WILCOX

Synthesis of Amido-Acids

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THE SYNTHESIS OF AMIDO-ACIDS

...BY...

Burton B Wilcox

THESIS

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Burton B. Wilcox (Under Dr. W. M. Dehn).

ENTITLED The Synthesis of Amido-acids.

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE

OF Bachelor of Arts in Chemistry

H. S. Grindley.

HEAD OF DEPARTMENT OF

The Synthesis of Amido-Acids.

The amido-acids may be considered to be derived from the oxy-acids by the replacement of the hydroxyl groups, -OH, by the amido group, $-NH_2$, thus, glycollic acid, $CH_2(OH)\ CO_2H$, becomes, by such replacement, glycocoll or amido-acetic acid, $CH_2(NH_2)\ CO_2H$. The simpler view is that the amido-acids are acids in which a hydrogen atom other other than the acid hydrogen is replaced by the amido group. In this light, propionic acid, $CH_3CH_2CO_2H$, becomes alphaamido propionic acid, $CH_3CH(NH_2)\ CO_2H$.

The structure of the amido-acids is still an open question in respect to the inter-relation of the amido-acid carboxyl groups. It is generally conceeded, however, that in most acids, that the amido group is in union with the carboxyl. So in the common texts the structural formulas are written in one of two ways or in both. Thus alpha-amido propionic acid is indicated by both of the formulas, $CH_3-CH(NH_2)\ CO_2H$ and $CH_3-CH(\overline{NH_3})-COO$. In the latter formula, the amido nitrogen is united to the oxygen of the hydroxyl of the acid, the hydrogen of which has been transfered to the nitrogen. This assumption necessitates a penta-valent nitrogen atom, but at least explains, and indeed owes its existence, to the fact that the amido-acids are neutral in reaction. Otherwise there is nothing peculiar in the structure of the amido-acid not existing in the acids themselves.

Among the organic compounds the amido-acids form a distinct class, as regarding both properties and occurence. They constitute almost all of the hydrolitic decomposition products of the proteids, thus furnishing a means of study which may lead to the solution of that vast field of chemical compounds-the proteids. Not only do these amido-acids come into existence as the products of the splitting of the proteid substances in the laboratory, but they occur widely distributed in nature as the result of metabolism in the bodies of plants and animals. Betaine, trimethyl-glycocoll, is found already formed in the sugar beet, Beta vulgaris, in the leaves and stalks of Lycium barbarum, in cotton-seed, in malt, and in wheat sprouts. Taurine, amido-ethyl-sulphonic acid, occurs in ox bile in combination with colic acid and also in different animal secretions. Creatine, methyl-guanido-acetic acid, was discovered by Chevreul as early as 1834 in meat extract. It occurs chiefly in the juices of the muscle. Leavo-aspartic acid, is found free in vinasse obtained from beet root, and its half-amid, asparagine is found in many plants especially in the seeds, in asparagus, Asparagus officiallis, in beet root, in peas, in beans and in vetch roots from which it is obtained on a large scale. Occuring with asparagine in vetch roots and molasses, is glutaminic acid, amido-glutaric acid. Of the ordinary fatty acids glycocoll and leucine occur free. Glycocoll was found by Chittenden in Pecten irradians. Leucine

alpha-amido-caproic acid, is found in many different animal fluids where its presence is of physiological importance. It is formed in the pancreas, in the spleen, in the lymph glands, and in typhoid, is found in the liver. It is formed by the decay of albuminoids, and fibrin is converted into it by pancreatic digestion.

It has been observed by a large number of investigators that amido-acids constitute a large part of the decomposition products of proteids. In 1820 Braconnot obtained glycocoll by the action of boiling sulphuric acid upon glue. Since that time the hydrolysis of proteids has offered a tempting field of research for chemists interested in the chemistry of life. The principle substance bringing about hydrolytic splitting are boiling alkalies and acids, super-heated steam, fused alkalies, ferments and bacteria. The products of decomposition of the proteids by concentrated hydrochloric acid are the following- glycocoll, leucine, aspartic acid, glutaminic acid, diamido-acetic acid, lysine, arginine, histidine $C_6H_9N_3O_2$ of unknown composition, phenylalanine, tyrosine, para-oxy-phenyl-amido-propionic acid, cystin the disulphid of amido-lactic acid and many other substances which are not amido-acids. The treatment with concentrated alkalies gives a slightly different list of substances; leucine, amido-valeric acid, amido-butyric acid, amido-propionic acid, aspartic acid, glutaminic acid and tyrosine. In the splitting of the various proteids we have essentially the same

compounds formed, and it is now the aim of the chemists who are doing research in the proteid chemisrty to learn something of the combination and relative postion of these amido-acids in the proteid molecule. It is only recently that anything whatsoever has been known in regard to this matter and it is due to the work of Emil Fisher that we have our present knowledge, and he beleives that they are combined in the molecule after the type of acido-amides. Fisher succeeded in condensing in this manner various amido-acids, and designates them as peptides. They resemble in a degree the true peptones and he has obtained similar bodies by the partial hydrolysis of proteids. In the Berichte 36, 2982, 1903, Fisher describes his synthesis of the polypeptides. Such dipeptides as glycylglycine and leucylleucine had previously been formed and it was his object to form higher polypeptides from these bodies. Chloracetylglycylglycine by the action of alkalies yields the acid, Cl $CH_2CO\cdot NHCH_2CO\ NHCH_2CO$-OH, which on warming with strong ammonia gives diglyclglycine having a structural formula NH $CH_2CO\cdot NHCH_2CO\cdot NHCH_2CO$ OH, the first representative of the class of tripeptides.- Other polypeptides formed were alanyl-glycylglycine $CH_3CH(NH_2)\cdot CO\cdot NHCH_2CO\ NHCH_2$-CO OH and by the action of phenyl-isocyanate on leucylglycylglycine he obtained phenyl-carbamido-leucylglycylglycine, C_6H_5-NH CO NH $CH(C_4H_9)$ CO $NHCH_2CO\ NHCH_2COOH$. These formulas verify the assumption of Schiff, which he derived from the biuret-

reaction, that the proteid molecule has two $CONH_2$-groups attached to a single carbon or nitrogen atom.

