THE ISOLATION AND SYNTHESIS OF THE NATURALLY OCCURRING α-AMINO ACIDS

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I. Introduction

Within the past decade, interest in the α-amino acids has greatly quickened and has turned from a concern primarily with the structure of proteins to the more mundane consideration of amino acids as nutritive agents. This change of view has shifted interest to the large-scale preparation of amino acids either by isolation from proteins or other natural sources or by organic synthetic methods. It is hoped in this review to describe the methods which are being used or which show promise of use for the preparation of the naturally occurring α-amino acids for large-scale production. Some of the procedures employed in the past for proof of structure may be mentioned briefly while others, as yet limited to small-scale laboratory preparations, will be described in greater detail where the author is of the opinion that they offer future possibilities.

Table 1 lists the amino acids which will be considered in this review. The great similarity in chemical composition, coupled with high melting points, precludes the separation of most of the naturally occurring amino acids by the conventional methods of organic chemistry—i.e., fractional distillation and crystallization. As will be seen below, successful separation has been found worthwhile in a few cases but it appears that, for practical purposes, reliance on synthetic methods will be the choice of the future for the majority of amino acids.

II. Isolation from Natural Sources

The literature pertaining to the isolation of amino acids by the elaborate methods whose primary purpose concerns their quantitative determination in proteins, etc., has been recently summarized by Block and Bolling (48) and will not be considered here.

Before an amino acid can be separated from a protein or protein concentrate, it is necessary to hydrolyze the protein into its constituent parts. This can be accomplished by heating with aqueous acid or alkali or by digestion with proteolytic enzymes. The conditions employed can be subjected to wide variations, depending on the starting material and on the particular amino acid desired. In common practice, 20 to 50 per cent aqueous sulfuric acid or 20 to 38 per cent hydrochloric acid at atmospheric or preferably at increased pressure is usually employed (*cf.* 41, 42, 43, 44, 45, 48, 50 for details). The excess mineral acid is then removed from the resulting amino acid mixture by the addition of an aqueous suspension of calcium hydroxide in the case of sulfuric acid, while distillation *in vacuo* is used to remove excess hydrochloric acid. Anion-exchange resins can also be advantageously employed in this connection (*cf.* 42, 44). In many cases, it is worthwhile to adsorb humin and other impurities by means of

activated carbon. Alkaline and enzymic hydrolysis are employed only in special cases, which will be given below.

A. ARGININE, HISTIDINE, AND LYSINE

The presence of extra nitrogen atoms in the molecules of these three amino acids has permitted their separation from the other constituents of protein hydrolysates.

TABLE 1

AMINO ACID	FORMULA	OPTICAL ROTATION	MOLECULAR WEIGHT	CHEMICAL COMPOSITION					MELTING POINT
				C	H	N	O	S or I	
									°C.
Alanine	$C_3H_7O_2N$	+	89.07	40.42	7.93	15.73	35.92		297
Arginine	$C_6H_{14}O_2N_4$	+	174.14	41.35	8.10	32.18	18.37		220 (as HCl)
Aspartic acid	$C_4H_7O_4N$	−	133.07	36.08	5.31	10.53	48.11		251
Asparagine	$C_4H_8O_3N_2$	−	132.08	36.36	6.11	21.22	36.36		226–227
Citrulline	$C_6H_{13}O_3N_3$		175.12	41.11	7.48	23.99	27.41		
Cystine	$C_6H_{12}O_4N_2S_2$	−	240.23	29.97	5.03	11.66	26.64	26.69	256–258
Diiodotyrosine	$C_9H_9O_3NI_2$	−	432.91	24.97	2.10	3.24	11.09	58.63	
Glutamine	$C_5H_{10}O_3N_2$	−	146.10	41.07	6.90	19.18	32.85		254–256 (*dl*)
Glutamic acid	$C_5H_9O_4N$	+	147.08	40.80	6.17	9.50	43.51		197–198
Glycine	$C_2H_5O_2N$		75.05	31.98	6.71	18.67	42.64		225–330
Histidine	$C_6H_9O_2N_3$	−	155.09	46.42	5.85	27.10	20.63		270
Hydroxylysine	$C_6H_{14}O_3N_2$		162.13	44.08	8.70	17.28	29.61		225 (as picrate)
Hydroxyproline	$C_5H_9O_3N$	−	131.08	45.77	6.92	10.69	36.62		270
Isoleucine	$C_6H_{13}O_2N$	+	131.11	54.92	9.99	10.69	24.41		280
Leucine	$C_6H_{13}O_2N$	−	131.11	54.92	9.99	10.69	24.41		293–295
Lysine	$C_6H_{14}O_2N$	+	146.13	49.27	9.66	19.17	21.90		263–264 (as HCl)
Methionine	$C_5H_{11}O_2NS$	−	149.15	40.23	7.43	9.39	21.45	21.50	283 (uncorrected)
Phenylalanine	$C_9H_{11}O_2N$	−	165.09	65.41	6.72	8.49	19.38		283
Proline	$C_5H_9O_2N$	−	115.08	52.14	7.88	12.17	27.81		220–222
Serine	$C_3H_7O_3N$	−	105.08	34.27	6.72	13.33	45.68		228
Threonine	$C_4H_9O_3N$	−	119.08	40.31	7.62	11.74	40.31		251–253
Thyroxine	$C_{15}H_{11}O_4NI_4$	−	776.82	23.17	1.43	1.80	8.24	65.35	235–236
Tryptophan	$C_{11}H_{12}O_2N_2$	−	204.11	64.67	5.93	13.72	15.68		289
Tyrosine	$C_9H_{11}O_3N$	−	181.09	59.64	6.12	7.74	26.50		314–318
Valine	$C_5H_{11}O_2N$	+	117.10	51.24	9.47	11.96	27.33		315

1. Electrolytic separation

In 1912, Ikeda and Suzuki (168) obtained a patent for separating arginine, histidine, and lysine based on placing a protein hydrolysate, from which the greater part of the mineral acid had been removed, in a three-compartment electrodialysis apparatus and subjecting it to electrolysis. Electrolytic separation of arginine, histidine, and lysine has been further investigated by Foster and Schmidt (133), Cox, King, and Berg (82), and others (*cf.* 48). In all cases, the

migration of the basic amino acids to the cathode compartment is utilized. The more acidic amino acids are excluded because of the alkalinity of the cathode compartment. Figure 1 is a drawing of the apparatus used by Cox *et al.* (82), employing graphite electrodes.

2. Selective adsorption

Sadikov *et al.* (260) reported that permutit removed 93 per cent of the diamino acids and 50 per cent of the monoamino acids from a casein hydrolysate. The

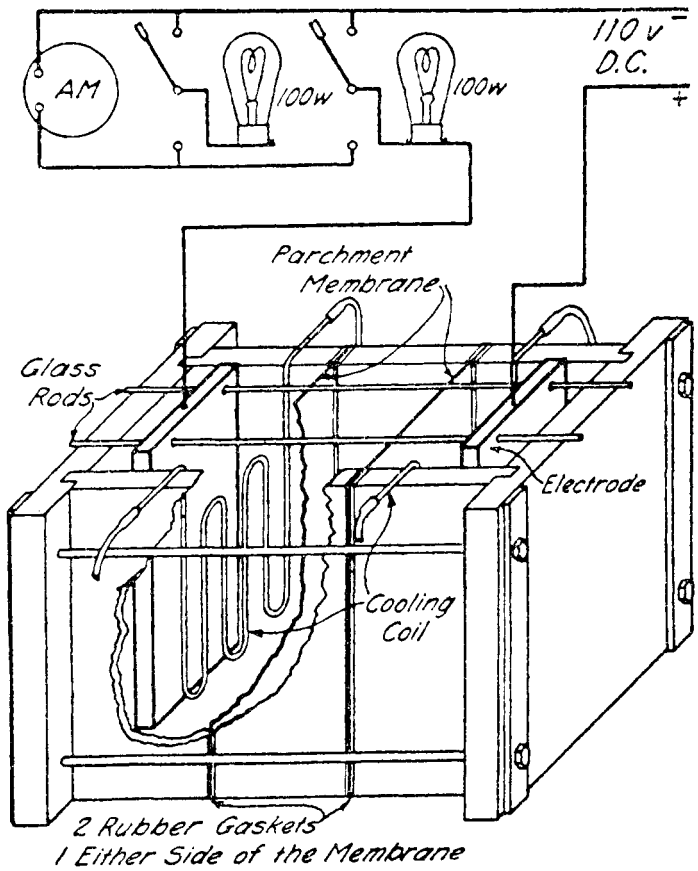


FIG. 1. Electrolytic separation of amino acids (J. Biol. Chem. **81**, 755–64 (1929))

amino acids could then be eluted with 30 per cent sulfuric acid. Turba (317) used bleaching earths (Filtrol, Floridin, etc.) to separate arginine, histidine, and lysine from other amino acids and from one another. However, his published work has been designed for their quantitative separation rather than for large-scale preparation. Turba uses 2500 mg. of bleaching earth to separate 25 mg. of lysine. Furthermore, it is not clear whether the adsorbent can be used over and over again.

Block (41, 42, 43, 45) has used the commercially available cation-exchange resins (polymerized sulfonated phenol–formaldehyde resins), such as Amberlite

IR-1, Amberlite IR-100, Duolite C-1, Duolite C-3, Amberlite XE-17, Ionac C-284, and others, for the concentration of polyamino acids with respect to monoamino acids. Thus, a single passage of a blood hydrolysate, prepared from 50 g. of blood meal (80 per cent protein), through a 50-g. column of cation-exchange resin in the hydrogen cycle resulted in the complete adsorption of arginine, histidine, and lysine and a partial adsorption of some of the monoamino acids. After washing the column with water, the adsorbed amino acids were eluted with constant-boiling hydrochloric acid or exchanged against 4 per cent aqueous ammonia. If desired, histidine and monoamino acids may be removed from the resin by elution with aqueous pyridine before elution of arginine and lysine with dilute ammonia. Whereas the original blood hydrolysate contained approximately twice as much monoamino as polyamino acids, the elutriates contained twice as much diamino as monoamino acids. Other sources of amino acids, such as soybean meal, fish meal, shark meat, etc., can be used with equal facility. The ion exchanger after acid elution is ready for the next cycle without further treatment; if ammonia exchange is used to recover the polyamino acids, it is better, although not necessary, to convert the exchanger to the hydrogen form before the next run. The life of these exchangers exceeds 500 cycles by laboratory test.

Block has also found (42, 43, 45) that not all cation-exchange materials are suitable for the separation of polyamino acids; thus, the sulfonated coals, which are utilized extensively for water softening and other purposes, have not been found operative under the above-described experimental conditions.

3. Individual separation of arginine, histidine, and lysine

Although both the electrolytic and the adsorption methods can be used to separate the three polyamino acids from one another, for large-scale preparation of arginine, histidine, and lysine it seems preferable first to employ one of the group separations given above with one or more of the methods designed for isolation of a specific polyamino acid to be given below.

(a) Arginine *via* arginine flavianate

Kossel and Gross (190, 191) observed that arginine could be readily and practically quantitatively precipitated from acid hydrolysates of arginine-rich proteins, such as gelatin, by the addition of a considerable excess of flavianic acid (2,4-dinitro-1-naphthol-7-sulfonic acid). The resulting arginine mono(or di)flavianate was then decomposed by dissolving in dilute ammonium hydroxide and precipitating the flavianic acid as the barium salt with baryta. Arginine carbonate was isolated from the filtrate after removal of the excess barium with carbon dioxide (*cf.* 166). An alternative method was to decompose the finely powdered arginine flavianate with hot 33 per cent sulfuric acid, cool, filter off the flavianic acid, and isolate arginine carbonate after removal of the sulfuric acid with baryta. Pratt (249) extracted the flavianic acid from sulfuric acid-decomposed arginine flavianate with butyl alcohol and subsequently isolated arginine carbonate according to Kossel and Gross (191). Cox (80) modified the Kossel methods by using hydrochloric acid for hydrolysis and throughout the procedure.

He decomposed the arginine flavianate with hot concentrated hydrochloric acid, cooled the solution to crystallize out flavianic acid, and precipitated arginine dihydrochloride from the concentrated filtrate with alcohol. The dihydrochloride was then converted into arginine monohydrochloride by the addition of aniline. Aniline hydrochloride is soluble in alcohol, while arginine monohydrochloride is not. Block (46) decomposed arginine flavianate by stirring an aqueous suspension with an anion-exchange resin (Amberlite IR-4B).

Arginine flavianate can be recrystallized from dilute ammonium hydroxide by acidification with hydrochloric or acetic acid (*cf.* 48).

(b) Arginine *via* the benzylidene derivative

Bergmann and Zervas (32, 33) found that benzaldehyde will condense with arginine in strongly alkaline solution (barium hydroxide or sodium hydroxide) at 0–5°C. to yield the insoluble sodium or barium benzylidenearginine. The salt is then washed with ice water, and dried with alcohol and ether. The benzylidenearginine is decomposed by heating with 5 *N* hydrochloric or nitric acid and, after extraction of the benzaldehyde with ether, arginine is isolated as the hydrochloride or nitrate.

(c) Separation of histidine by means of metallic derivatives

Kossel (189, 192) in 1898 utilized the insolubility of histidine mercuric chloride or histidine silver at neutrality as the means of separating this amino acid from protein hydrolysates. Since that time Vickery and Leavenworth (322), Jones (172), and others have used either or both of these salts, with modifications in detail (*cf.* 48, 134, 142, 152, 172, etc.), for the isolation of histidine from hemoglobin or blood meal. Kapfhammer and Spörer (174) found that histidine was precipitated along with the other heterocyclic amino acids, proline and hydroxyproline, by Reinecke acid [4-tetrarhodanato-2-amminochromic acid] and suggested that use of this reagent, followed by precipitation with mercuric chloride at pH 7, would be a good way of preparing histidine. However, Reinecke acid does not appear to offer any advantage over the original Kossel methods.

(d) Isolation of histidine by aromatic sulfonic acids

The successful application by Kossel and Gross (191) of flavianic acid (2,4-dinitro-1-naphthol-7-sulfonic acid) for the separation of arginine prompted Bergmann and his associates (92, 301) to study the specific precipitability of other aromatic sulfonic acids. From a large number of these, Vickery (321) found that the easily prepared 3,4-dichlorobenzenesulfonic acid was an excellent precipitant for histidine. When blood cells are hydrolyzed with hydrochloric acid and the excess mineral acid is removed by repeated concentration *in vacuo*, histidine can be readily isolated from the decolorized hydrolysate by the addition of 5–6 moles of 3,4-dichlorobenzenesulfonic acid. The resulting histidinedisulfonate is readily recrystallized from boiling water. Histidine can then be isolated from the pure salt by precipitation of the 3,4-dichlorobenzenesulfonic acid with barium hydroxide. The excess barium hydroxide is removed with carbon dioxide, and histidine is isolated as the free base from the concentrated aqueous solution.

(e) Lysine picrate

Up to 1939, lysine was only isolated from protein hydrolysates by precipitation with the costly phospho-24-tungstic acid (*cf.* 48), followed by formation of lysine picrate. In that year, Rice (257) in W. C. Rose's laboratory reported that lysine picrate could be prepared directly from blood hydrolysates from which the excess sulfuric acid had been previously removed by precipitation with cream of lime to pH 6. The resulting lysine picrate, contaminated with histidine and other picrates, was purified by repeated crystallization from hot water. The lysine picrate was then decomposed by hot hydrochloric acid, the picric acid was removed by crystallization and with charcoal, and the lysine was isolated directly as the dihydrochloride or, after treatment with pyridine, as lysine monohydrochloride (*cf.* Section II,A,3,(a) above).

Unfortunately, this simple procedure does not appear to be generally applicable to crude commercial protein hydrolysates (46, 197, 257) but it gives excellent results when applied to polyamino acid concentrates prepared by ion-exchange adsorption (*cf.* Section II,A,2, above).

(f) Benzoyllysine copper

Kurtz (197) evolved a novel method for preparing lysine. The amino acids from a neutralized protein hydrolysate (pH 6) are converted into their copper salts by boiling with excess cupric carbonate. The excess cupric carbonate is filtered off, and the clear blue filtrate is treated with benzoyl chloride at 0°C. The ε-benzoyllysine copper which precipitates is washed with ice water and alcohol and dried in air. The copper is removed by hydrogen sulfide, and ε-benzoyllysine is allowed to crystallize from aqueous solution. Lysine is recovered from the benzoyl derivative following hydrolysis with 8 *M* hydrochloric acid for 10 hr.

B. GLUTAMIC ACID AND ASPARTIC ACID

1. Precipitation as the calcium or barium salts

The dicarboxylic amino acids can be precipitated from protein hydrolysates as the calcium or barium salts from strongly alkaline solution by the addition of 5–10 volumes of ethanol. Details of this procedure, which was first used as a quantitative method by Ritthausen and by Foreman, are given in reference 48.

2. Electrolytic separation

Foster and Schmidt (133, 168) also suggested that the dicarboxylic amino acids could be concentrated in the anodic compartment of the three-cell electrodialysis apparatus described in Section II,A,1. This suggestion has apparently not been developed further.

3. Selective adsorption

Turba and Richter (318; *cf.* 48) found that glutamic and aspartic acids are quantitatively adsorbed by Brockmann's standardized aluminum oxide. Glutamic acid is then eluted with *N* acetate–acetic acid buffer, after which the aspartic acid is removed with dilute alkali. It requires 5000 mg. of aluminum oxide to adsorb 10 to 15 mg. of dicarboxylic amino acids.

