" Col.vol. I, John Wiley and Sons, N.Y. (1941), p. 241.

40. Carter, J.Am.Chem.Soc. 50, 2299 (1928).

41. Claisen and Moritz, Ber. 13, 2121 (1880).

42. Curtius, J.prakt.Chem. 26, 175 (1882).

43. Dannenberg and Fittig, Ann. 331, 123 (1904).

44. Darmstaedter and Lifschutz, Ber. 29, 2890 (1896).

45. Darzens, Compt.rend. 152, 443 (1911).

46. Darzens and Levy, Compt.rend. 204, 272 (1937).

47. Dieckmann, Ber. 43, 1032 (1910).

48. Ellinger and Matsuoka, Z.physiol.Chem. 109, 259 (1920).

49. Erlenmeyer, Jr,Ber. 35, 2483 (1902).

50. Erlenmeyer, Jr., Ber. 33, 3001 (1900).

51. Erlenmeyer, Jr., Ann. 333, 160 (1904).

52. Erlenmeyer, Jr., and Arbenz, Ann. 333, 228 (1904).

53. Erlenmeyer, Jr., and Arbenz, Ann. 337, 302 (1904).

54. Erlenmeyer, Jr., and Frustück, Ann. 284, 48 (1895).

55. Erlenmeyer, Jr., and Stadlin, Ann. 337, 283 (1904).

56. Fenton and Jones, J.Chem.Soc. 77, 77 (1900).

57. Fichter and Steinbuch, Helv.Chim.Acta. 26, 695 (1943).

58. Fichter and Willmann, Ber. 37, 2384 (1904).

59. Fischer and Baer, Ber. 65, 337 (1932).

60. Fischer and Fourneau, Ber. 34, 2868 (1901).

61. Fischer and Otto, Ber. 36, 2106, 2982 (1903).

62. Fittig and Dannenberg, Ann. 331, 123 (1904).

63. Fittig and Kaehlbrandt, Ann. 305, 52 (1899).

64. Flatow, Z.physiol.Chem. 64, 367 (1910).

64a. Ford, Thompson and Marvel, J.Am.Chem.Soc. 57, 2619 (1935).

65. Forster and Muller, J.Chem.Soc. 97, 126 (1910).

66. Freudenberg, Dürr, and Hochstetter, Ber. 61, 1735 (1928).

67. Freylon, Ann.chim. and phys. (8), 20, 101 (1910).

68. Gault and Weick, Bull.soc.chim. iv 31, 867 (1922).

69. Glucksman, Monats, 10, 771 (1889).

70. Goldschmidt and Strauss, Ann. 471, 1, (1929).

71. Greenstein, J.Biol.Chem. 118, 321 (1937); 121, 9, (1937).

72. Groanacher, Gero, and Schelling, Helv.Chim.Acta. 7, 575 (1924).

73. Hahn and Hansel, Ber. 71B, 2163 (1938).

74. Hahn and Rumpf, Ber. 71B, 2141 (1938).

75. Hahn and Stiehl, Ber. 69B, 2627 (1936).

76. Hahn and Werner, Ann. 520, 123 (1935).

77. Hall, Hynes, and Lapworth, J.Chem.Soc. 107, 132 (1915).

77a. Hamlin, University of Maryland, Doctors Diss. (1941).

78. Hamlin and Hartung, J.Biol.Chem. 145, 349 (1942).

79. Hartung, J.Am.Chem.Soc. 50, 3370 (1928).

80. Hemmerle, Ann.chim. (9) 7, 226 (1917).

81. Herbst and Engel, J.Biol.Chem. 107, 505 (1934).

82. Herbst and Shemin, "Organic Synthesis" Col.Vol. 2, John Wiley and Co. New York, (1943), p. 519.

83. Hida, J.Shanghai Sci. Inst. IV, 1, 201 (1935).

84. Hoff-Jorgensen, Zphysiol.chem. 265, 77 (1940).

85. Howard and Fraser, "Organic Synthesis" Col. Vol. I. John Wiley and Sons New York, 1941, p. 475.

86. Hubner, Ann. 120, 330 (1861).

87. Jackson, J.Biol.Chem. 84, 1 (1929).

88. Janny, Ber. 16, 170 (1883).

88a. Jones and Major, J.Am.Chem.Soc. 52, 669 (1930).