Properties of the Amido-Acids.

The amido-acids are crystalline bodies with usually a sweet taste and are readily soluble in water. In general they are insoluble in alcohol and ether. Their esters having comparatively low boiling points, furnishes a means for their separation.

The amido-acids form metallic salts with the metallic oxides which are often characteristic and serve as a means for the isolation and identification of the acids. Thus, lysatinine gives a salt $AgNO_3 + C_6H_{13}N_3O_2HNO_3$ and lysine the salt $PtCl_4C_6H_{14}N_2O_2 2HCl + C_2H_5OH$. The carboxylic hydrogen is replaceable by alcoholic radicals with the formation of esters which are unstable. However, the hydrogen of the NH_2 group is replaceable by both acid and alcoholic groups forming stable bodies. The acid derivatives are formed by the action of acid chlorides;

$NH_2OH_2CO_2H + C_2H_3O\,Cl = C_2H_3O \cdot NH \cdot CH_2CO_2H + HCl$ and the alcohol derivatives are obtained by the action of amines in saturated fatty acids;

$$Cl\text{-}CH_2\text{-}CO_2H + NH(CH_3)_2 = N(CH_3)_2CH_2CO_2H + HCl$$

Dimethyl glycocoll.

The alkylation process may be continued so far as to split off the amido group entirely, forming unsaturated acids. Thus

alpha-amido-propionic acid yields acrylic acid and alpha-amido butyric acid yields crotonic acid.

The amido-acids may be changed to fatty acids by heating with hydroiodic acid at 200° C.

The amido group is very stable and is not displaced by boiling alkalies, but when fused with alkalies they are converted into salts of the fatty acids, amines, and ammonia. By dry distillation, with baryta they yield amines and Co_2, thus lysine was found to yield cadaverine and carbonic oxide, a fact which established its constitutions.

$$NH_2CH_2CH_2CH_2CH_2CH(NH_2)CO_2H = NH_2CH_2CH_2CH_2CH_2CH_2NH_2 + CO_2$$

lysine cadaverine

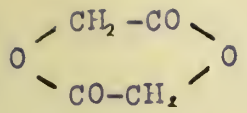
Nitrous acid by its regular action on amines converts the amido-acids into oxy-acids;

$$NH_2CH_2CO_2H + NO_2H = CH_2(OH)CO_2H + N_2 + H_2O$$

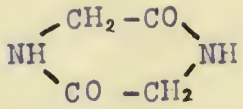
The esters of the diazo-acids result when potassium nitrate acts upon the hydrochlorides of the amido fatty acid esters.

$$HCl(NH_2)CH_2CO_2C_2H_5 + NO_2K = N_2\ CH\ CO_2C_2H_5 + KCl + H_2O.$$

This reaction serves as a test for minute quantities of the amido-acids. One of the chief characteristics of the amido-acids is the formation of cyclic anhydrides which are similar to the corresponding bodies of certain aliphatic oxy-acids. The alpha-amido acids condense to form double acid amides, similar to the lactides.



GLYCOLLIDE



GLYCOCOLL ANHYDRIDE

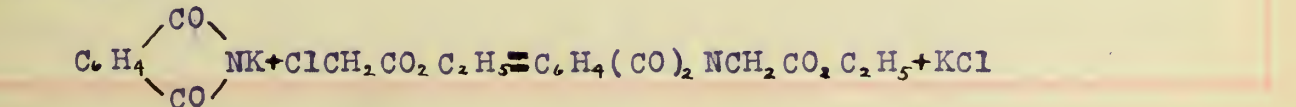
A characteristic property of the gamma; and delta-amido acids is their tendency, when heated, to split off water and form within their own molecule simple acid amides, or lactams. These bodie correspond to the lactams, and as the lactones with caustic alkalies yield oxy-acids, so the lactams on digesting with alkalies and acids yield salts of the amide-acids. The amido-acids themselves are not poisonous, but their lactams are violent strychnine-like poisons.

General Methods of Synthesis of Amido Acids

There are certain general methods which serve to form most of the amido-acids. These methods of synthesis are as follows:-

1. The esters of halogen acids on heating with ammonia yield amido-acids esters by the replacement of the halogen atoms by the group NH_2. The free halogen-substituted acids are apt to form imido-acids on treatment with ammonia and aretherefore little used.

2. The above reaction is aided by the use of potassium phthalimide. The halogen acid estersare made to react with that substance, after wich the amido-acid is split off by hydrochloric acid at 200° C:-



and $C_6H_4(CO)_2NCH_2COC_2H_5+HCl+2H_2O=HCl\cdot NH_2-CH_2CO_2C_2H_5+C_6H_4(CO_2H)_2$

3. Nitro and iso-nitroso acids on reduction by nascent hydrogen yield amido-acids:

$CH_2(NO_2)\ CO_2C_2H_5+HH=CH_2NH_2CO_2H+H_2O+C_2H_5OH$ and $CH_3-C(=NOH)\ CO_2H+4H=CH_3-CN(NH_2)-CO_2H$

4. The cyanogen group of cyan-acids reduces to an amido group by means of nascent hydrogen, evolved from zinc and hydro-chloric acid or formed on heating with hydroiodic acid.

$CN-CO_2H+4H=CH_2(NH_2)\ CO_2H$

This reaction furnishes an acid having one more carbon atom in the chain than the original acid.