A superior analytical and preparative method has been devised by Cannan and Kibrick (67, 179). This consists in adsorbing the dicarboxylic amino acids on one of the polyamine phenol–formaldehyde resins such as Amberlite IR-4, Duolite A-3, De-Acidite, etc. Cannan (67) has described the conditions ensuing when an acidic protein hydrolysate is treated with sufficient anion-exchange resin to raise the pH to 6–7.

"The neutral amino acids will be present almost entirely in the form of dipolar ions with zero net charge, the acidic amino acids in the form of monoanions, and the ammonia and the basic amino acids (except histidine) in the form of monocations. The histidine will be partially cationic and partially dipolar. The chief anions present will, therefore, be those of the hydrochloric acid and of the dicarboxylic amino acids; and it is these alone which should be adsorbed by the resin. Let a, b, and c represent the equivalents of acidic amino acids, bases (basic amino acids and ammonia), and chloride respectively in the original hydrolysate. Then, if α is the fraction of the total anions which is bound by the resin, and if α_a and α_c are the fractions of the total dicarboxylic amino acids and of chloride respectively which are bound, electrical neutrality in the solution requires that

$$a + c - b = (a + c)\alpha = a\alpha_a + c\alpha_c$$

$$\therefore \alpha = 1 - \frac{b}{a + c} \quad "$$

The α is determined by the ratio of bases to acids. Therefore, for complete removal of the dicarboxylic amino acids, hydrochloric acid must be present in very large excess relative to the bases, or the greater part of the bases must be removed from solution prior to treatment with the resin. In fact, the effluent from the cation adsorption of the basic amino acids has proved an excellent source for the adsorption of the dicarboxylic acids (unpublished experiments of the author and G. P. Taylor, 1941) (*cf.* Section II,A,2).

C. GLUTAMIC ACID, GLUTAMINE, ASPARTIC ACID, AND ASPARAGINE

1. Glutamic acid

Probably over 1,000,000 lb. of glutamic acid are prepared per year in the United States for use in the form of monosodium glutamate as a condiment, especially in soups. This is usually prepared from hydrolysates of wheat gluten, corn gluten, casein, soy bean proteins, or beet sugar wastes. However, wheat gluten is by far the largest source.

The protein is hydrolyzed with concentrated hydrochloric acid under pressure for several hours, the black hydrolysate is then decolorized with carbon, and the yellow-brown filtrate is concentrated to a small volume to permit the crystallization of glutamic acid hydrochloride. The glutamic acid hydrochloride is removed, dissolved in water, and the pH is adjusted to 3.2–3.3, whereupon glutamic acid crystallizes. The amino acid is then neutralized with sodium hydroxide to form monosodium glutamate ($C_5H_8O_4NNa$), which is recovered by crystallization. The monosodium glutamate is approximately 99 per cent pure. There are numerous publications and patents embodying minor modifications of the above method, a few of which are given (12, 24, 38, 79, 181, 205, 281, 342).

Apparently the most economical procedure for the preparation of monosodium glutamate is its isolation from the beet sugar by-product known as Steffen house

waste (227). This dilute waste liquid is concentrated to a fairly high specific gravity and subjected to mild alkaline hydrolysis to liberate the glutamic acid. Further steps are similar to those employed for the isolation of monosodium glutamate from wheat gluten hydrolysates.

2. Glutamine

The glutamine present in beet juice is precipitated by mercuric nitrate at pH 7. After decomposition of the precipitate with hydrogen sulfide and sulfuric acid, glutamine is crystallized from the inorganic ion-free solution with the aid of alcohol (323).

3. Aspartic acid

This amino acid is best isolated, as the copper salt, from the dicarboxylic amino acid fraction prepared by one of the methods given in Section II,B, after precipitation of glutamic acid as the free base or the hydrochloride (*cf.* 48).

4. Asparagine

White lupin seedlings are extracted with water and the soluble proteins are removed. Asparagine crystallizes out of the aqueous solution on concentration *in vacuo* (248, 324).

D. ISOLATION OF CYSTINE AND CYSTEINE

1. Isoelectric precipitation

A cystine-rich protein, such as horse hair, rabbit fur, wool scraps, etc., is hydrolyzed for several hours with hydrochloric acid. The humin is removed by filtration and activated carbon. Cystine is then precipitated by neutralization of the light yellow hydrolysate with sodium hydroxide, ammonium hydroxide, or sodium acetate to pH 4.5–4.8. The crude cystine so obtained is recrystallized from hot dilute hydrochloric acid or hydrochloric acid–acetic acid, followed by neutralization to pH 4.5–4.8 with ammonium hydroxide (48, 131, 237, 238, 270, 330).

2. Precipitation of cuprous cysteine mercaptide

Lucas and Beveridge (214), using the observation of Hopkins and others (*cf.* 48) that cysteine is quantitatively precipitated by cuprous ions, found that when a protein hydrolysate containing cystine is treated with a suspension of cuprous oxide at pH 5, a quantitative precipitation of $CuSCH_2CHNH_2COOH$ results. The copper is then removed with hydrogen sulfide and cysteine hydrochloride is isolated.

Cystine and cysteine are prepared in commercial quantities by these general procedures and are being used in increasing quantities in the food (41, 49) and cosmetic industries.

E. TYROSINE

Tyrosine is usually obtained as by-product in the manufacture of both cystine and glutamic acid. It is very insoluble at its isoelectric point, *ca.* pH 5.7, a fact which is utilized in its preparation (*cf.* 22, 25, 48, 90, 161).

F. TRYPTOPHAN

Tryptophan is best prepared by synthesis, but for the isolation of the naturally occurring form, a protein, preferably lactalbumin or blood fibrin, is digested for several weeks with commercial pancreatin at pH 8 (sodium bicarbonate) in the presence of toluene or chloroform as preservatives (81). The tryptophan is precipitated with mercuric sulfate in 10 per cent sulfuric acid (48, 81). After decomposition of the mercury precipitate with hydrogen sulfide, the tryptophan is extracted with butyl alcohol (86) and crystallized from the solvent (*cf.* 262, 327).

G. LEUCINE, ISOLEUCINE, AND VALINE

These three amino acids are also obtained as by-products in the preparation of glutamic acid and cystine. Most of the commercial "leucine" contains considerable quantities of isoleucine, valine, and even methionine. Barnett (22, 23) isolated leucine as dileucine hydrochloride from a neutralized casein hydrolysate at pH 1.7–2.8 in the presence of a large excess of sodium chloride, while Stein *et al.* (301) used 2-bromotoluene-5-sulfonic acid to precipitate leucine from a leucine-rich, isoleucine-poor protein hydrolysate (hemoglobin).

A mixture consisting of approximately 50 per cent leucine and 50 per cent isoleucine is obtained as a by-product in the manufacture of monosodium glutamate from beet sugar waste. Isoleucine can be separated from leucine by making use of Ehrlich's observation that its copper salt is considerably more soluble in methanol than that of leucine (*cf.* 48, 102). Specific organic reagents may also be developed for this purpose. Town (314) reported that it is possible to separate leucine and valine by fractional precipitation.

H. MISCELLANEOUS

1. Alanine

This amino acid has been obtained from silk fibroin hydrolysates after preliminary removal of glycine as the insoluble azobenzene-*p*-sulfonic acid salt (301) and as the insoluble alanine calcium picrate (124).

2. Diiodotyrosine

Iodogorgoic acid was obtained by Wheeler and Mendel (335) from hydrolysates of the common bath sponge by precipitation with silver nitrate and ammonium hydroxide, followed by phospho-24-tungstic acid.

3. Glycine

Glycine is readily isolated from glycine-rich protein hydrolysates by precipitation of the ester hydrochloride (*cf.* 2, 48). It also forms an insoluble salt with calcium picrate $NH_2CH_2COOH \cdot [C_6H_2(NO_2)_3O]Ca \cdot 2H_2O$ (124) and with nitranilic acid (2,5-dihydroxy-3,6-dinitro-*p*-benzoquinone) (313). Inorganic ions, ammonia, and basic amino acids also form insoluble nitranilates (313; *cf.* 48).

4. Hydroxylysine

This amino acid has been isolated in small quantities from gelatin and isinglass by Schryver *et al.* (9, 28) and Van Slyke *et al.* (320). The method used is based upon the Kossel–Kutscher procedure for lysine (*cf.* 48), followed by fractional crystallization of lysine and hydroxylysine picrates.

5. Hydroxyproline

This amino acid is precipitated along with proline by Reinecke acid (4-tetrarhodanato-2-amminochromic acid) (174). Hydroxyproline is not soluble in absolute ethanol, which dissolves proline (*cf.* 184, 215, 312).

6. Methionine

Methionine is isolated from protein hydrolysates only with considerable difficulty. Pirie (246) used butyl alcohol extraction, followed by precipitation with mercuric acetate. Approximately 30 per cent of the methionine present in casein was obtained by this method.

7. Phenylalanine

This compound has been obtained by the Fischer ester distillation method (*cf.* 48), by fractional crystallization from phenylalanine-rich protein hydrolysates in the presence of large quantities of sodium chloride (18), and by precipitation with 2,5-dibromobenzenesulfonic acid (301).

8. Proline

Proline can be isolated from protein hydrolysates, especially gelatin and zein, because, of all the monoamino acids, it alone is soluble in absolute alcohol (*cf.* 48). Other methods which have been used are based on the fact that proline copper is soluble in ethanol (132) and methanol (182, 184, 312), and that proline is precipitated with Reinecke acid (174) or rhodanilic acid (tetrathiocyanatodianilidochromiato acid, $[Cr(CNS)_4(C_6H_5NH_2)_2]H$) (30, 299).

9. Serine

This substance can only be isolated with ease from silk fibroin by precipitation with *p*-hydroxyazobenzene-*p'*-sulfonate after previous removal of tyrosine, glycine, and alanine (301).

I. GENERAL ISOLATION METHODS

Besides the often-described Fischer ester method, Dakin's butyl alcohol extraction procedure and fractional crystallization (*cf.* 48), high-vacuum distillation of butyl esters (148, 149), differential adsorption on charcoal (48, 71), fractional extraction with organic acids (250) and specific organic reagents (92, 232, 233, 301, etc.) have been used for separating α-amino acids.

Free amino acids can be recovered from their sodium salts by treating with carbon dioxide under pressure in the presence of an equimolar amount of ammonium bicarbonate and removing the precipitate of sodium bicarbonate (143)

or from their hydrochlorides by adding an excess of an amine such as aniline, pyridine, γ-methylbutylamine, etc., or pinene, and extracting the amine hydrochloride or bornyl chloride (16) with chloroform or ethanol (29, 311).

III. Synthesis

Many methods for synthesizing α-amino acids have been proposed since 1850, when A. Strecker (302) prepared alanine by treating acetaldehyde with hydrogen cyanide and ammonia, followed by hydrolysis of the resulting aminocyanohydrin.

It is hoped to present the general methods of synthesis of amino acids with a brief historical picture, although neither the experimental details nor the complete literature will be given. The reader is referred to the excellent reviews by Dunn (94, 95), Clarke (74), and Carter (68) for other details of this subject.

A. Amination of α-halogen acids

1. From monocarboxylic acids

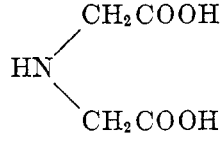
(a) Preparation of glycine

In 1858, Cahours (66) prepared glycine by treating chloroacetic acid with 2 moles of ammonia. This preparation was confirmed by Perkin and Duppa (245) by the synthesis of glycine starting with acetic acid.

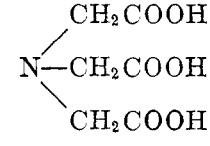
$$CH_3COOH + Br_2 \xrightarrow{150°C.} CH_2BrCOOH + HBr \xrightarrow{2NH_3} CH_2NH_2COOH + NH_4Br$$

Glycine

Since that time, amination of chloroacetic acid has been one of the best methods for preparing glycine. The formation of by-products such as



and



is minimized by the use of a large excess of ammonium hydroxide, sixty times theory (13, 53, 70, 240, 258, 310), liquid ammonia (283), ammonia containing ammonium carbonate (72), or ammonia plus ammonium chloride (109). Glycine is separated from ammonium chloride formed in the reaction by isolation through the copper salt (93) or by precipitation with methanol (13, 53, 240, 310). Krause (193) prepared glycine by amination of the copper salt of chloroacetic acid, eliminating both by-products and ammonium chloride simultaneously.

(b) Synthesis of alanine, valine, leucine, norleucine, etc.

Synthesis of amino acids other than glycine by direct amination of the α-halogen acid is limited by the availability of the halogen acid and by the increasing resistance of higher and branched α-halogen acids to amination. How-

ever, alanine has been synthesized in 70–80 per cent yield from bromopropionic acid and in lower yields from chloropropionic acid (157, 309).

$$CH_3CH_2COOH + \text{red P} + Br_2 \rightarrow CH_3CH_2COBr + H_3PO_4$$

$$+ HBr \xrightarrow[40\text{–}50°C.]{Br_2} CH_3CHBrCOBr \xrightarrow{H_2O} CH_3CHBrCOOH$$

$$\xrightarrow{NH_4OH} CH_3CHNH_2COOH$$

Alanine

Benzoyl-*dl*-alanine is readily resolved by forming the strychnine salt (242). Norleucine has been made from *n*-caproic acid (1, 73, 225) as follows:

$$CH_3(CH_2)_4COOH \xrightarrow[PCl_3 \text{ or red P}]{Br_2} CH_3(CH_2)_3CHBrCOOH \xrightarrow{NH_4OH}$$

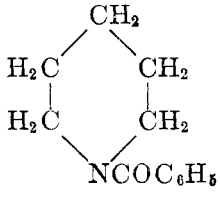
$$CH_3(CH_2)_3CHNH_2COOH$$

Norleucine

Leucine (222) and valine (220) have also been successfully made by this general reaction (*cf.* 72, 283). The large-scale synthesis of the higher fatty acids from hydrocarbons (156; and other papers by Hass) may permit a much further application of the Cahours–Perkin procedure. Phenylalanine (279) and aspartic acid (177, 326) have also been prepared by this general method.

(c) Synthesis of lysine, proline, and other amino acids

v. Braun prepared lysine from benzoylpiperidine by the following reactions (60):



$$\xrightarrow{PCl_5} C_6H_5CONH(CH_2)_5Cl \xrightarrow{KCN}$$

$$C_6H_5CONH(CH_2)_5CN \xrightarrow{KOH} C_6H_5CONH(CH_2)_5COOH \xrightarrow[\text{red P}]{Br_2}$$

$$C_6H_5CONH(CH_2)_4CHBrCOOH \xrightarrow{NH_3}$$

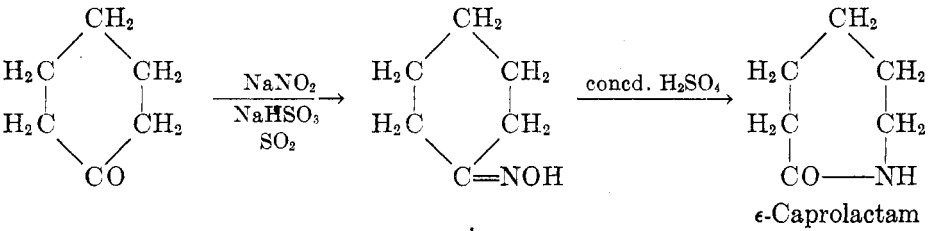
$$C_6H_5CONH(CH_2)_4CHNH_2COOH \xrightarrow{\text{concd. HCl}}$$

$$HCl \cdot NH_2CH_2CH_2CH_2CH_2CHCOOH$$

$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad NH_2 \cdot HCl$$

dl-Lysine dihydrochloride

Eck and Marvel (101) used a modification of the v. Braun (60) method for the synthesis of lysine, starting with cyclohexanone and making use of the Beckmann–Wallach rearrangement.



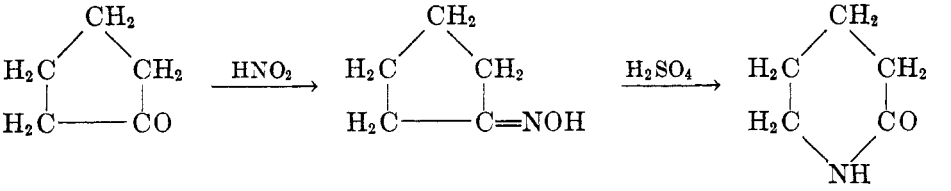
ε-Caprolactam

$$\xrightarrow[\text{heat}]{\text{NaOH}} NH_2(CH_2)_5COOH \xrightarrow{C_6H_5COCl} C_6H_5CONH(CH_2)_5COOH$$

$$\xrightarrow[Br_2]{\text{red P}} RCHBrCOOH \xrightarrow{NH_4OH} RCHNH_2COOH \xrightarrow{HCl}$$

$$HCl\cdot NH_2(CH_2)_4CHCOOH$$
$$|$$
$$NH_2\cdot HCl$$

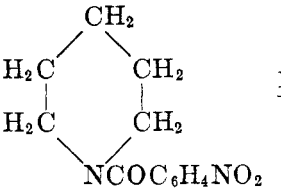
Schniepp and Marvel (273) employed a somewhat similar method for preparing ornithine and arginine, starting with cyclopentanone through the oxime.