89. Karrer, "Organic Chemistry", Nordemann Publishing Co., Inc., New York, (1938), p. 240.

90. Karrer and Hoffman, Helv.Chem.Acta. 22, 654 (1939).

91. Keagle, University of Maryland Thesis (1944).

92. Knoop and Oesterlin, Z. Phys.Chem. 148, 294 (1925).

93. Kohn, Monats. 19, 522 (1898).

94. Kohn and Franke, Monats. 20, 288 (1899).

95. Kondo, Biochem. Z. 38, 407 (1912).

96. Krebs, Enzymologia 7, 53 (1939).

97. Krebs, Z.Phys.Chem. 217, 191 (1933).

98. Krestinsky, Ber. 55, 2762 (1922).

99. Kuwata, J.Am.Chem.Soc. 60, 559 (1938).

100. Lapworth, J.Chem.Soc. 91, 1133 (1907).

101. Locquin, Bull.soc.chim. iii, 35, 962 (1906).

102. Locquin, Bull.soc.chim. iii, 31, 1147 (1904).

103. Lowry, Browning, and Farmery, J.Chem.Soc. 117, 552 (1920).

103a. Marvel and Hager, "Organic Synthesis" Col.Vol. 1, John Wiley & Company, New York, 1941, p. 248.

104. Mebus, Monat. 26, 483 (1905).

105. Moritz, J.Chem.Soc. 39, 13 (1881).

106. Neubauer, Zentrablatt 1909 II, p. 50.

107. Neuberger and Sanger, Biochem.J. 38, 119 (1944).

108. Novello, Harrow and Sherwin, J.Pharmacol. 27 Proc. 242 (1926).

109. Parrod, Compt.rend. 215,146 (1942).

110. Perkins and Simonsen, Proc.Chem.Soc. 25, 165 (1909).

111. Plattner, Monats. 36, 899 (1915).

112. Plöchl, Ber. 16, 2815 (1883).

113. Plöchl and Wolfrum , Ber. 18, 1183 (1885).

114. Przewalsky, J.prakt.Chem. 88, 495 (1913).

115. Ramart-Lucas, Compt.rend. 154, 1617 (1912).

116. Rambaud, Bull.soc.chim. (V) 1, 1342 (1934).

117. Rassow and Bauer, J.prakt.Chem. 80, 97 (1909).

118. Richard, Ann.chem.phys. (8) 21, 360 (1910).

119. Richter, "Organic Chem." Vol. 1. Nordemann Publishing Company, New York (1934), p. 462.

120. Ruhemann and Stapleton, J.Chem.Soc. 77, 239 (1900).

121. Shemin and Herbst, J.Am.Chem.Soc. 60, 1951 (1938).

122. Smedley, MacLean, and Pearce, Biochem.J. 28, 486 (1934).

123. Sorenson, Katschioni-Walther, Compt.rend.trav.lab.Carlsberg, 17, 1 (1928).

124. Sumerford and Dalton, J.Am.Chem.Soc. 66, 1330 (1944).

125. Tipson and Levene, J.Biol.Chem. 129, 575 (1939).

126. Tistchenko, Bull.Soc.Chim. iv, 37, 623 (1925).

127. Tschelinzeff and Schmidt, Ber. 62, 2210 (1929).

128. Van der Sleen, Rec.Trav.Chim. 21, 233 (1902).

129. Waelsch and Borek, J.Am.Chem.Soc. 61, 2252 (1939).

129a. Walden, Z.physik.Chem. 59, 384 (1907).

130. Waldschmidt-Leitz and Künster, Z.Physiol.Chem. 171, 70 (1927).

131. Wallach, Ann. 346, 220 (1906).

132. Westerkamp, Biol.Chem.Zeit. 263, 239 (1933).

133. Wichelhaus, Ann. 143, 1 (1867).

134. Windaus and van Schoor, Z.Physiol.Chem. 161, 143 (1926).

135. Wislicenus, Ann. 246, 306 (1888).

136. Wislicenus, Ber. 20, 589 (1887).

137. Wislicenus, and Silberstein, Ber. 43, 1825 (1910).

138. Wolffenstein, Ber. 26, 2991(1893).