5. Aldehydes and ketones add hydrocyanic acid directly forming alpha-oxy compounds, the hydroxyl group of which is easily replaced by ammonia in alcoholic solution. The amido nitrile thus formed yields on saponification with acids the amido-acid.

$CH_3-CHO+HCN=CH_3CH(CN)OH+NH_3=CH_3(CN)NH_2+H_2O$

$CH_3(CN)NH_2+HCl=CH_3(NH_2)CO_2H+NH_4Cl$

A similar reaction is the formation of the cyan-amide from aldehydes and ammonium cyanide, and the subsequent saponification of the nitrile by acids.

$CH_3CHO+NH_4CN=CH_3CH(CN)NH_2$

CH CH(CN)NH H O CH CH(NH)CO H NH

The preceeding method serves only for the formation of alpha-amido acids. Other methods which are used for the synthesis of amido-

acids having the amido group further removed from the carboxyl, that is, the beta, gamma, delta, epsilon acids are as follows:-

6. Even as the bases $C_nH_{2n+3}N$ are prepared by the reduction of the phenyl-hydrazine derivatives of the ketones and aldehydes, so by the reduction of the phenyl-hydrazine derivatives of the ketonic acids by sodium amalgam and acetic acid in the cold, the amido-acids are formed.

$$CH_3\ C(N_2H\ C_6H_5)CH_2CH_2CO_2H+H_2=CH_3-CH(NH_2)-CH_2-CH_2CO_2H+C_6H_5NH_2$$

7. Amido-acids are formed by heating the imides of the di-basic acids with KBrO and potassium hydroxide at 50°.-

$$\begin{array}{l} CH_2-CO \\ \quad\quad\ NH+KBrO+3KOH= \\ CH_2-CO \end{array}\quad \begin{array}{l} CH_2-CO\ NBrK \\ \quad\quad\quad\quad\quad\quad +2KOH+H_2O=NH_2CH_2\ CH_2\ CO_2H \\ CH_2-CO_2K \end{array}$$

$$+K_2CO_3+KBr+H_2O$$

8. Ammonia acts at the double bond of unsaturated acids forming saturated amido acids.

9. Amido ketones or oxidation yield amido-acids, as diacetone-amine oxidized by chromic acid mixture, yields amido-iso-butyric $(CH_3)_2C(NH_2)\ CO_2H$, propalanine and amido-iso-valeric acid $(CH_3)_2-C(NH_2)\ CH_2CO_2H$.

10. Cylicimides yield, on oxidation compounds that can be transformed into amido-acids. Thus piperidine yields amido-valeric aldehyde which on further oxidation yields the amido-valeric acid.

Important Amido Acids and their Methods of Synthesis.

I. Amido-acetic acids.

Glycocoll, glycine, amido-acetic acid, was first obtained by Braconnot, 1820, by boiling glue with sulphuric acid. Dessaignes obtained it by boiling hippuric acid with hydrochloric acid and glycocholic acid was converted into it by Strecker. It has since been found to be the decomposition product of almost all proteids.

The first synthetic method(1858), for the preparation of glycocoll is the action of ammonia on brom-acetic acid. This reaction also gives di- and triglycolamidic acid at the same time. It is formed by heating chlor-acetic acid with dry ammonium carbonate, and by running cyanogen gas into boiling hydroiodic acid.

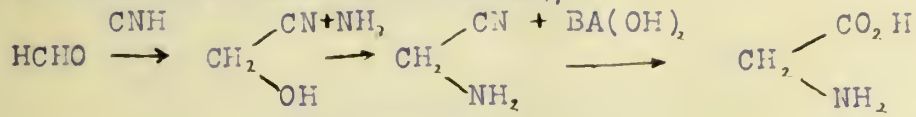
$$CN-CN+5HI+2H_2O=CH_2(NH_2)CO_2H+NH_4I+4I$$

Zinc and hydrochloric acid reduce cyan-formic ester in alcoholic solution, thus:-

$$CN-CO_2C_2H_5+4H+H_2O=CH_2(NH_2)CO_2H+C_2H_5OH$$

It is also prepared by the general methods from; nitro-acetic acid by reduction; methylene amido-acetic nitrile by heating with alcoholic hydrochloric acid, when it changes into the hydrochloride of the glycine ester; and methylene cyanhydrine, the product of the union of formaldehyde and prussic acid upon which ammonia acts to form the glycocoll nitrile and by boiling with

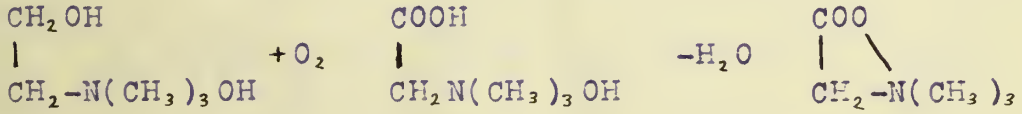
baryta water the base is obtained,



Glycocoll Derivatives.

Methyl-glycocoll, $CH_2(NHCH_3)\,CO_2H$ or $CH_2(COO)-NH_2-CH_3$. Sarcosine, was obtained by Liebig 1847, as a decomposition product of creatine found in beef extract. Volkard, 1862, made it synthetically from chlor-acetic acid and a concentrated water solution of methylamine at 120-130°. Its nitrile is obtained from methylene-cyan-hydrin and methyl amine.

Trimethyl Glycin, Betaine, Lycine $CH_2N(CH_3)_3(COO)$ is formed by the careful oxidation of choline.