α-Piperidone

$$\longrightarrow NH_2(CH_2)_4COOH \longrightarrow \text{etc.}$$

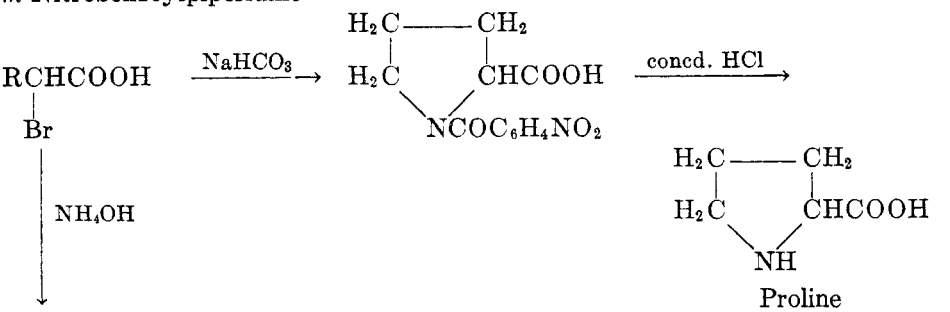
Fischer and Zemplén (130) also used a modification of v. Braun's method to prepare ornithine and proline.



$$\xrightarrow{KMnO_4} NO_2C_6H_4CONH(CH_2)_4COOH$$

$$\xrightarrow{P,\ Br_2} RCHBrCOOH$$

m-Nitrobenzoylpiperidine



Proline

$$O_2NC_6H_4CONH(CH_2)_3CHCOOH \xrightarrow{\text{concd. HCl}}$$
$$|$$
$$NH_2$$

$$HCl\cdot NH_2CH_2CH_2CH_2CHCOOH$$
$$|$$
$$NH_2\cdot HCl$$

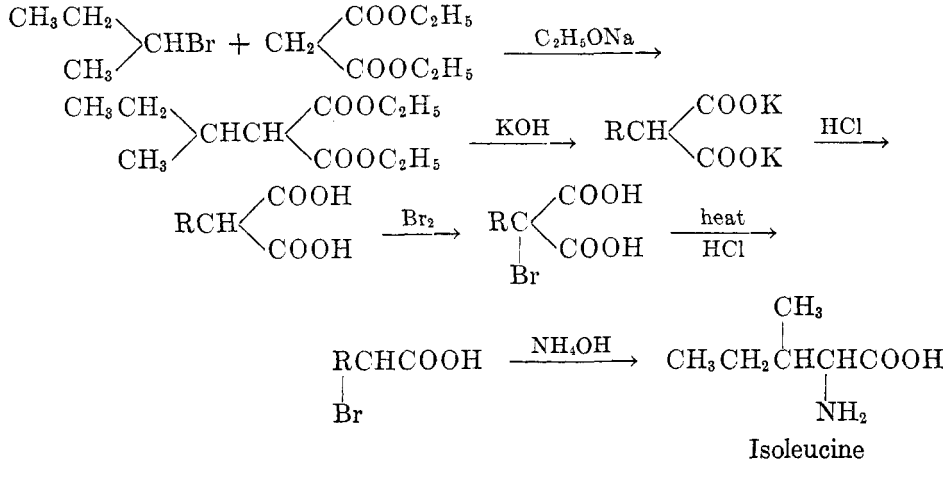
Ornithine

2. From substituted malonic acids

Direct halogenation of monocarboxylic fatty acids is often difficult or impracticable, while the corresponding malonic (1,1-dicarboxylic) acids halogenate readily.

(a) Preparation of valine, isoleucine, norleucine, and phenylalanine

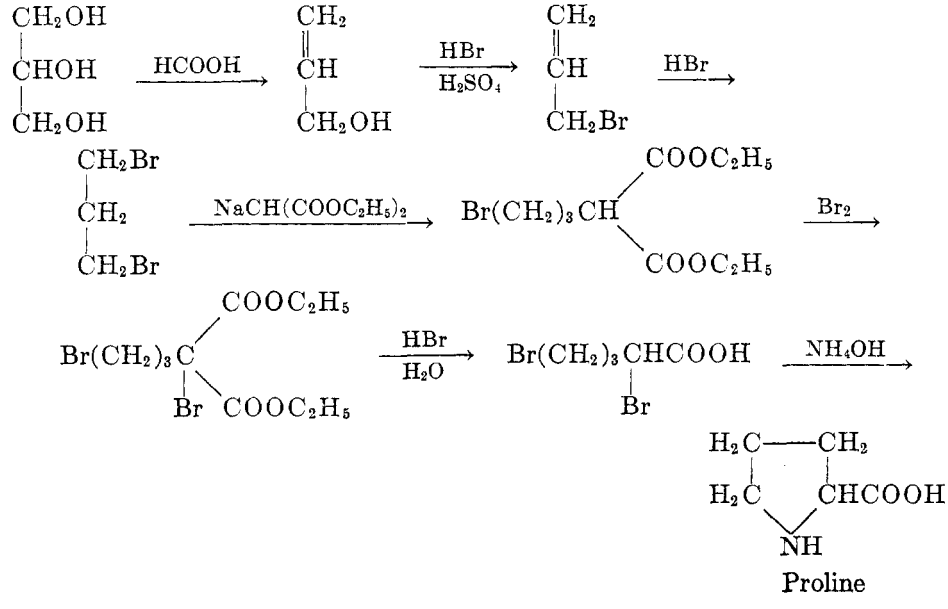
Marvel and associates have developed this procedure for the practical preparation of the above-mentioned amino acids (4, 221, 223, 226). Thus isoleucine (221) is prepared as follows:



Isoleucine

(b) Preparation of proline and hydroxyproline

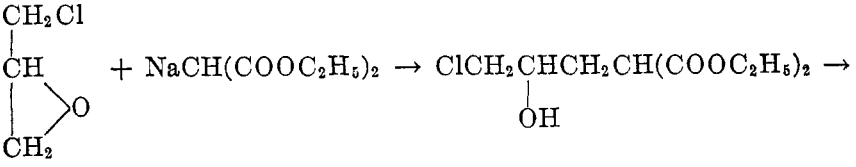
Willstätter's (136, 336, 337) synthesis of proline from trimethylene bromide and sodiomalonic ester antedates the above use of malonic acid. Proline was synthesized as follows:



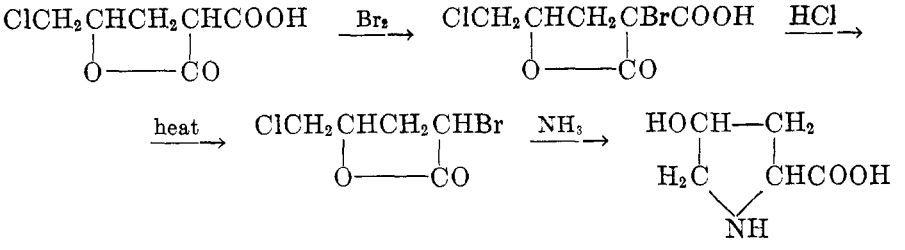
Proline

γ-Chlorobutyronitrile (158) can also be used to synthesize proline.

Leuchs and Brewster (200, 201, 202) prepared hydroxyproline from Traube's (316) δ-chloro-γ-valerolactone-α-carboxylic acid as follows:



Epichlorohydrin



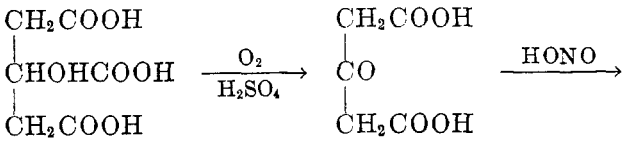
α-Bromo-ϑ-chloro-γ-valerolactone

Hydroxyproline

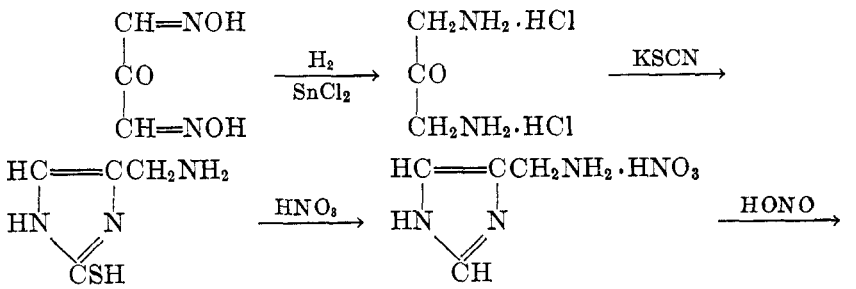
Traube (315) later made some modifications of the Leuchs–Brewster synthesis.

(c) Synthesis of histidine

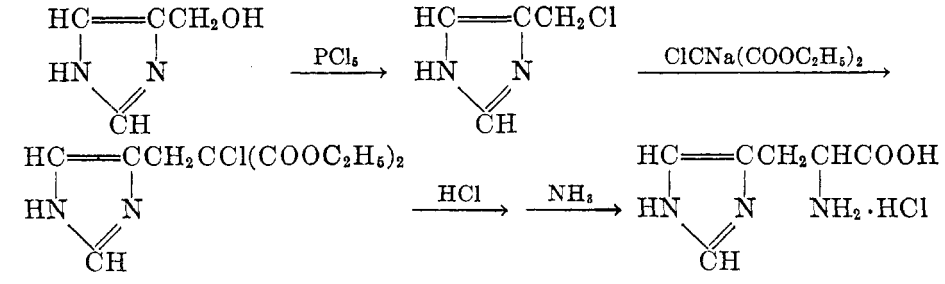
The brilliant synthesis of histidine by Pyman (253) in 1911 is of theoretical rather than practical interest, although with improved intermediary compounds certain features may prove of practical importance.



Citric acid



2-Thiol-4(or 5)-amino-methylimidazole



Histidine hydrochloride

(d) Preparation of methionine and homocystine

Windus and Marvel (338) prepared methionine by the following series of reactions:

$$CH_3SH + ClCH_2CH_2OH \xrightarrow{C_2H_5ONa} CH_3SCH_2CH_2OH \xrightarrow{SOCl_2}$$

$$CH_3SCH_2CH_2Cl \xrightarrow{NaCH(COOC_2H_5)_2} CH_3SCH_2CH_2CH(COOC_2H_5)_2 \longrightarrow$$

$$CH_3SCH_2CH_2CH(COOH)_2 \xrightarrow{Br_2} CH_3SCH_2CH_2CBr(COOH)_2 \xrightarrow{NH_4OH}$$

$$CH_3SCH_2CH_2C(COONH_4)_2 \xrightarrow{HCl} \xrightarrow{heat} CH_3SCH_2CH_2CHNH_2COOH$$
$$\quad NH_2$$

Methionine
(24 per cent yield)

Patterson and du Vigneaud (244) condensed sodium benzyl mercaptan with ethylene chloride and prepared benzylhomocysteine essentially according to the method of Windus and Marvel (338); the benzyl group was then replaced by methyl, utilizing sodium in liquid ammonia for both debenzylation and methylation.

$$C_6H_5CH_2SNa \xrightarrow{ClCH_2CH_2Cl} C_6H_5CH_2SCH_2CH_2Cl \xrightarrow{NaCH(COOC_2H_5)_2}$$

$$C_6H_5CH_2SCH_2CH_2CH(COOC_2H_5)_2 \xrightarrow{KOH} \xrightarrow{Br_2} \xrightarrow{NH_3} \xrightarrow{HCl} \xrightarrow{heat}$$

$$C_6H_5CH_2SCH_2CH_2CHNH_2COOH \longrightarrow$$

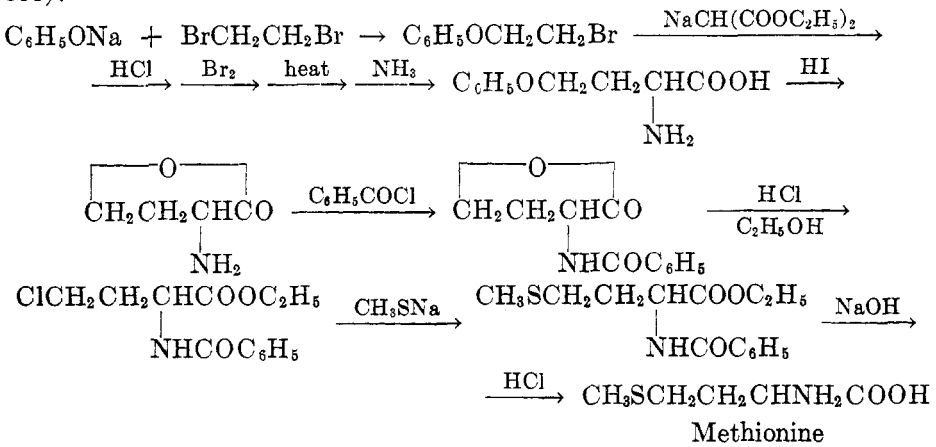
$$CH_3SCH_2CH_2CHNH_2COOH$$

Methionine

$$\xrightarrow[\text{liquid } NH_3]{\text{Na in}} \begin{cases} \xrightarrow{CH_3I} CH_3SCH_2CH_2CHNH_2COOH \\ HSCH_2CH_2CHNH_2COOH \xrightarrow[FeCl_3]{O_2} \begin{matrix} SCH_2CH_2CHNH_2COOH \\ | \\ SCH_2CH_2CHNH_2COOH \end{matrix} \end{cases}$$

Homocystine

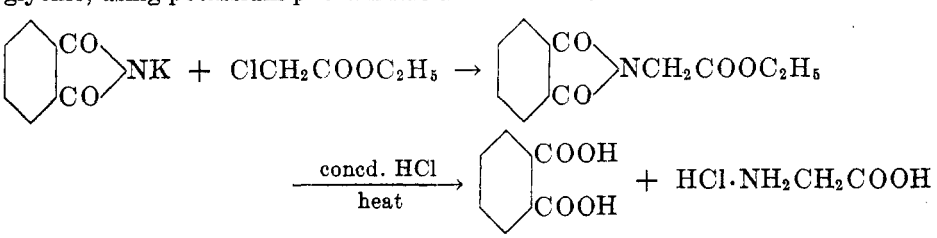
Hill and Robson (162) prepared methionine[1] *via* α-amino-γ-butyrolactone, the intermediate steps being essentially according to the following reactions (244, 338):



3. From potassium (sodium) phthalimide

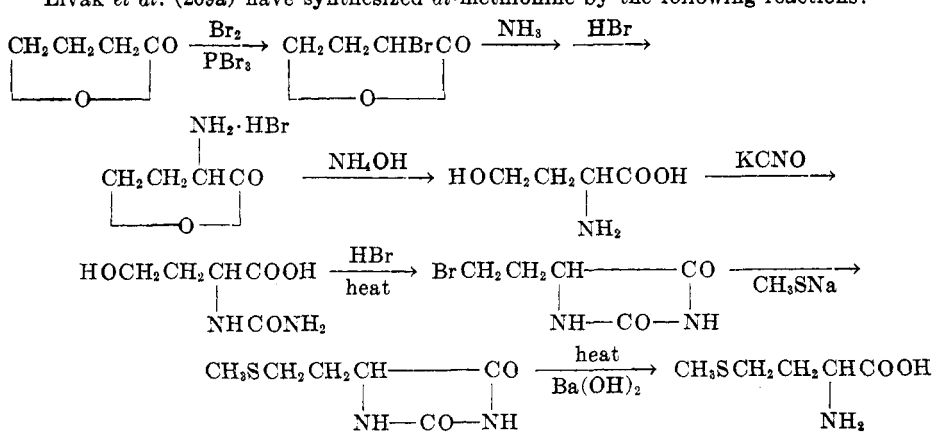
(a) Gabriel synthesis

In 1888 Goedeckemeyer (145), working in Gabriel's laboratory, synthesized glycine, using potassium phthalimide and chloroacetic ester.