It can be obtained by the action of mono-chlor-acetic acid on trimethyl amine, and by heating glycocoll with methyliodide, caustic-potash and methyl alcohol.-

Creatine, methyl guanidine acetic acid, $-NH\,C(NH_2)\,N(CH_3)-CH_2-CO_2H$, was prepared by J. Volkard in 1861, from sarcosine and cyan-amide;

$CN-NH_2+CH_3\,NH-CH_2-CO_2H=NH\,C(NH_2)-N(CH_3)-CH_2CO_2H$, with baryta water it forms urea and sarcosine.

$NH-C(NH_2)\,N(CH_3)-CH_2CO_2H+H_2O=NH_2-CO-NH_2+CH_3-NH-CH_2COOH$.

By loss of water creatine forms creatinine, methyl glycocyamidine, $NH=C(NH-CO)N(CH_3)-CH_2$,

which occurs constantly in the urine.

II. Amido-propionic acids.

Alpha-amido-propionic acid, Alpha Alanine, $CH_3-CH(NH_2)CO_2H$, was prepared by Strecker, in evaporating a mixture of acet-aldehyde ammonia, hydrocyanic acid and hydro-chloric; by Kolbe from alpha-brom- propionic acid and alcoholic ammonia. Alpha-alanine is not a general decomposition product of the proteids, but it has been found in gelatine and Weyl obtained it by boiling fibrin of silk with sulphuric acid.

Beta Alanine, $NH_2-CH_2-CH_2-COOH$, the isomer of alpha-alanine is not found as a decomposition product of proteids. It has been made synthetically by Heintz in heating beta-iodpropionic acid with ammonia; and by Engel by the action of zinc and sulphuric acid on cyan-acetic acid. The ethyl ester is formed by heating for ten hours at 110 -115°, acrylic acid ester with alcoholic ammonia. It is also prepared by heating succinimio at 50 - 60 with KBrO and KOH.

Diamido-propionic acid was prepared by Kolbe by heating di-brom-propionic acid with ammonia.-

Serin and Iso-serin.

Serin discovered by Cramer, 1865, as a product of the hydrolytic splitting of silk glue as the first oxy-amido acid of the aliphatic series, became from both a chemical and a physio -

logical standpoint an object of interest. Serin and its isomer were first prepared by Melikow from alpha-chlor-lactic acid ester, also by Forcher from glycide acid, $CH-CO_2H$ / $O-CH_2$. At this time it was beleived that the amido group of serin was in the alpha-position, which fact was verified in subsequent synthesis by Emil Fisher and Erlenmeyer.

Emil Fisher and Hermann Leuchs made serin from glycol-aldehyde, $CHO-CH_2OH$ obtained after the method of Fenton from dioxy-maleic acid.- Dioxy-maleic acid was heated with water at 60°-70° for 1/2 hr. until the evolution of carbon dioxide ceased, then the solution was evaporated under strongly diminished pressure. The glycol-aldehyde remaining was treated with alcoholic-ammonia and let stand two days, and after treating again with hydrocyanic acid was let stand 24 hrs. To this solution was added hydrochloric acid (1.19) and on cooling with ice was saturated with gaseous hydrochloric acid. After removing the ammonium chloride with lead oxide and the excess of lead with sulphuric acid, the liquor was evaporated and the acid precipitated by means of absolute alclhol. The serin obtained was pure, but gave only a 9% yield.

In view of the poor yield of Fisher, E. Erlenmeyer attempted a synthesis. The method consists in condensing ethyl formate $HCO_2C_2H_5$ and hippuric acid ester $C_6H_5CO\ NH-CH_2CO_2C_2H_5$ in the presence of sodium ethylate. It gave a yield of 60% of the sodium salt $C_6H_5CO-NH-C(=CH-ONa)-CO_2C_2H_5$,

which gives an oil that will not crystallize. Upon reduction in ether solution with aluminium-amalgam it gave the n-benzoly-serin ester in colorless crystals melting 80%.-

I. $HCOOC_2H_5 + NaOC_2H_5 = NaO-HC(OC_2H_5)_2$

II. $NaO-CH(OC_2H_5)_2 + H_2C(NHCOC_6H_5)(COOC_2H_5) = NaO-CH=C(COOC_2H_5)(NHCOC_6H_5) + 2C_2H_5OH$

III. $HO-CH=C(COOC_2H_5)(NHCOC_6H_5) + H_2 = HO-CH_2-CH(COOC_2H_5)(NHCOC_6H_5)$

n-benzoylserinester

Emil Fisher and Leuchs, repeated the synthesis of iso-serin and verified its constitution. They obtained the chlor-acetic acid from epichlorhydrine.

III. Amido-butyric acids.

The alpha and beta-amido-butyric acids are prepared by the general method of heating the alpha-and beta-chlor-butyric acids with ammonia. They are not found as decomposition products of proteids.

Gamma-amido-butyric acid, piperidic acid, $NH_2-CH_2-CH_2-CH_2-CO_2H$, is prepared from piperyl-urethan $C_5H_{10}-N-CO_2-C_2H_5$, by treating with cold fuming nitric acid, whereby the acid ester $C_4H_8O_2-N\ CO_2\ C_2H_5$(?) is formed. By heating this ester with concentrated hydrochloric acid at 100 it breaks down into C_2H_5Cl, CO_2

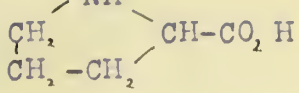
and the gamma-amido-butyric acid. By heating beta-phthalimido-ethyl-malonic-diethyl ester with hydrochloric acid at 170-180° the amido-acid results.

$C_6H_4(CO)_2NH\text{-}CH_2CH_2CH(CO_2C_2H_5) + HCl = HCl\cdot NH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CO_2H$

By heating the gamma-amido-butyric acid, to its melting point (184°)it forms an anhydride, pyrrolidone $\begin{matrix} CH_2\text{-}CH_2 \\ | \quad\quad \backslash \\ CH_2\text{-}CO \quad\quad NH \end{matrix}$, which is the basis of acids formed by the hydrolysis of proteids—pyrrol-idinecarbonic acids.

Emil Fisher in the hydrolysis of gelatine found a small amount of a substance which he considered to be an oxy-pyrrolidine-carbonic acid. In the hydrolysis of casein pyrrolidine-carbonic acid is formed. From the work of Fisher it is almost certain that pyrrolidine-carbonic acid is a primary decomposition product of the proteid molecule. In 1903, Willstaetter and E.H.Linger synthesized alpha-pyrrolidine-carbonic acid. Delta-brom-propyl-malonic-ethyl-ester $CH_2Br\text{-}CH_2\text{-}CH_2CH(CO_2C_2H_5)_2$ is prepared by treating malonic ester with a little more than a molecule of trimethlene bromide, at room temperature, the product is converted into the alpha-delta-dibrom-propyl-malonic-ethyl ester by action of bromine in chloroform solution. By heating this compound with ammonia at 140° the diamid of alpha-pyrrolidine-carbonic acid is formed, $\begin{matrix} NH \quad\quad CONH_2 \\ | \quad\quad\quad | \\ CH_2\text{-}C\text{-}CONH_2 \\ | \quad\quad\quad | \\ CH_2\text{-}CH_2 \end{matrix}$, which on heating with alkalies splits off two molecules of ammonia and forms the alpha-pyrrolidine carbonic acid.

Fisher in his synthesis of alpha-delta-diamido-valeric acid by treating



phthalimido-propyl-brom-valeric-ester with ammonia, formed the alpha-pyrrolidine-carbonic acid.

IV. Amido-valeric Acids.

Alpha-amido-valeric acid, $CH_3-CH_2-CH_2-CH(NH_2)\,CO_2H$, is formed by treating butyric-aldehyde-ammonia with hydrocyanic acid and hydrochloric acid, and by replacing the bromine of alpha-brom-valeric acid by ammonia. Benzoyl-conine yields a benzoyl derivative of that acid when oxidized with potassium permanganate It is a decomposition product of proteids by alkalies.

Gamma-amido-valeric acid, $CH_3-CH(NH_2)-CH_2-CH_2CO_2H$, is formed by reducing action of sodium-amalgam on phenyl-hydrazine-laevulinic acid at temperature below 15°.

$$CH_3\,C(N_2H\,C_6H_5)\,CH_2CH_2CO_2H + 4H = C_6H_5NO_2 + C_6H_5NH_2$$

Delta-amido-valeric acid, $-NH_2-CH_2-CH_2-CH_2-CH_2-CO_2H$, a product of the putrefaction of flesh and fibrin, is formed by heating gamma-phthal-imido-propyl- malonic-ethyl-ester with hydrochloric acid at 180°-190°.

In 1877, Jaffe prepared a base, $C_5H_{12}N_2O_2$ from the ornithine acid extracted from the excrement of fowls fed on benzoic acid. It was found to have two amido groups. In 1901, Ellinger by causing some of it to ferment with the formation of putrescine, proved it to be the alpha-delta-diamido-valeric acid.

$NH_2 CH_2-CH_2-CH_2-CH(NH_2)-COOH = NH_2-CH_2-CH_2-CH_2-NH_2+CO_2$
putrescine

year
The following, 1902, Emil Fisher, synthesized ornithine by brominating phthalimido--propyl-malonic-diethyl ester to the compound $C_6H_4(CO)_2N-(CH_2)_3 \cdot C\ Br \cdot (CO_2C_2H_5)_2$ which on heating in a closed tube with hydrobromic acid splits off one of the carboxyl groups. The bromine is now replaced by heating with ammonia and the phthalic acid split off by hydrochloric acid.

Alpha-amido-gamma-oxy-valeric acid, was prepared by Fisher, 1902, from Aldol, $CH_3-CH(OH)\ CH_2CHO$, by treating with ammonia in the cold, then adding hydrocyanic acid and finally vaporifying out hydrochloric acid, when the oxy-amido acid is obtained. $CH_3-CH(OH)-CH_2-CH(NH_2)\ COOH$.

V. Amido-Caproic Acids.

Alpha-amido-caproic acid, Leucine, $CH_3(CH_2)_3-CH(NH_2)\ CO_2H$, as previously described is an important decomposition product of the proteids. It was made synthetically by Huefner in treating alpha-brom-caproic acid with concentrated ammonia at 120°.

Epsilon

Amido-caproic acid, $NH_2(CH_2)_5-COOH$, has been formed by Gabriel, by heating with hydrobromic acid, (density 1.49) the phthalimido-butyl-malonic ester $C_6H_4(CO)_2N(CH_2)_4-CH(CO_2C_2H_5)$ formed by the union of delta-brom-butyl-phthal-imid, and sodium-malonic ester.

Alpha-epsilon-diamido-caproic acid, Lysine, $NH_2(CH_2)_3CH(NH_2)-CO_2H$.

This base discovered by Drechsel, 1891, has been found by Siegfried, Hidin, Schulze and A. Kossel and his workers in many plant and animal albuminoid substances especially those rich in Histone and Sturine. Henderson investigated these substances in order to prove whether or not the lysines from the different sources were identical. In using lysines prepared by mineral acids from peptone, spoyine, glue and caseine, he obtained salts melting from 192° to 193°. The specific rotation gave numbers from 14.03 to 15.00. The Kjeldahl method gave nitrogen from 9.7% to 10.3%, theory being 12.8%. Volumetric analysis gave nitrogen, 13% to 13.03%. From these data it is concluded that the lysines obtained from the different sources are identical and also the Kjeldahl method is not reliable. Drechsel stated that lysine was alpha-epsilon-diamido-normal-caproic acid. Liebig found that leucine on heating with KOH gave valeric acid, thus;

$$C_6H_{13}NO_2 + 3KOH = C_5H_9O_2K + K_2CO_3 + NH_3 + 2H_2.$$

By analogy one could expect that the diamido-acid would give glutaric acid. He found that heating lysine with KOH in a nickel crucible gave a mixture of acetic and propionic. But later it was found that acid under these same conditions gives the same products as lysine. Emil Fisher completed the establishment of the constitution by decomposing it into cadaverine as ornithine is decomposed into putrescine.