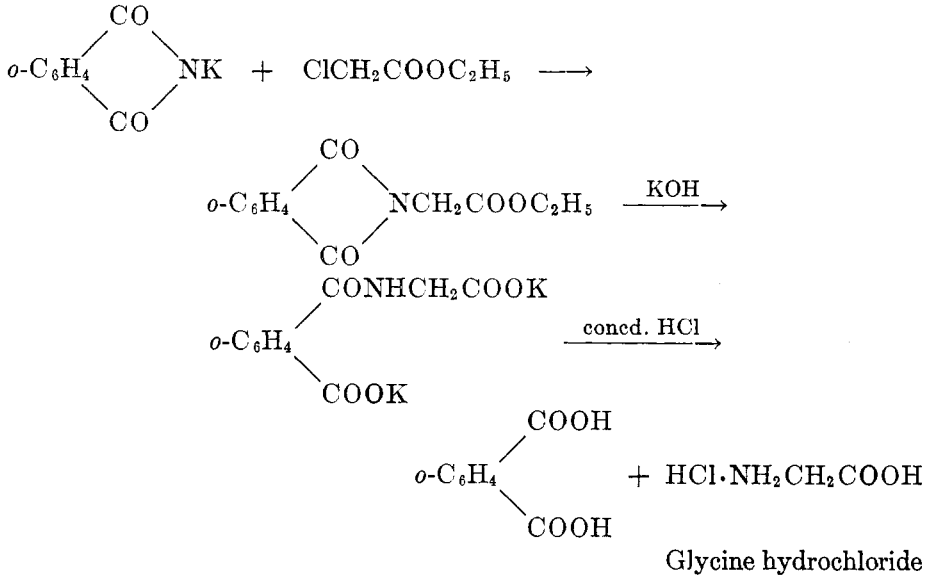
Glycine hydrochloride

[1] Livak *et al.* (209a) have synthesized *dl*-methionine by the following reactions:



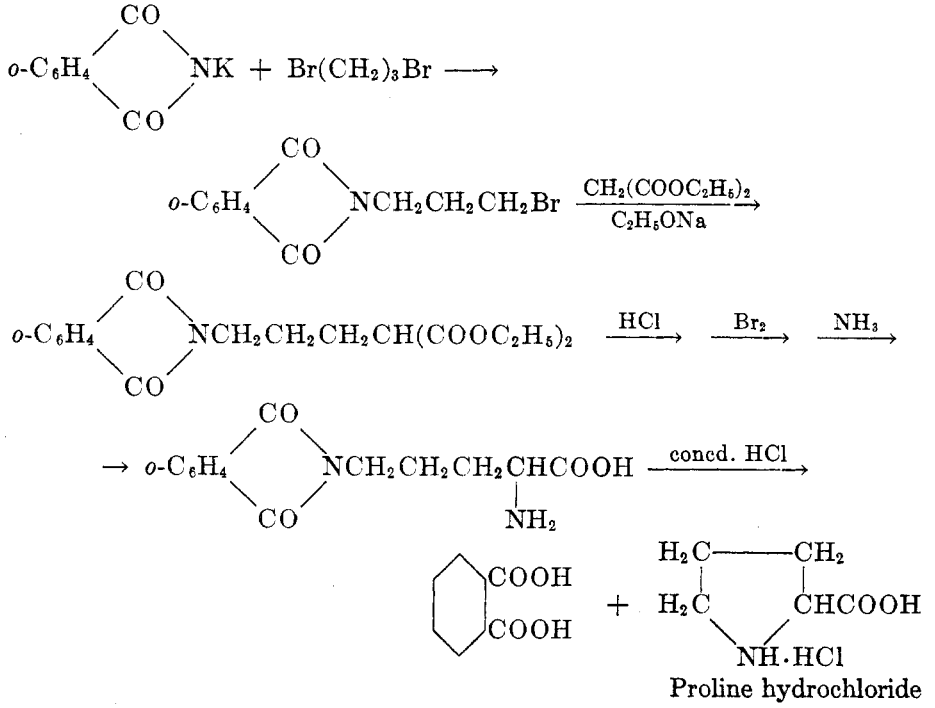
dl-Methionine

The conditions were somewhat changed by Gabriel and Kroseberg (138; *cf.* 274) the following year. This reaction was to become the basis of the Sørensen phthalimidomalonic ester method and similar methods which will be described later in this paper.

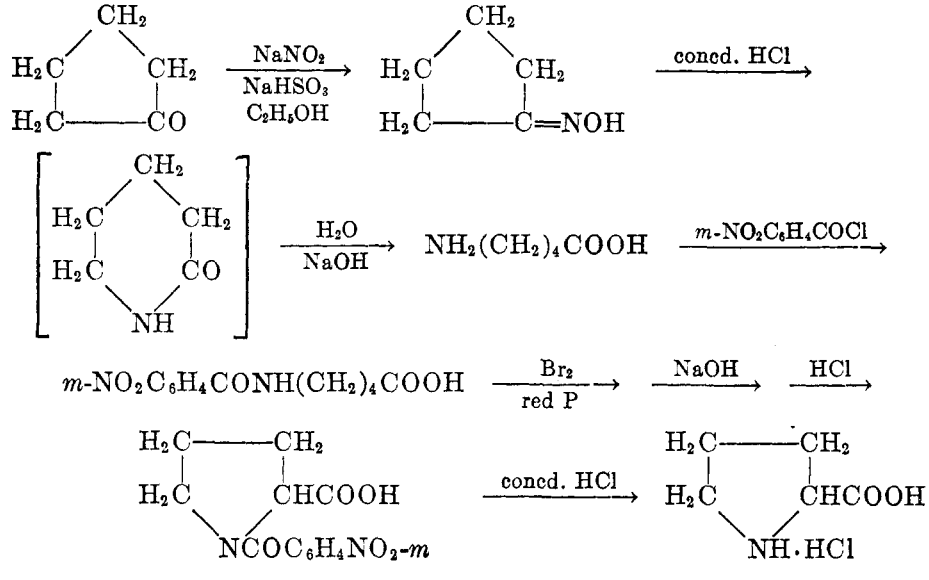


4. Miscellaneous methods

Fischer (125) utilized both potassium phthalimide and malonic ester in his synthesis of proline.



Dunn (95) used a combination of Marvel's (101, 273) cyclopentanone method and Fischer's method (130) to prepare proline.



Proline hydrochloride

Schroeter (276, 277) used double decomposition of *p*-toluenesulfonamide and α-halogen acids for the preparation of amino acids. The toluenesulfonamide is easily recovered.

$$p\text{-}CH_3C_6H_4SO_2NH_2 + ClCH_2COOH \xrightarrow{NaOH}$$

$$p\text{-}CH_3C_6H_4SO_2NHCH_2COOH \xrightarrow[ClCH_2COCl]{CH_3COCl\ or}$$

$$p\text{-}CH_3C_6H_4SO_2Cl + NH_2CH_2COOH$$

Glycine

Alanine is similarly prepared from α-bromopropionic acid.

Gaudry (140, 140a) has recently prepared phenylalanine and tyrosine from benzenediazonium and *p*-methoxybenzenediazonium chlorides and acrylonitrile, all easily available compounds. This reaction appears promising.

$$C_6H_5N_2Cl + CH_2{=}CHCN \rightarrow C_6H_5CH_2CHClCN \xrightarrow{H_2SO_4}$$

$$C_6H_5CH_2CHClCONH_2 \xrightarrow[HCl]{heat} C_6H_5CH_2CHClCOOH \xrightarrow[(NH_4)_2CO_3]{NH_4OH}$$

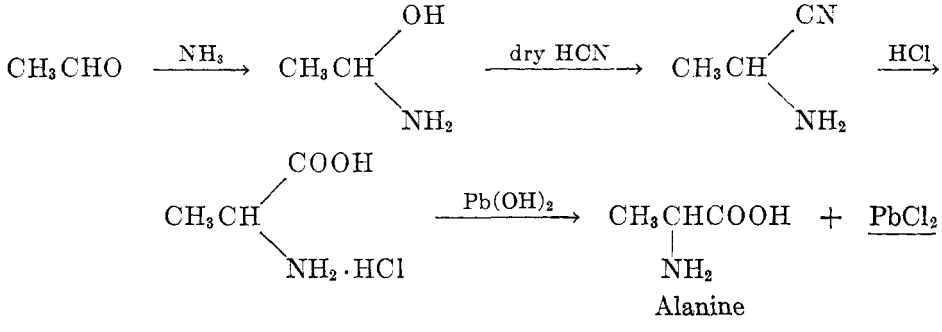
$$C_6H_5CH_2CHNH_2COONH_4$$

B. HYDROLYSIS OF AMINOCYANOHYDRINS (STRECKER SYNTHESIS)

In contrast to the many modifications which the synthesis of amino acids by the amination of α-halogen fatty acids has undergone, Strecker's aminocyanohydrin synthesis has been changed in minor detail only during the past 100 years.

1. Alanine

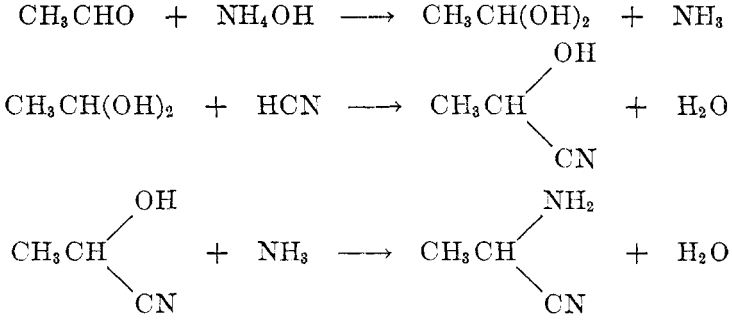
In 1850, A. Strecker (320) treated the addition product of acetaldehyde and ammonia with anhydrous hydrogen cyanide. He hydrolyzed the resulting aminocyanohydrin with hydrochloric acid and, after removing the bound hydrochloric acid with lead hydroxide, recognized that the resulting α-amino acid was the next higher homologue of glycine. Although the structure of glycine had been elucidated a few years previously, this acid was not to be synthesized until eight years later by Cahours (66). Thus the first synthetically prepared α-amino acid was named *alanine* after its mother substance, aldehyde.



The order of adding ammonia and hydrogen cyanide may be reversed.

Lubavin (210) treated acetaldehyde with ammonium cyanide to obtain the aminocyanohydrin in one step, while Barker (21) and Kendall (178) used a mixture of ammonium chloride and sodium cyanide to achieve the same result. Liquid hydrogen cyanide and concentrated ammonium hydroxide (76) or ammonia gas (167) or liquid ammonia (229) have also been used.

The mechanism of the Strecker synthesis has been studied by Sannie (263), who formulates the reaction as follows:



Other mechanisms and by-products are also given. Brodkorb (62) saponifies the aminonitriles with just enough sulfuric acid to form ammonium bisulfate and RCHCOOH.

$$\begin{array}{l} | \\ NH_2 \cdot H_2SO_4 \end{array}$$

Bucherer (63, 64) treated the aldehyde or the aldehyde bisulfite addition product with ammonium carbonate and hydrogen cyanide in hot ethanol and isolated the hydantoin, which was then hydrolyzed to the amino acid.

$$CH_3CH\begin{matrix} OH \\ \diagdown \\ \diagup \\ SO_3Na \end{matrix} + (NH_4)_2CO_3 + HCN \xrightarrow[C_2H_5OH]{\text{heat}}$$

$$CH_3CH\text{——}CO \quad \text{(HN, NH, CO)} \xrightarrow[H_2SO_4]{\text{heat}} CH_3CHCOOH + CO_2 + (NH_4)_2SO_4$$

$$\text{(NH}_2\cdot H_2SO_4\text{)}$$

Methylhydantoin

2. Glycine

Jay and Curtius (169) observed that potassium cyanide and ammonium chloride reacted with 2 moles of formaldehyde rather than 1 mole, as expected from Strecker's experience with alanine. This product, methyleneaminoacetonitrile, was, however, readily hydrolyzed to glycine or glycine ester hydrochloride.

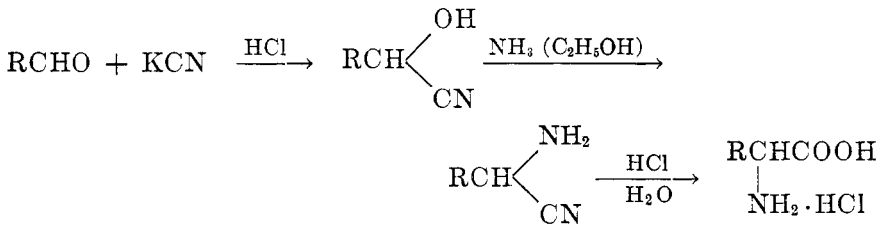
$$2HCHO + KCN + NH_4Cl \xrightarrow{CH_3COOH}$$

$$CH_2{=}NCH_2CN \xrightarrow[\text{or } Ba(OH)_2,\ \text{heat}]{HCl + C_2H_5OH} \underset{\text{Glycine}}{CH_2NH_2COOH}$$

This reaction has been used to prepare glycine or glycine ester hydrochloride in relatively large quantities by Cocking (77), Ling (208), Anslow *et al.* (13), and others.

3. Leucine, valine, isoleucine, and phenylalanine

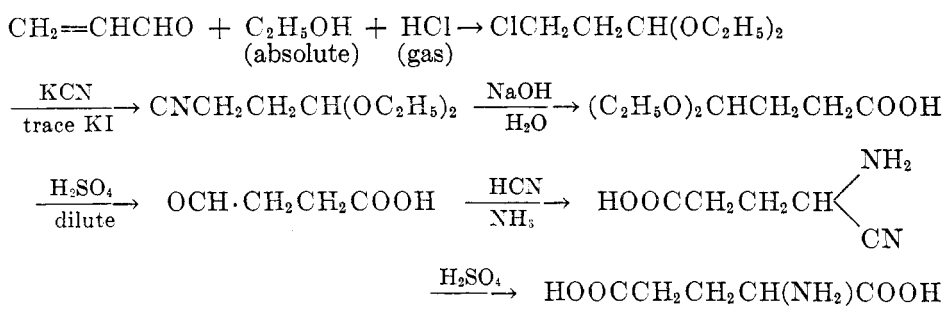
Fourteen years after the synthesis of alanine by A. Strecker, his brother Hermann Strecker (303) prepared leucine hydrochloride from valeraldehyde by the same method. Lipp (209) synthesized valine from isobutyraldehyde by Strecker's original procedure (*cf.* 183), while Ehrlich (103) and Tiemann (307, 308) prepared isoleucine and phenylalanine, respectively, from the corresponding aldehydes as follows:



Bucherer and Steiner (64) also synthesized phenylalanine from benzaldehyde, ammonium carbonate, and hydrogen cyanide *via* the hydantoin.

4. Glutamic acid

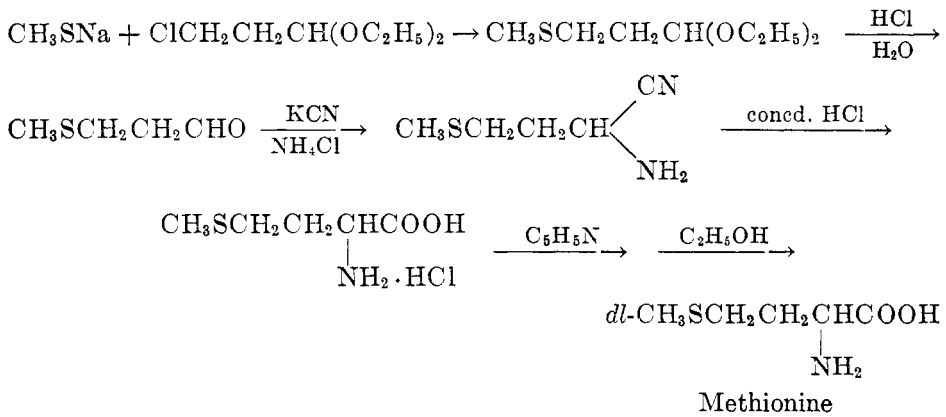
Keimatsu and Sugasawa (176) synthesized glutamic acid from acrolein. This synthesis has academic interest only, as it is far simpler to prepare *dl*-glutamic acid from natural *l*-glutamic acid by racemization.



Glutamic acid

5. Methionine

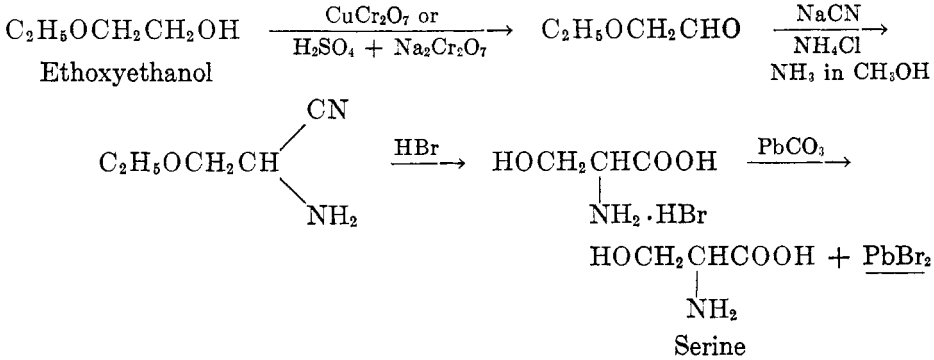
The synthesis of methionine from β-methylthiolpropionaldehyde diethylacetal by Barger and Coyne (19) established the constitution of this amino acid.



Methionine

6. Serine

Dunn and Redemann (97, 256) synthesized this amino acid from the readily available ethylene glycol monoethyl ether (Cellosolve).



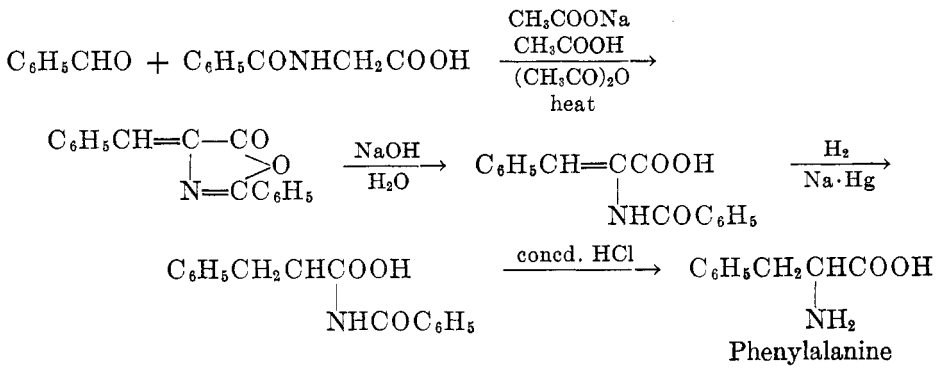
C. CONDENSATION OF AN ALDEHYDE WITH AN ACTIVE METHYLENE GROUP: THE PERKIN REACTION (ERLENMEYER'S SYNTHESIS)

This synthesis is useful for the preparation of the four aromatic amino acids—phenylalanine, tyrosine, tryptophan, and thyroxine, especially the first two.

1. Phenylalanine and tyrosine

(a) By condensation with hippuric acid

Although Erlenmeyer and Lipp (114) synthesized tyrosine from *p*-aminophenylalanine by deamination of the *p*-NH_2 group with nitrous acid anhydride as early as 1883, a general method for the ready preparation of phenylalanine and tyrosine was not described until 10 years later (111, 112). This was accomplished by condensing benzaldehyde or *p*-hydroxybenzaldehyde with hippuric acid by heating with a mixture of sodium acetate, acetic acid, and acetic anhydride, to form the lactimide (azlactone). The azlactone ring was opened with alkali, the unsaturated linkage reduced, and the benzoyl group was removed by acid hydrolysis.

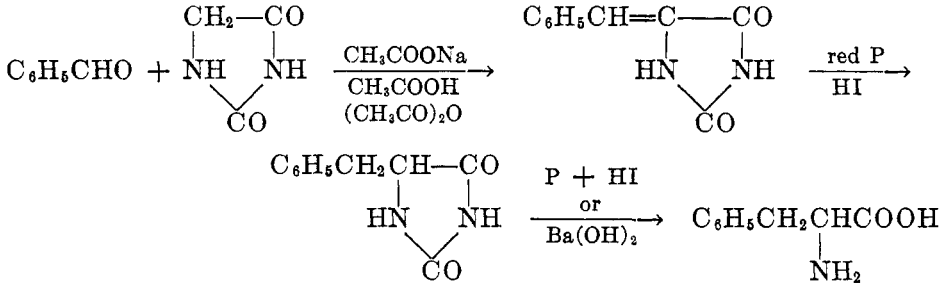


Each step in the original Erlenmeyer hippuric acid synthesis has been modified by various investigators. Thus, Erlenmeyer and Halsey (112) omitted acetic acid, while du Vigneaud *et al.* (325) were unable to obtain efficient condensation (between furfural and hippuric acid) except when fused sodium acetate *alone* was employed.

Various agents have been employed for the reduction of the substituted acrylic acids. Thus, Erlenmeyer (111, 112), Deulofeu (91), and others used sodium amalgam; Harington (155), Lamb (198), and others (163) employed hydrogen iodide, red phosphorus, and glacial acetic acid or acetic anhydride; Johnson used tin and hydrochloric acid (170); Bergmann (31) reduced the double bond catalytically; while Boyd and Robson (51) used ammonium sulfide or sodium hydrosulfide.

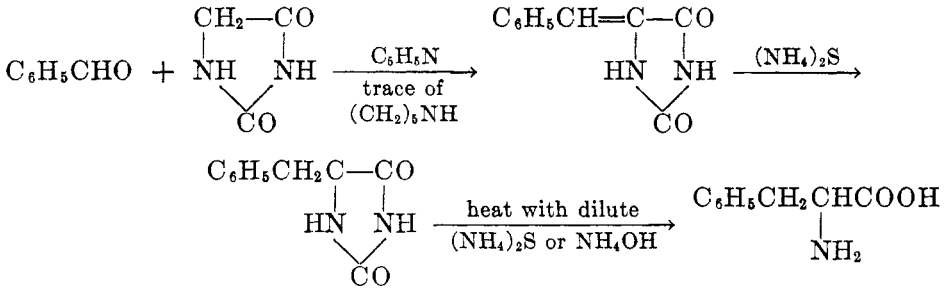
(b) By condensation with hydantoins

Wheeler and Hoffman (333) condensed benzaldehyde or anisaldehyde (*p*-methoxybenzaldehyde) with a preformed cyclic compound containing an active methylene group, i.e., hydantoin.



Phenylalanine

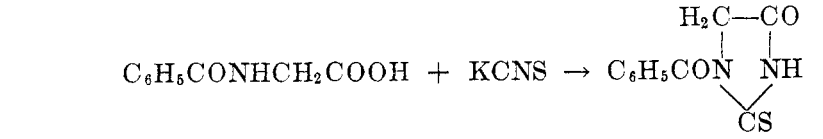
Boyd and Robson (55, 56) replaced Erlenmeyer's acetate condensing agent with an alkaline condensing agent, such as pyridine containing traces of piperidine or diethylamine.

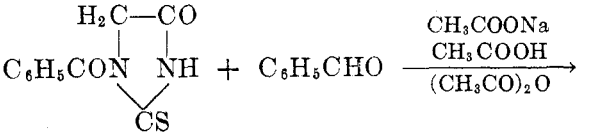


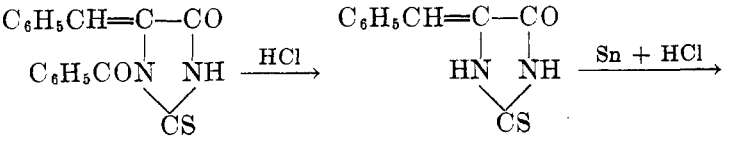
Phenylalanine

(c) By condensation with thiohydantoin

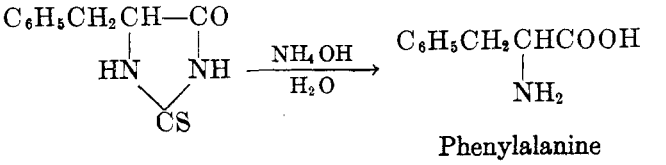
Johnson and O'Brien (170) prepared a thiohydantoin from hippuric acid and then used the Erlenmeyer–Wheeler method.







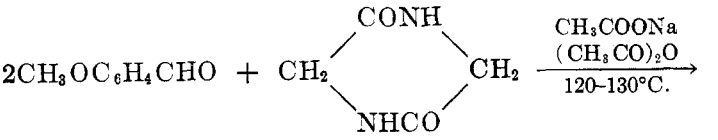
Benzoylbenzylthiohydantoin



Phenylalanine

(d) By condensation with diketopiperazine (glycine anhydride)

Sasaki (228) used diketopiperazine as the ring structure containing an active methylene group.



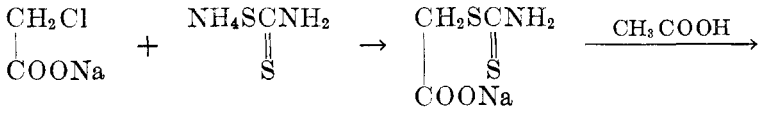
3,6-dianisylidene-2,5-diketopiperazine $\xrightarrow[HI]{\text{red P}}$

p-$HOC_6H_4CH_2CHNH_2COOH + CH_3I$

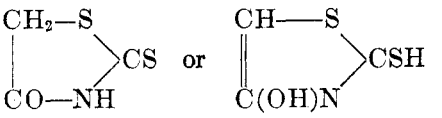
Tyrosine

(e) By condensation with rhodanine (2-mercapto-4-oxythiazole)

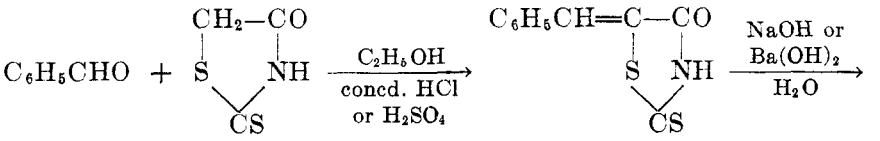
Gränacher (146, 147) used the well-known reactivity of rhodanine with aromatic aldehydes to prepare amino acids.

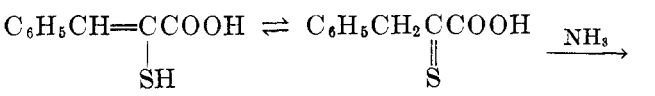


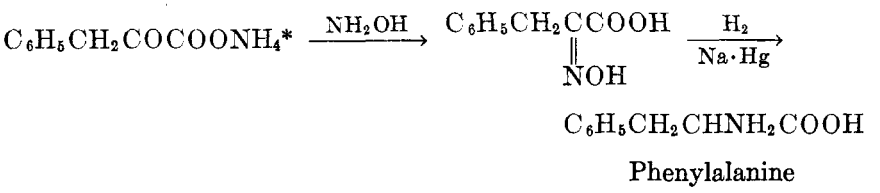
Ammonium dithiocarbamide



Rhodanine







Phenylalanine

* If NH_2OH is used on sulfhydrylcinnamic acid, the oxime of phenylpyruvic acid is formed directly.

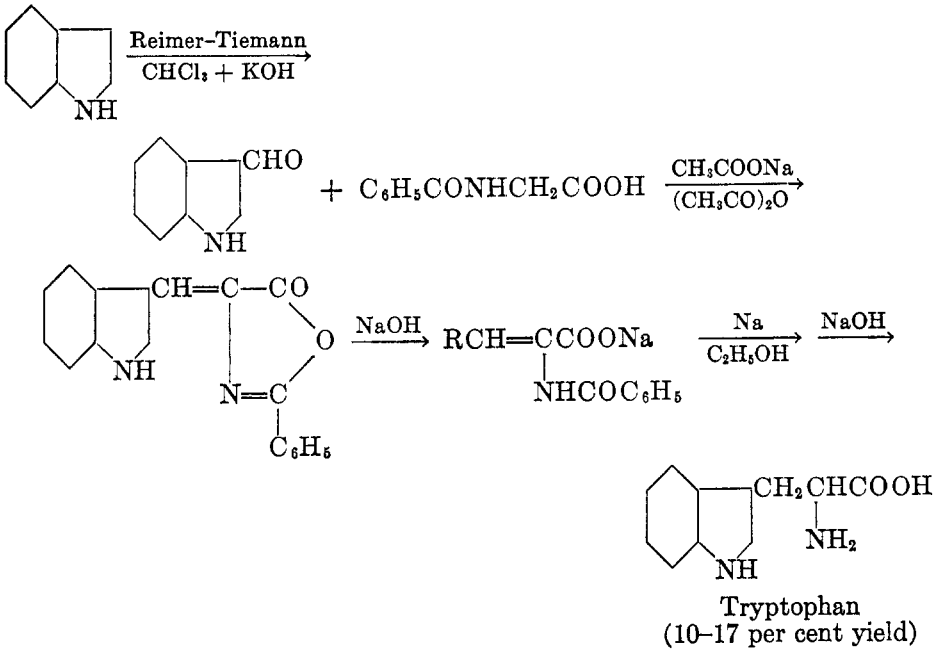
(f) By condensation with acetylglycine

Herbst and Shemin (159) used acetylglycine rather than benzoylglycine (hippuric acid) for the synthesis of phenylalanine.

2. Tryptophan

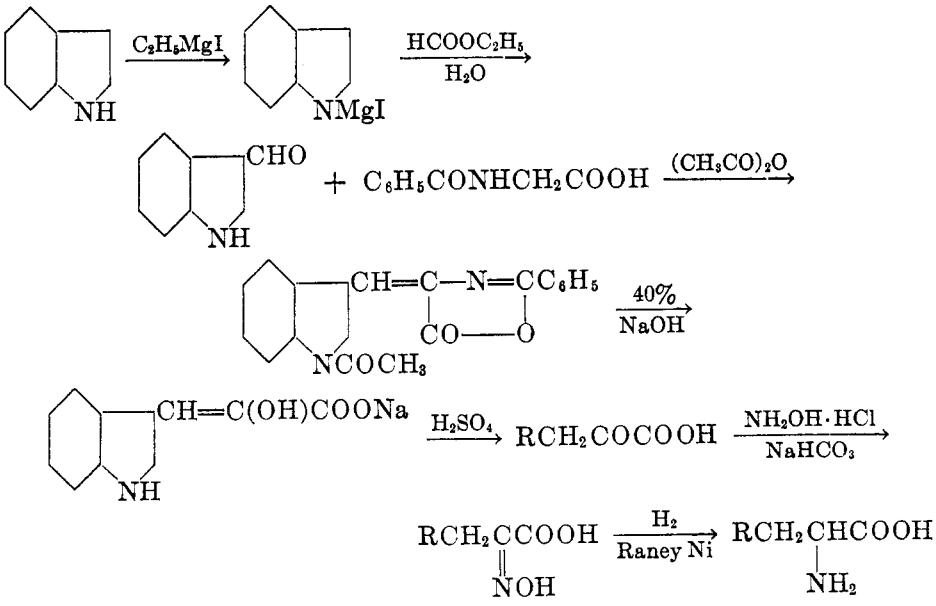
(a) By condensation with hippuric acid

This important amino acid was first synthesized by Ellinger and Flamand (105) according to Erlenmeyer's original method except, of course, avoiding treatment with hot mineral acids.



Tryptophan
(10–17 per cent yield)

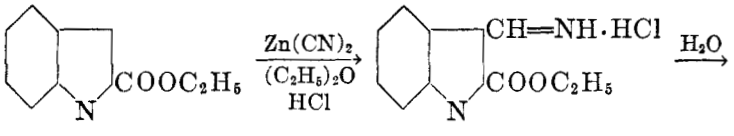
Baugess and Berg (26) prepared indolepyruvic acid by the azlactone method and then made the amino acid by reduction of the oxime. The indolealdehyde was prepared from indole by Majima and Kotake's method (217).



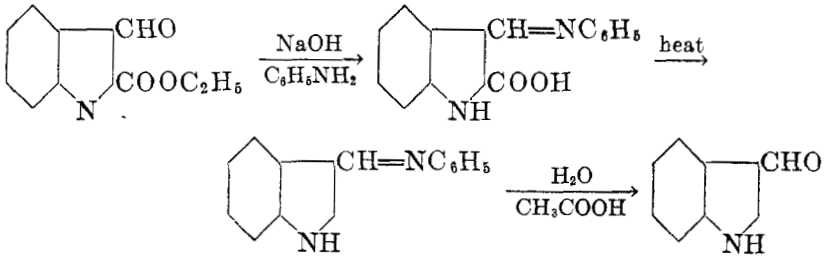
(b) By condensation with hydantoin

Majima and Kotake (217) condensed indolealdehyde, prepared from indolemagnesium iodide (*cf.* above) and ethyl formate, with hydantoin according to Wheeler and Hoffman (333). The β-indolalhydantoin was reduced with sodium amalgam and the hydantylskatole was hydrolyzed with baryta.

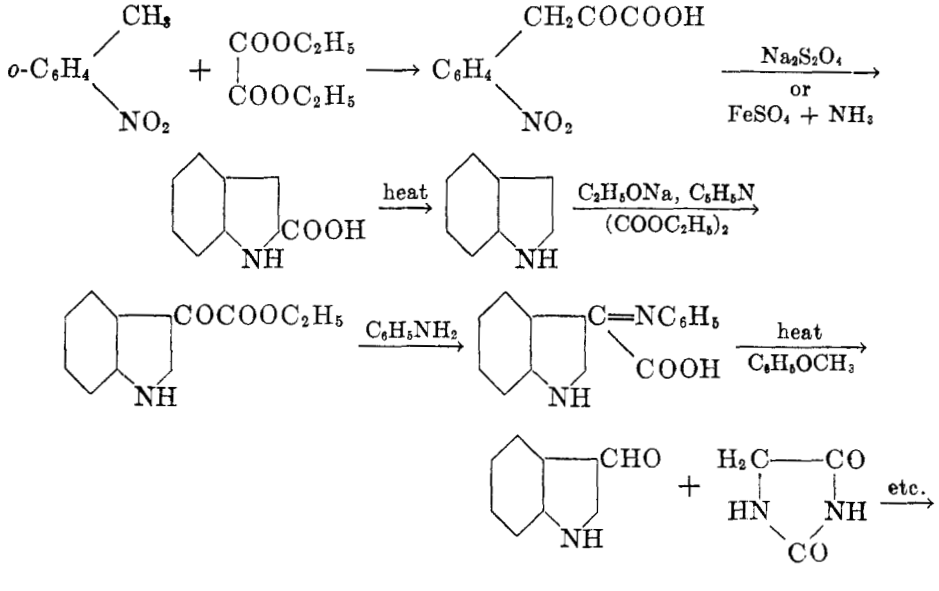
Boyd and Robson (58) condensed indole-3-aldehyde with hydantoin, using pyridine–piperidine, and obtained tryptophan from the resulting indolalhydantoin by simultaneous reduction and hydrolysis with ammoniacal ammonium sulfide. Indolealdehyde was prepared either by the Reimer–Tiemann reaction or *via* the Gatterman synthesis (57).