In 1902, Emil Fisher, synthesized lysine by the following method:-

He condensed gamma-chlor-butyric-nitrile with sodium-malonic ester, and obtained the compound, gamma-cyan-propyl-malonic-diethylester, $CN-CH_2-CH_2-CH_2-CH(CO_2C_2H_5)_2$

This compound was transformed into alpha-oximido-delta-cyan-valeric-ethyl-ester by the action of ethy-nitrite, which on reduction with sodium amalgam gave the diamido-caproic acid.

$CN-CH_2-CH_2-CH_2-CH(=N-OH)\ CO_2C_2H_5 + 7H = NH_2-CH_2-CH_2-CH_2-CH_2-CH(NH_2)-$

$-CO_2H + H_2O$

Experimental Part

In the earlier part of the experimental work, it was thought best to repeat one important synthesis before beginning the original research. The synthesis chosen was that of lysine made by Emil Fisher in 1902, since it is quite difficultly prepared and was the most important of the recent synthesis of the amido-acids. Fisher started his synthesis with gamma-chlor-butyro-nitrile, $Cl-CH_2-CH_2-CH_2-CN$,. This substance was not available, so it was necessary to form it from lower compounds. The synthesis was started with glycerine from which was formed allyl alcohol.

1. Allyl Alcohol, $CH_2 = CH-CH_2OH$, was made from glycerine and oxalic acid according to a method given in LeVy-Bistrzycki

Equation:-

$$\begin{array}{l} CH_2OH \\ CHOH \\ CH_2OH \end{array} + (COOH)_2 = \begin{array}{l} CH_2O.CO\ H \\ CHOH \\ | \\ CH_2OH \end{array} + CO_2 + H_2O$$

$$\begin{array}{l} CH_2O\ CO\ H \\ | \\ CHOH \\ | \\ CH_2OH \end{array} = \begin{array}{l} CH_2 \\ \| \\ CH \\ | \\ CH_2OH \end{array} + CO_2 + H_2O$$

Allyl Alcohol

In a tubulated retort 1400 grams of glycerine were mixed with 700 grams of oxalic acid and to this mixture was added 3 grams of salammoniac to aid in the reaction. The retort was connected to a Liebig condenser. An asbestos mat was placed under the retort and heated with a large Bunsen burner. At 130°, CO_2 begins to be evolved energetically and the liquid remains at that temperature for some time. The gas evolution diminishes as the temperature reaches 180°. At 195° the distillate contains a large percentage of formic acid and this fraction up to 215° is collected in separate flask. At 200°-210° the CO_2 evolution begins again, and the second stage of the reaction begins- the formation of allyl alcohol from the mono-formyl-glyceride. About 215° a yellow oily substance condensed which is allyl alcohol. The temperature is kept at 220°-230° as long as possible. When finally the temperature reaches 240°, which is after several hours heating, the distillation was interuppted. The contents of the flask was allowed to cool and 420 grams more of oxalic acid were added. The distillation was repeated as above, only letting the temperature rise finally to 260°. The mixture was again cooled and 140 grams of oxalic acid was added and the temperature run up to 260° again.

The mixture in the retort was much diminished in volume, although the reaction is by no means a quantitative one. The collected distillates above 195° contained besides allyl alcohol, some allyl formate, glycerine, and acrolein . By distilling the mixture all of the allyl alcohol was obtained with a little formic acid and acrolein . The distillation is kept up until a portion on saturation with solid K_2CO_3 gave no oily drops. It began to distill at 80°, most passing over at 150°. The temperature was allowed to rise however to 115-120°. The allyl alcohol was obtained by salting out with solid K_2CO_3 and separating the two layers , water and alcohol, with the separatory funnel. The crude alcohol was treated with 12% of dry KOH and let stand 24hrs. by which means the acrolein was mostly removed. The alcohol became very dark colored. After separating from the alkaline layer at the bottom of the flask the alcohol was distilled first in the water-bath and then with a free flame until the temperature reached110°, it having begun at 89°. The resultant distillate was slightly yellow. It was freed from water by solid K_2CO_3 and redistilled. The distillation began at 90.5° and rose to 110°, remaining mostly below 96°, the boiling point of the pure substance. The resultant liquid is perfectly colorless and has a slight sharp odor. It was used to form allyl chloride.

Allyl chloride, $CH_2 = CH-CH_2Cl$.

Equation:

$CH_2 = CH-CH_2OH + HCl \rightarrow CH_2 = CH-CH_2Cl + H_2O$

The allyl alcohol was transformed into allyl chloride according to the method of Eltekow, by placing the allyl alcohol in closed saponification flasks with three times its weight of hydro-chloric acid, sp. gr. 1.20 and heating one hour on water bath . The above amount of HCl constitutes about two molecules of HCl to each molecule of $CH_2 = CH-CH_2OH$. The flasks were opened after cooling and the dark layer which separated in the top was removed and distilled on the water bath. It began to distill at 46° and rose to 50 . (Boiling point pure 46°.) It is a pleasant-smelling liquid bearing a slightly yellow color. About 100 grams were obtained.