2-Carboxyethylindole



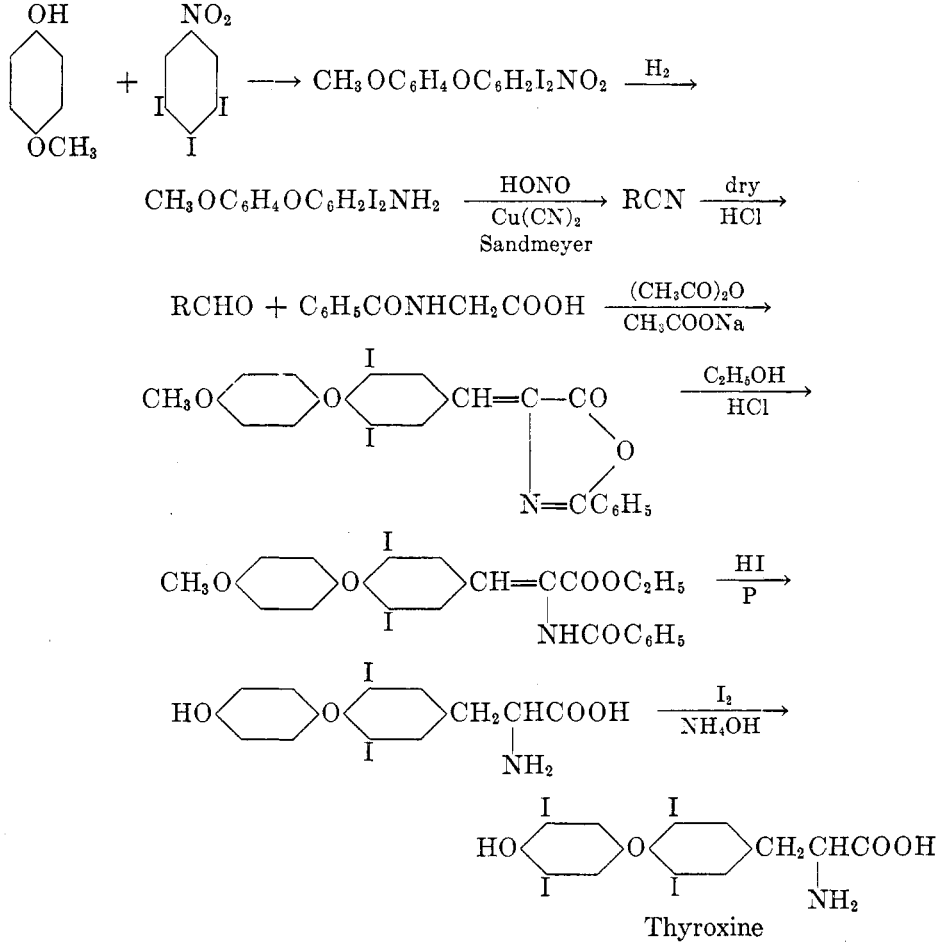
Elks *et al.* (104) prepared indole-3-aldehyde from *o*-nitrotoluene and condensed the former with hydantoin in the usual fashion.



3. Thyroxine

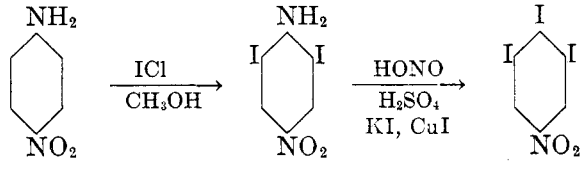
(a) By condensation with benzoylglycine

Harington and Barger (154, 265) prepared thyroxine from the proper aldehyde according to Erlenmeyer's hippuric acid method.



Thyroxine is [β-(3,5-diiodo-4-(3′,5′-diiodo-4′-hydroxyphenoxy)]phenyl-α-amino-propionic acid.

Triiodonitrobenzene can be prepared from *p*-nitroaniline as follows (265):



4. Histidine

(a) By azlactone synthesis

Pyman (254) used this reaction for his second synthesis of histidine. Glyoxaline formaldehyde was prepared by nitric acid oxidation of the corresponding imidazolylmethyl alcohol. The remainder of the synthesis was essentially according to Erlenmeyer.

5. Lysine

(a) By condensation with malonic acid

Sugasawa (304) has synthesized *dl*-lysine from acrolein in an elaborate procedure involving pyridine–piperidine condensation with malonic acid (*cf.* Section III,B,4 and Section III,C,1,(b)).

$$CH_2{=}CHCHO \xrightarrow[HCl]{C_2H_5OH} ClCH_2CH_2CH(OC_2H_5)_2 \xrightarrow[KCN]{KI}$$

$$CNCH_2CH_2CH(OC_2H_5)_2 \xrightarrow[Na + C_2H_5OH]{H_2} NH_2CH_2CH_2CH_2CH(OC_2H_5)_2$$

$$\xrightarrow[KOH]{C_6H_5COCl} C_6H_5CONH(CH_2)_3CH(OC_2H_5)_2 \xrightarrow[HCl]{H_2O}$$

$$RCHO + CH_2(COOH)_2 \xrightarrow[\text{trace } C_5H_{11}N]{C_5H_5N} RCH{=}C(COOH)_2 \xrightarrow{H_2} \xrightarrow{\text{heat}}$$

$$RCH_2CH_2COOH \xrightarrow{Br_2} RCH_2CHBrCOOH \xrightarrow{NH_4OH}$$

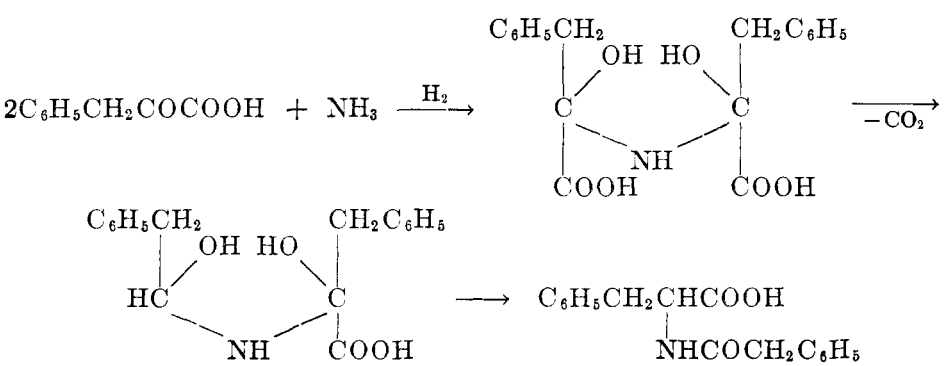
$$RCH_2CHNH_2COOH \xrightarrow[\text{heat}]{HCl} \underset{\cdot HCl}{NH_2CH_2CH_2CH_2CH_2CHNH_2COOH} \cdot HCl$$

Lysine dihydrochloride

D. REDUCTION OF α-KETO GROUPS AND THEIR DERIVATIVES

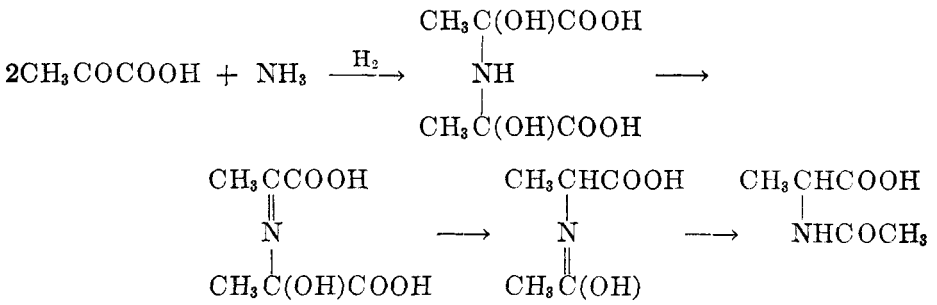
1. Reducion of α-keto groups in the presence of ammonia: alanine, leucine, isoleucine, glutamic acid, etc.

In 1899, Erlenmeyer and Kunlin (113) made the interesting observation that when phenylpyruvic acid was reduced in the presence of ammonia, *N*-phenylacetylphenylalanine was formed.



The phenylacetylphenylalanine could then be hydrolyzed to phenylalanine and phenylacetic acid.

De Jong (173) prepared acetylalanine by reduction of ammonium pyruvate. He formulated the reaction as follows:



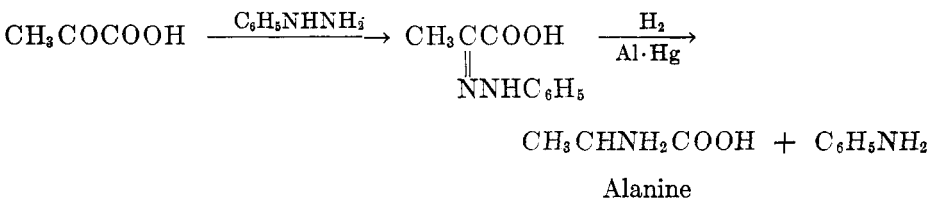
Schoenheimer and Ratner (274), using the active palladium method of Knoop and Oesterlin (186, 187), prepared alanine, phenylalanine, norleucine, tyrosine, aspartic acid, and glutamic acid fron their respective α-keto analogues. When ammonia containing an excess of isotopic nitrogen was used, the resulting amino acids contained an excess of N^{15} in the α-NH_2 group.

$$RCOCOOH + NH_3 \xrightarrow[Pd]{H_2} RCHNH_2COOH + H_2O$$

Other investigators have used palladium or platinum for preparing glycine, alanine, and other amino acids (15, 90, 284). This then appears to be a useful general method for the preparation of α-amino acids provided the corresponding keto acids are available. In fact, Knoop and Oesterlin (186, 187) and others have pointed out that this may be the reaction mechanism for the formation in the body of α-amino acids from α-keto acids.

2. Reduction of phenylhydrazones: alanine, leucine, isoleucine, valine, phenylalanine, tyrosine, etc.

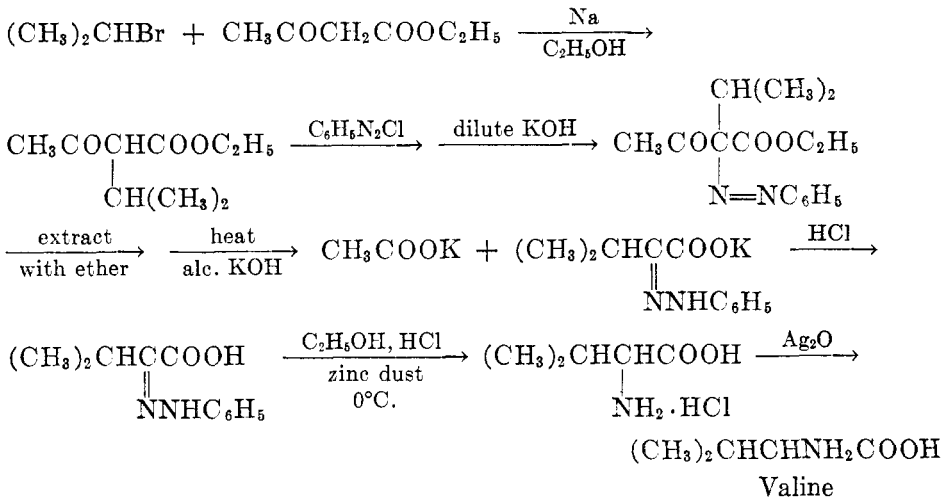
Fischer and Groh (127) obtained a small yield of alanine by reduction of pyruvic acid phenylhydrazone with aluminum amalgam.



Alanine

Feofilaktov and collaborators (116, 117, 118, 121, 122, 123) have modified the Fischer–Groh method into a general procedure for the synthesis of α-amino acids. They have successfully prepared alanine, leucine, isoleucine, valine, phenylala-

nine, tyrosine, and norleucine from the corresponding substituted acetoacetic esters.

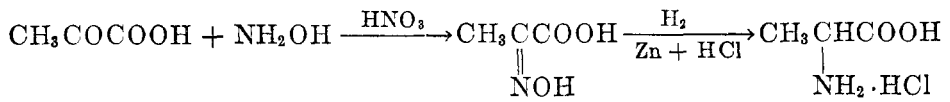


Very high yields are reported.

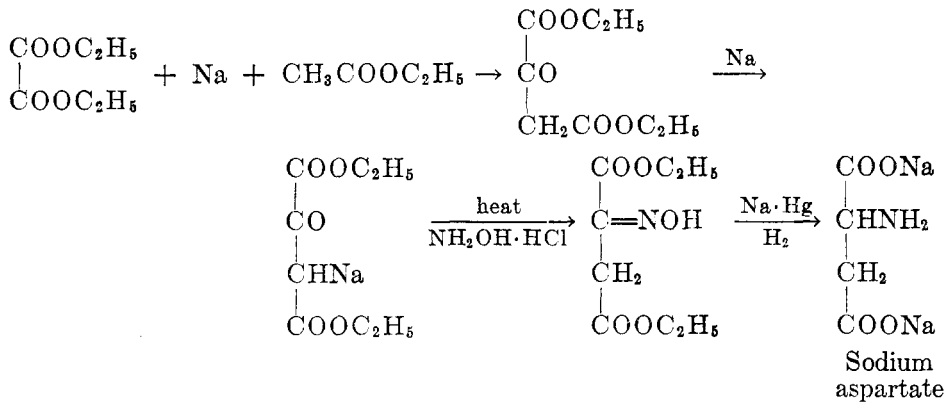
3. Reduction of oximes

(a) Preparation of oxime with hydroxylamine: aspartic acid, asparagine, glutamic acid, alanine, phenylalanine, tyrosine, etc.

Gutknecht (150) in 1880 prepared alanine by the reduction of pyruvic acid oxime (*cf.* 280).

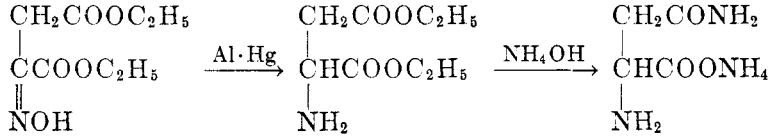


A few years later, Piutti (247) used this observation in his classical synthesis of aspartic acid from oxalacetic ester.



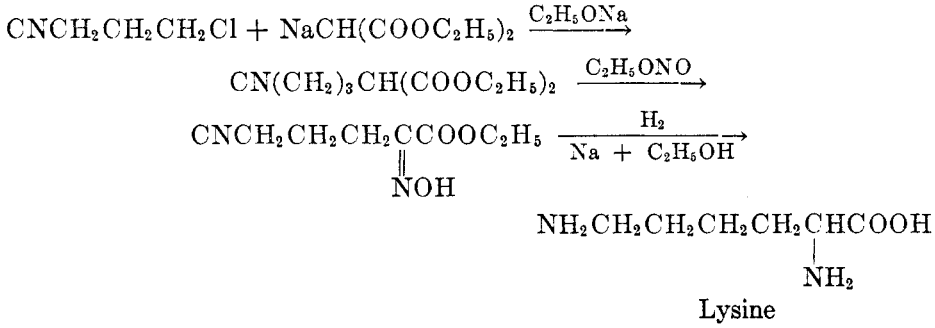
Granacher (146, 147) also used sodium amalgam to reduce ketoximes, Baugess and Berg (26) and Shemin and Herbst (280) used nickel and platinic oxide, respectively, while β-amino acids have been prepared by the electrolytic reduction of substituted β-oximinoacetoacetic esters (14, 259, 267, 332).

Cocker (75) utilized Piutti's (247) method to prepare asparagine.



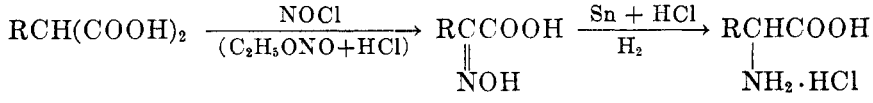
(b) Preparation of oxime by action of nitrite on substituted malonic acid or ester: lysine, leucine, phenylalanine

Fischer and Weigert (129) synthesized lysine from γ-chlorobutyronitrile and malonic ester by means of ethyl nitrite.



Lysine

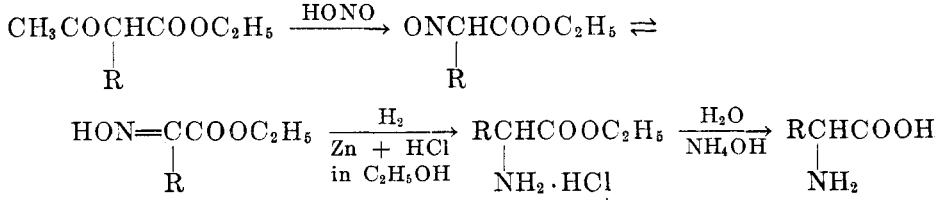
Onishchenko (239) modified Fischer's method to prepare leucine and phenylalanine in good yields.



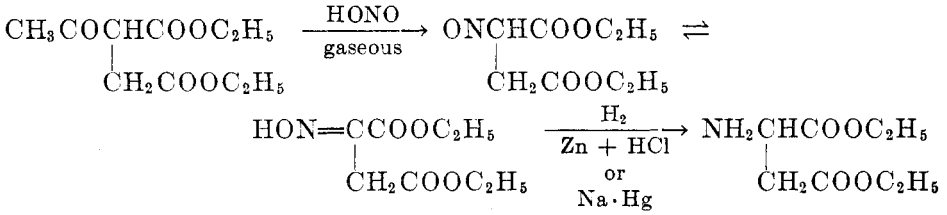
(c) Preparation of oxime by action of nitrite on substituted acetoacetic ester: methionine, threonine, hydroxyproline, aspartic acid, glutamic acid, leucine, isoleucine, etc.

Bouveault and Locquin (54) used acetoacetic ester rather than malonic ester in the preparation of amino acids. Since their publication in 1906, numerous investigators have used this method for the synthesis of many amino acids.

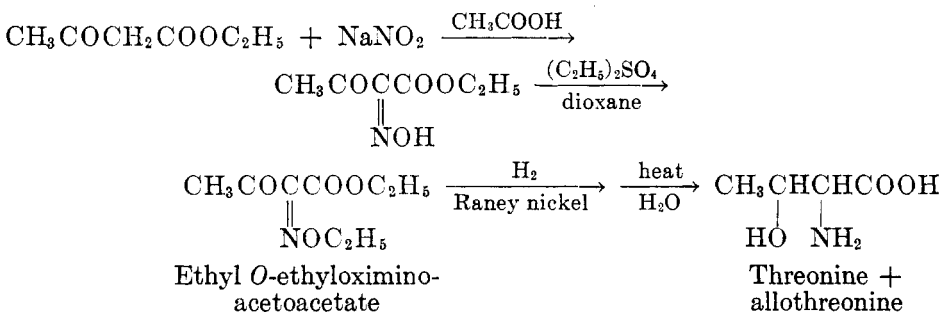
(1) *Alanine, valine,* and *isoleucine* by Bouveault and Locquin (54).



(*2*) *Aspartic acid* by Schmidt and Widmann (271).



(*3*) *Threonine* by Adkins and Reeve (6).



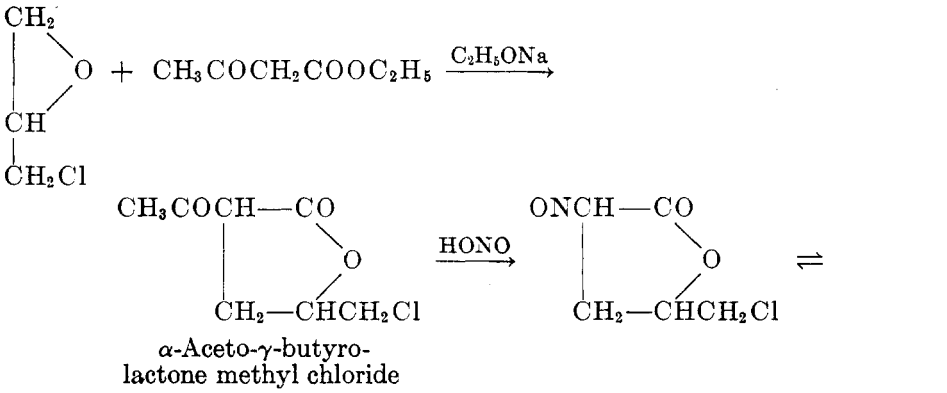
It should be noted that this synthesis does not involve a substituted acetoacetic ester and that the acetyl group is not removed by nitrosation.

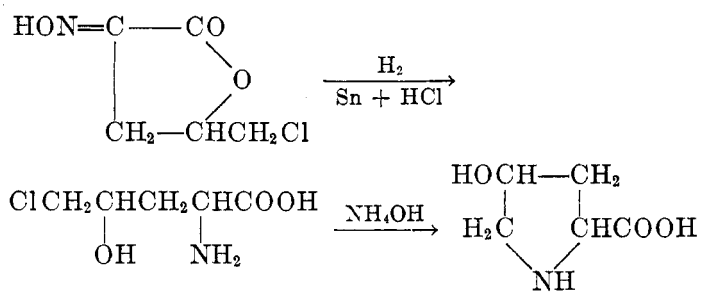
(*4*) *Other amino acids:* McIlwain and Richardson (228) used the Bouveault–Locquin method for glutamic acid (good yield) and hydroxyproline (poor yield). They were unable to prepare threonine, as the hydroxyl group was reduced by their platinic oxide catalyst.

Hamlin and Hartung (151) prepared tyrosine, isoleucine, and a number of other amino acids by treatment of substituted acetoacetic esters with butyl nitrite (*cf.* 236) and sulfuric acid. The α-oximino esters were reduced with hydrogen and palladium. The yields were very good.

(d) Syntheses from α-oximino-γ-butyrolactone

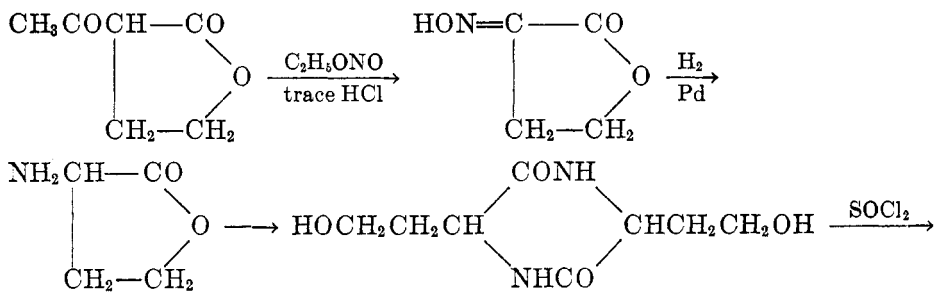
(*1*) *Hydroxyproline:* Feofilaktov (119, 120) used the action of nitrous acid on an acetobutyrolactone to prepare hydroxyproline.



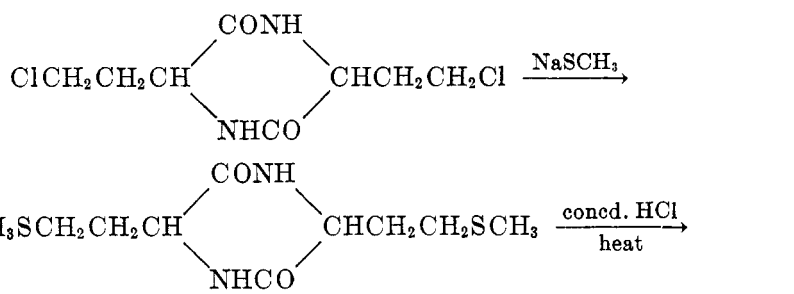


Hydroxyproline

(2) *Methionine:* Snyder *et al.* (285, 286, 287) used α-aceto-γ-butyrolactone from ethylene oxide and acetoacetic ester to prepare methionine, essentially as follows:



3,6-Bis(β-hydroxyethyl)-2,5-diketopiperazine



Methionine

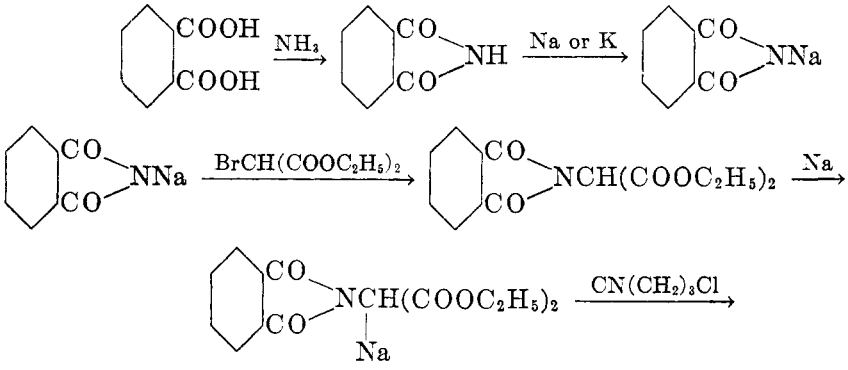
E. ALKYLATION OF AMINOMALONIC ACIDS

1. Phthalimidomalonic ester

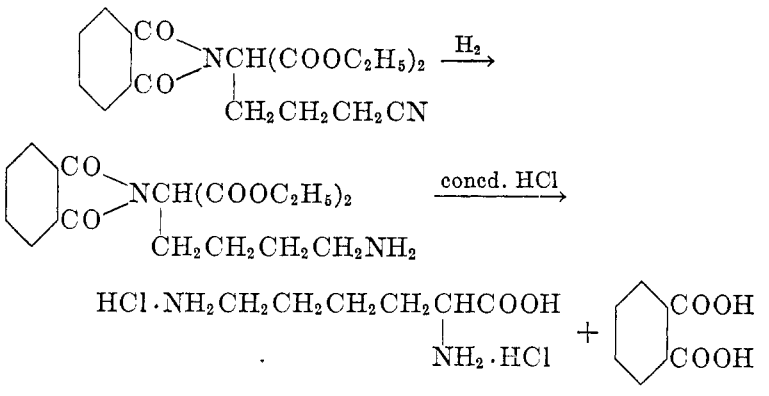
(a) Lysine

In 1889, Gabriel and Kroseberg (138) prepared glycine by treating chloroacetic ester with potassium phthalimide and hydrolyzing the resulting phthalimido-

acetic ester (*cf.* Section III,A,3). Sørensen (292) used an interesting modification of this procedure for the synthesis of lysine (241, 293).



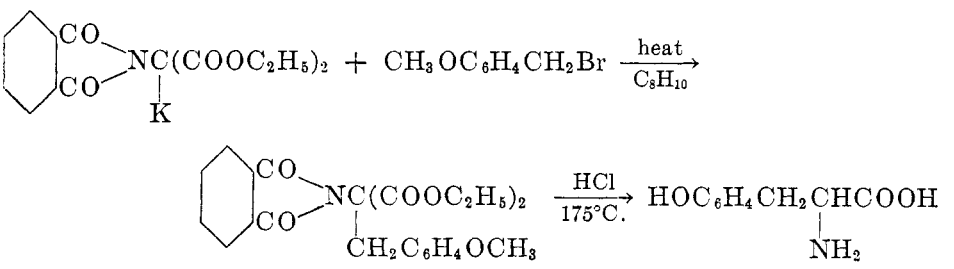
Diethyl sodiophthalimidomalonate



Lysine dihydrochloride

(b) Phenylalanine and tyrosine

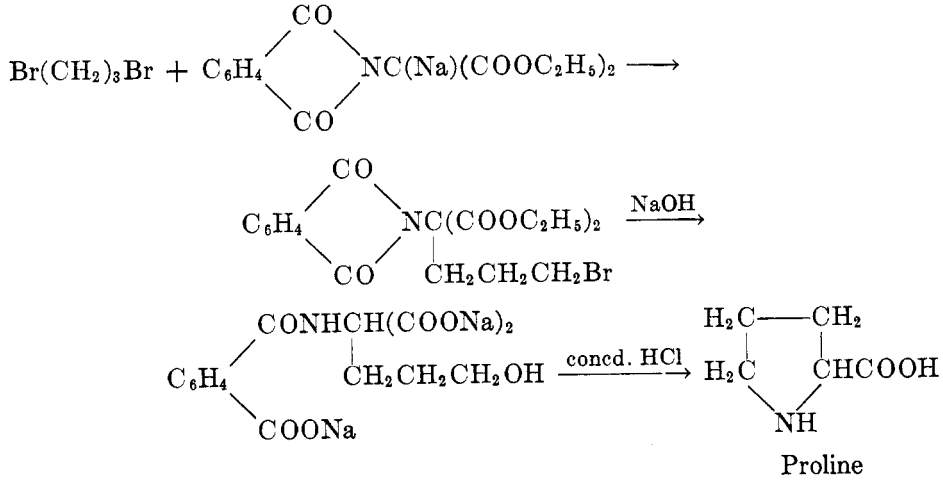
Sørensen (293) used the sodium phthalimidomalonic ester method for the synthesis of phenylalanine from benzyl chloride, while Stephen and Weizmann (298) synthesized tyrosine by the same general method from *p*-methoxybenzyl bromide.



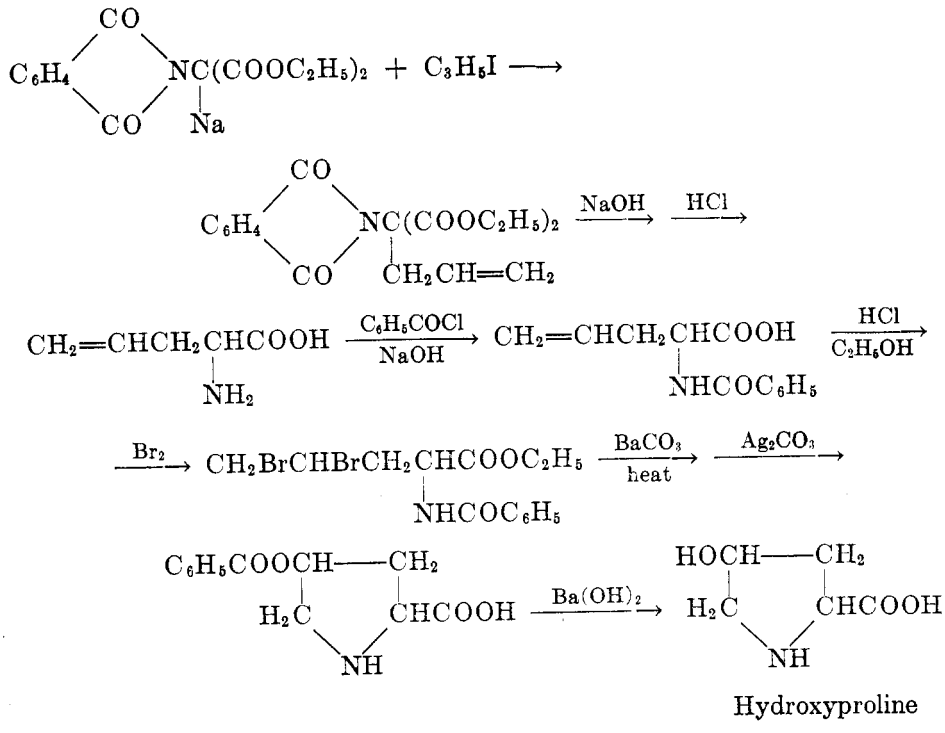
Tyrosine

(c) Proline and hydroxyproline

These amino acids were synthesized as follows (294, 297):



Proline

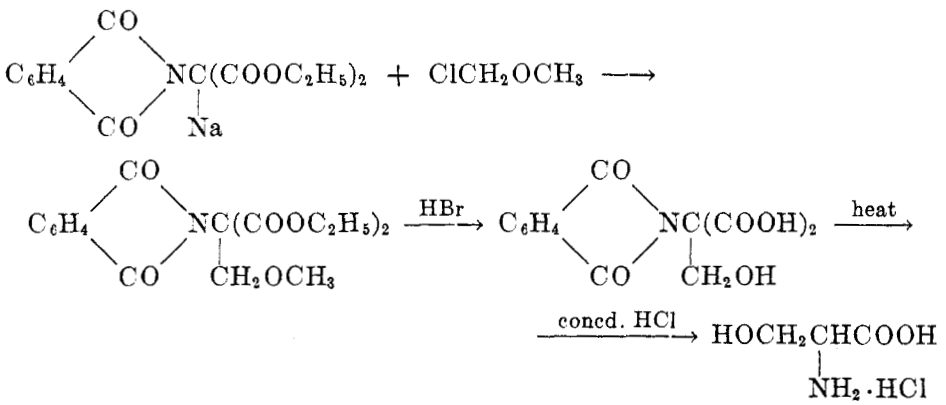


Hydroxyproline

The migration of the benzoyl group from nitrogen to oxygen is to be noted.

(d) Serine and β-hydroxynorvaline

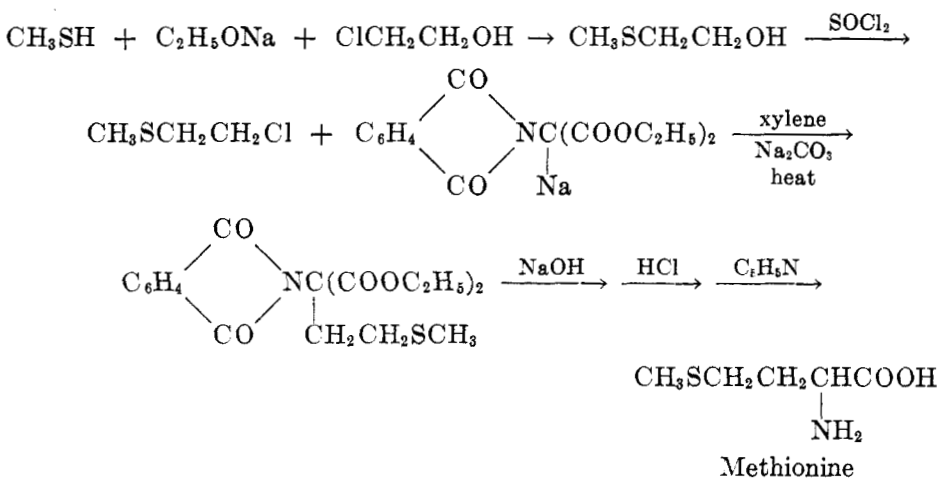
Serine has been synthesized from chloromethyl ether in the absence of a solvent (216, 231).



β-Hydroxynorvaline (3) has been prepared in a similar fashion from α-chloro-*n*-propyl ethyl ether.

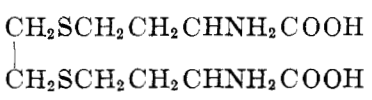
(e) Methionine and cystine

Barger and Weichselbaum (20; *cf.* 51) used Sørensen's method to synthesize methionine.



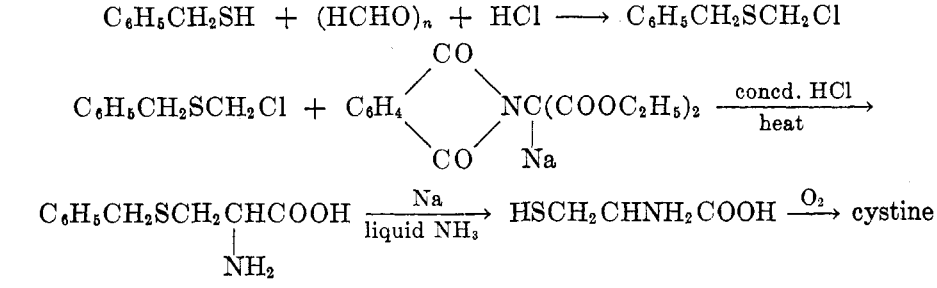
Methionine

Snyder *et al.* (288) obtained ω, ω′-bimethionine as a by-product of the Barger-Weichselbaum method.



ω,ω′-Bimethionine

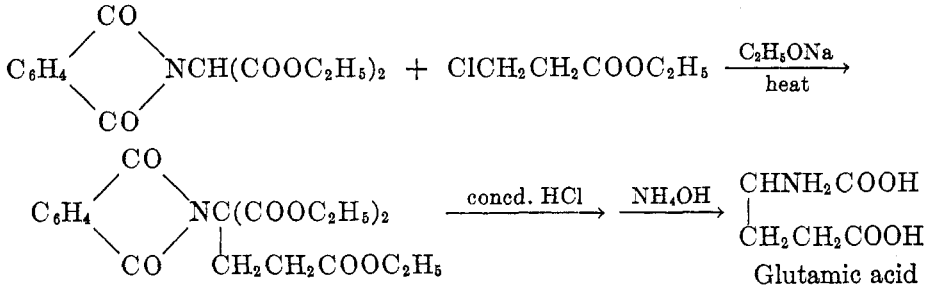
Wood and du Vigneaud (340) prepared cystine from chloromethyl benzyl sulfide.