3. Timethylene-chlor-bromide. $CH_2Cl-CH_2-CH_2Br$.

Equation:

$$CH_2Cl-CH = CH_2 + HBr \rightarrow CH_2Cl-CH_2-CH_2Br.$$

Theunion of the allyl chloride and the hydro-bromic acid was brought about according to the method of Reboul. The allyl chloride was heated in closed tubes for seven or eight hours with hydro-bromic acid of a specific gravity 1.65 which contains 70% HBr. The hydrobromic acid of this strength was made by passing HBr gas evolved from sodium bromide and sulphuric acid, into water and saturating it at 12-15° C. It was first sought to bring about the reaction in saponification flasks, but almost all blew out their stoppers or exploded. It became necessary to resort to

sealed tubes. The only ones on hand were 3cm in diameter and one meter long, and were very difficult to seal. A 4 in. gas pipe closed at one end was used as a vertical water bath. In the tube the allyl-chloride was heated with enough hydro-bromic acid(1.65) to make two molecules to one of allyl chloride. The results were far from satisfactory; only a small amount of gamma-chlor-propyl bromide being obtained.

The operation was repeated, beginning with glycerine, with no better success. It is thought that if flasks sufficiently strong could be obtained the union could be made, for in one saponification flask which held almost as much tri-methylene-chlor bromide was obtained as in all the rest of the work. In the closed tubes standing vertically the lighter allyl chloride has a very small contact surface with the hydro-bromic acid. In all 16 grams of tri-methylene-chlor-bromide was obtained. It was used to make gamma-cyanpropyl bromide.

4. Gamma-cyanpropyl-chloride $-CN-CH_2-CH_2-CH_2Cl$

Equation:

$$Cl-CH_2-CH_2-CH_2Br + KCN \rightarrow CN-CH_2-CH_2-CH_2Cl + KBr.$$

The gamma-chlor-butyro-nitrile was formed after the method of Gabriel from tri-methylene-chlor-bromide and alcoholic potassium cyanide. 6.4 grams of KCN was dissolved in 10 grams of water and 40 cc of 96% alcohol. To this was added 16 grams of the chlor-bromide and heated 1 1/2 hrs. under a reflux condensor. The alcohol is then distilled off and water added to the residue, when

a small amount of oil was obtained which distilled at the proper temperature. Owing to a lack of starting materials in stock a repetition of the above was not made and the duplication of Emil Fisher's synthesis of lysine was not attempted.

Experiments on the Action of Potassium Cyanide on Dichlorhydrine.

After the above mentioned, unsuccessful attempt to replace the bromine of trimethylene-chlor-bromide with the cyanogen group, an effort was made to produce the same substitution in a similar compound. The compound chosen was dichlor-hydrine, Cl-CH -CH(OH)-CH Cl.

1. To replace one of the chlorine atoms by CN.

(a) A hot solution of 32 grams of KCN in 50 cc water was added to 200 cc alcohol in a 500 cc flask. To this was added 65 grams of dichlorhydrine (one molecule to one molecule KCN) and the mixture was heated on the water-bath. Violent action started almost immediately with the precipitation of KCl. The liquid was cooled to prevent too violent bumping and to check the reaction. The mixture was then heated for 25-30 min, when the reaction was apparently finished. The crystals of KCl were removed by filtration and the alcohol distilled off under reduced pressure. The dark brown residue was dissolved in ether, leaving only a crystalline mass-KCl. The ether solution was evaporated and the residue prepared for fractional distillation under reduced pressure.

Fractionation:

Temperature	Pressure in mm.	Fraction.
80-125°	35	1
125-147°	38	2
147-165°	25	3
165-210°	24	4
210-230°	24	5

Two other lots of dichlor-hydrine were treated with potassium cyanide in the same manner excepting the flask containing the mixture was kept cool to check the reaction. The fractionations were essentially the same as the first. In all 169 grams were treated with KCN. The three lots were placed together and fractionated. After two distillations the fractions boiled as follows:-

Temperature	Pressure in mm.	No.
75-110°	25	1
110-125°	25	2
120-140°	25	3
140-160°	25	4
160-170°	20	5
170-210°	22	6

The substance tended to pass into three fractions 1, 4, and 6. In treating epichlor-hydrine $CH_2\!-\!\overset{O}{\overset{/\ \backslash}{}}\!CH\!-\!CH_2Cl$ with hydrocyanic acid in a closed flask at 60°,

Lespien obtained the chlor-cyan derivative $CH_2CN-CH(OH)-CH_2Cl$ which at 15-20 mm pressure boiled at 140°. In the above synthesis the larger portion of the substance distilled at 140-160°, mostly 150°, at 25 mm., which indicated it is the same substance as formed by Lespien.

The residues left after the various distillations were disolved in alcohol and saponified with KOH. Copious fumes of ammonia were given off indicating saponification. The reaction mixture was diluted with water and made slightly acid when a brown floculent precipitate was formed. It was filtered out and boiled with animal charcoal to decolorize, but the substance reprecipitated was as brown as before. If it had been possible to purify the substance it would probably have been found to be beta-oxy-glutaric acid, formed by the saponification of the dicyanide $CH_2(CN)-CH(OH)-CH_2-CN$.

2. To condense cyan-chlor-hydrine with sodium malonic ester.

Equation:

$$CN-CH_2-CH(OH)-CH_2Cl + NAHC(CO_2C_2H_5)$$

$$= CN-CH_2-CH(OH)-CH_2-CH-(CO_2C_2H_5)_2 + NaCl.$$

Gamma-cyan-delta-oxy-propyl-malonic ester.

Cyan-chlor-hydrine was condensed with sodium malonic ester in the following manner. 6.9 grams of sodium were dissolved in 75 cc of absolute alcohol and poured while still hot into a saponification flask. Then 43 grams of freshly distilled malonic ester and 35 grams of the cyan-chlor hydrine, were added to the

then solid sodium. Action began immediately with the separation of a solid substance, and the evolution of heat. The mixture was cooled to check the reaction and then after some time heated on a water bath for 7 hrs. The result was an almost solid mass, which was transferred to a 250cc flask, and distilled with steam. The first portion of the distillate contained alcohol. Finally the water mixed with a heavy oil distilled over. The distillation was continued until no more oil came over. The aqueous distillate was extracted with ether, and the residue from the ether solution after drying distilled in vacuo. It distilled at 110-120°, 25mm. Malonic ester boils near that temperature. The water solution obtained from the steam distillation was extracted with ether, and after drying with $CaCl_2$, the ether was distilled off. An attempt was made to distill the residue under reduced pressure, 25mm, but it could not be done on account of decomposition which manifested itself in frothing. It was thought best to saponify to the acid, $CO_2H-CH_2-CH(OH)-CH_2-CH(CO_2H)_2$.

3.-To form gamma-caryboxylic-delta-oxy-propyl-malonic acid. The above substance after dissolving in alcohol was heated with dry KOH, as long as ammonia was liberated. The resultant liquid separated into two layers, the lower dark colored one being a concentrated water solution of KOH. After standing 48 hrs. a certain amount of crystals separated, which were slightly soluble in water and insoluble in alcohol. After filtering off the cry-

stals , the two layers were separated. A portion of the upper layer after being acidulated with sulphuric acid gave a ~~pate~~ precipitate which proved to be Na_2SO_4. An extraction with ether of the acidulated lower layer gave after evaporation only a trace of a residue, so after evaporating the water of, was extracted with alcohol. All was dissolved except crystals of sodium sulphate. Evaporation of the alcohol gave a brown syrup. Since it was probably a mixture of the ester and the free acid, it was dissolved in absolute alcohol and dry hydrochloric acid gas was passed into the solution so as to transform all into the ester. The alcoholic hydrochloric acid was distilled off and the residue distilled in vacuo. At 70mm it boiled at 100-170°. The distillate separated into two layers and was extracted with ether, the ether evaporated and the residue distilled in vacuo. It boiled at 44mm at 125°.

At ordinary pressure a few drops were found to boil at 200°. Malonic ester boils at 198°. Aside from the insignificant amount of crystals which separated upon saponification it is probable ~~th~~ that the reactions after the malonic ester condensation have miscarried through lack of knowledge of the proper conditions, or that the bodies were really formed and the methods of isolation were not of the proper nature for the separation of the end substance. It is however probable that the gamma-carboxylic-delta-oxy-malonic acid can be formed and its synthesis will result on a more careful study of the necessary conditions.

It was the object of this synthesis to replace the hydroxyl contained in the original glycerine by chlorine and then by ammonia, thus a triboxylic-amido acid of the following formula would be formed;

$$CO_2H-CH_2-CH(NH_2)-CH_2-CH(CO_2H)_2.$$

There being on hand no more alpha-dichlorhydrine it was impossible to attempt to repeat the experiments leading to the synthesis of this acid, or its decomposition product;

$$CO_2H-CH_2-CH(NH_2)-CH_2-CH_2-COOH$$

Action of Potassium Cyanide on Ethylene bromide.

In the latter part of the experimental work, the action of potassium cyanide was tried on ethylene bromide, $BrCH_2-CH_2Br$. For this work 500 grams of ethylene bromide was prepared by passing into bromine, ethylene gas made by heating alcohol and sulphuric acid.

66 grams of KCN(C.P.) was dissolved in 100 grams of water and added to 200 cc of alcohol. A solution of 200 grams of ethylene bromide, and 200cc of alcohol was placed in a 500cc flask and connected with a reflux condenser. The alcoholic solution of KCN was added slowly to the top of the condenser. Little heat was evolved indicating little or no immediate reaction. After adding 100cc of alcohol to insure complete solution of the ethylene bromide, the mixture was heated on the water bath for two or three hours.

A fairly large precipitation of KCl was observed. It was filtered off; the alcohol was removed by distillation and the residue was extracted with ether. After drying with fused calcium chloride and freeing from ether the residue was distilled. It boiled at 260-270°. Ethylene-cyanide or succinic nitrile boils at 265-267 The thermometer rose immediately to that temperature indicating that no mono-cyan compound had been obtained.

The experiment was repeated with 300 grams of ethylene bromide and 100 grams of potassium cyanide. This time the mixture was not heated but let stand two or three days and the reaction mixture kept very dilute with alcohol. This time very little of the ethylene cyanide was found and no mono-cyan compound. Most of the ethylene bromide was recovered from the alcohol by diluting with water.

The conclusions that may be drawn from these last two experiments are as follows:-

1. HCN does not react readily with $BrCH_2-CH_2Br$ in dilute and cold alcoholic solutions,

2. The reaction proceeds mostly if not entirely to the dicyanogen substitution products according to the reaction:-

$$BrCH_2-CH_2BR + 2KCN = NC-CH_2-CH_2-CN + 2KBr.$$

Had time permitted, experiments under different conditions and leading to the formation of the mono-cyan derivative would have been attempted, since I formed the mono-cyan compound of di-

chlorhydrine by this reaction with a good yield. It was the purpose of my latter experiment to effect the synthesis of gamma-amido-normal-propyl-malonic acid, by the following reactions.

1. $BrCH_2-CH_2Br + KCN = BrCH_2-CH_2CN + KBr$,

2. $NC-CH_2-CH_2Br + NaCH(COOR)_2 = NC-CH_2-CH_2-CH(COOR)_2 + NaBr$,

3. $NC-CH_2-CH_2-CH(COOR)_2 + 2H_2 = H_2N(-CH_2)_3-CH(COOR)_2$

However, time did not permit me to proceed to this desired end.

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