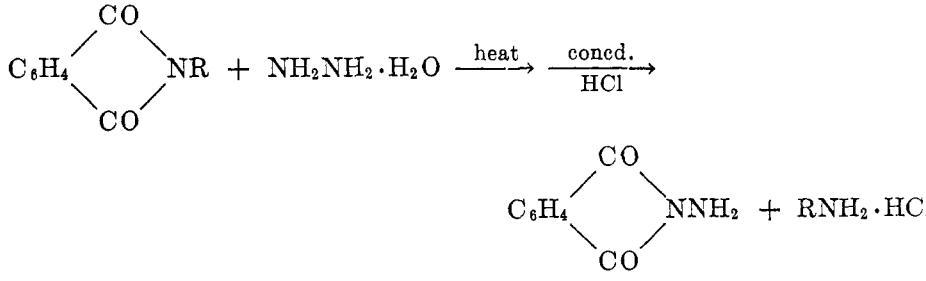
Kuhn and Quadbeck (195) synthesized lanthionine from dichlorodimethyl sulfide.

(f) Glutamic acid and aspartic acid

Dunn and Smart (98) used the Sørensen method for the synthesis of aspartic acid from chloroacetic ester, while Marvel and Stoddard (224) prepared glutamic acid from ethyl β-chloropropionate or from methyl acrylate.



One of the chief hindrances to the use of the Gabriel–Sørensen method is the difficulty of splitting off the phthalic acid. King and Robinson (180) have facilitated this reaction by the use of hydrazine.



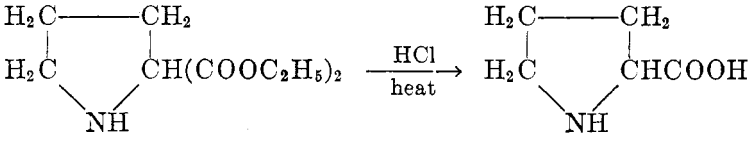
2. Aminomalonic ester

(a) Proline

Putochin (251) apparently was the first to use aminomalonic ester for the synthesis of an amino acid.

$$CH_2(COOC_2H_5)_2 + Na + CH_3ONO \xrightarrow[C_2H_5OH]{\text{absolute}}$$

$$HON{=}C(COOC_2H_5)_2 \xrightarrow[\text{or Pt sponge}]{H_2,\ Al\cdot Hg} NH_2CH(COOC_2H_5)_2 \xrightarrow[Br(CH_2)_3Br]{C_2H_5ONa}$$



Proline
(25 per cent yield)

Aminomalonic acid was first obtained by Baeyer (17) by reduction of nitrosomalonic acid, but it was not readily prepared until 1880 when Conrad and Bischoff (78) synthesized nitrosomalonic ester from diethyl sodiomalonate and nitrous acid. Nitrosomalonate can be reduced by Raney nickel (203).

(b) Glycine, leucine, and phenylalanine

These amino acids were synthesized by Locquin and Cerchez (211, 212, 213) from aminomalonic ester.

$$NH_2C(COOC_2H_5)_2 + RCl \xrightarrow{\text{isoamyl methyl ether}}$$
$$\quad Na$$

$$NH_2C(COOC_2H_5)_2 \xrightarrow[\text{superheated steam}]{H_2O} RCHCOOH + CO_2 + C_2H_5OH$$
$$\quad R \qquad\qquad\qquad\qquad\quad NH_2$$

3. Benzoylaminomalonic ester

(a) Glutamic acid, aspartic acid, glycine, alanine, leucine, valine, and phenylalanine

Dunn, Redemann, and their coworkers introduced diethyl benzamidomalonate as a general reagent for the preparation of α-amino acids (99, 243, 255). Good to excellent yields were obtained.

$$HON{=}C(COOC_2H_5)_2 \xrightarrow[H_2]{Al} NH_2CH(COOC_2H_5)_2 \xrightarrow[Na_2CO_3]{C_6H_5COCl}$$

$$C_6H_5CONHCH(COOC_2H_5)_2 \xrightarrow[BrCH_2CH_2COOC_2H_5]{C_2H_5ONa}$$

$$C_6H_5CONHC(COOC_2H_5)_2 \xrightarrow[\text{or HBr}]{HCl} \xrightarrow{Ag_2O} NH_2CHCOOH$$
$$\quad CH_2CH_2COOC_2H_5 \qquad\qquad\qquad\qquad\quad CH_2CH_2COOH$$

Glutamic acid
(52 per cent yield)

4. Acetylaminomalonic ester

(a) Leucine, norleucine, and phenylalanine

Dakin (87) treated acetylaminomalonic ester from aminomalonic ester and acetic anhydride with isobutylene oxide to prepare γ-hydroxyleucine, which may be a constituent of proteins, and proposed an analogous synthesis of hydroxyproline from epichlorohydrin. Snyder *et al.* (289) and Albertson *et al.* (7, 10) have recently prepared phenylalanine and leucine in from 30 to 60 per cent yields by the alkylation of diethyl sodioacetylaminomalonate.

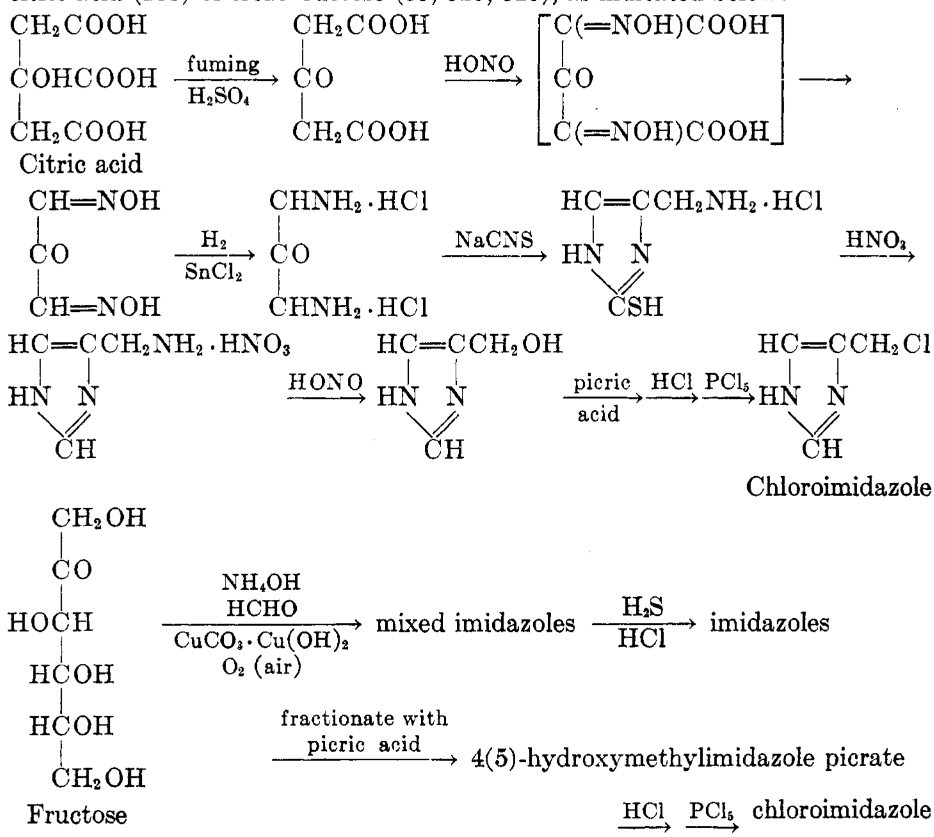
$$C_6H_5CH_2Cl + CH_3CONHCH(COOC_2H_5)_2 \xrightarrow{C_2H_5ONa}$$

$$\underset{\displaystyle CH_2C_6H_5}{CH_3CONHC(COOC_2H_5)_2} \xrightarrow{HCl} \xrightarrow{NH_4OH} \underset{\text{Phenylalanine}}{C_6H_5CH_2CHNH_2COOH}$$

Snyder *et al.* (289) were unable to prepare isoleucine or valine from *sec*-butyl bromide and isopropyl bromide, respectively, i.e., secondary halides are of little use in any variation of the Gabriel–Sørensen method.

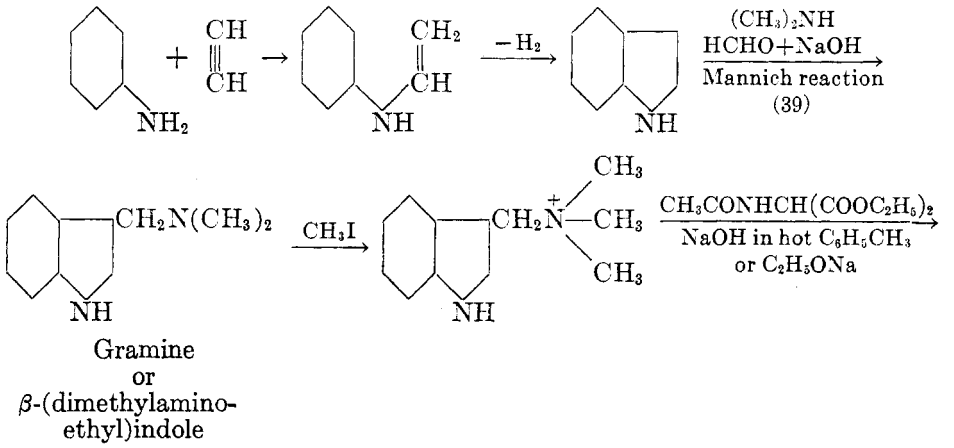
(b) Histidine

Albertson and Archer (7) synthesized histidine by the alkylation of acetylaminomalonic ester with 4(or 5)-chloroimidazole. The latter was prepared from citric acid (188) or from fructose (89, 328, 329), as indicated below:

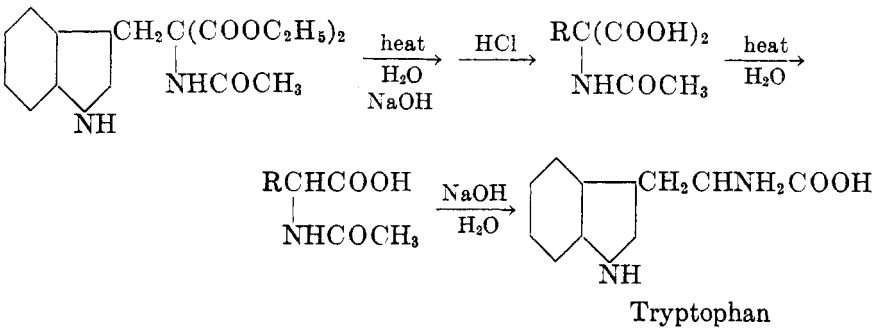


(c) Tryptophan

Snyder *et al.* (164, 290, 291) and Albertson *et al.* (8, 9) prepared tryptophan from aniline *via* indole (218) and gramine (194) in large quantities and excellent yield.



Gramine
or
β-(dimethylamino-
ethyl)indole

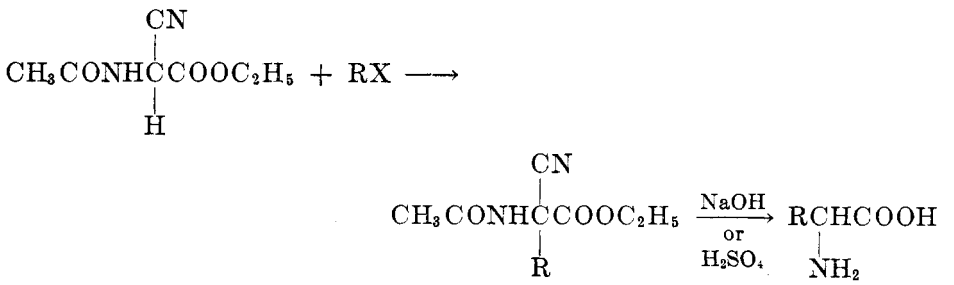


Tryptophan

5. Acetylaminocyanoacetic ester

(a) Valine, methionine, phenylalanine, tryptophan, histidine, etc.

Albertson and Tuller (10) have found that acetamidocyanoacetic ester can be used as a general method for preparing amino acids. The yields varied from 60 to 80 per cent.

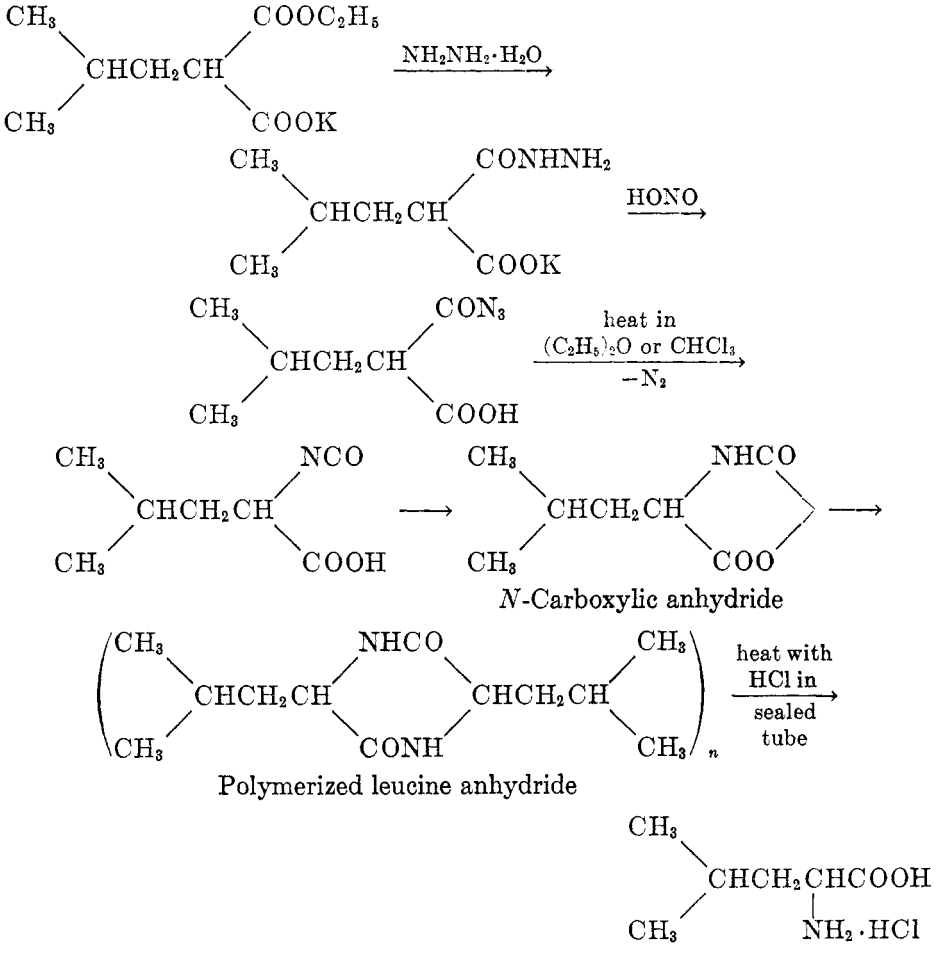


F. AZIDE SYNTHESIS

1. Potassium ethyl malonate: Curtius reaction

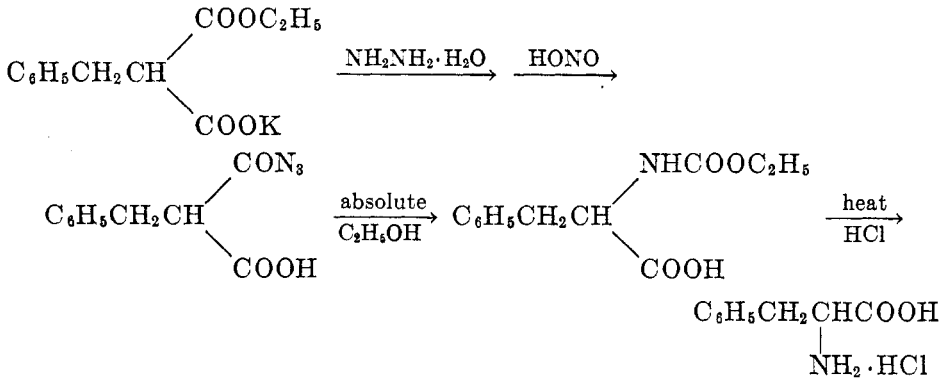
(a) Alanine, valine, leucine, and glycine

Curtius and Sieber (83, 84, 85) found that one of the carboxyl groups of malonic acid could be converted into an amino group by way of the azide. Thus, substituted malonic acids should be sources of α-amino acids. This method may be of special value when bromination of substituted malonic acids cannot be carried out owing to the reactivity of the substituent group (*cf.* Section III,A,2).



(b) Phenylalanine

If the azide is treated with absolute alcohol, the reaction takes a slightly different course and the amino acid is formed *via* the urethan.



Azides are toxic and explosive.

2. *Cyanoacetic ester: Curtius reaction*

(a) Glycine

Sah (261) proposed the use of the azides of cyanoacetic ester for the synthesis of α-amino acids. The paper which has come to the author's attention, however, only describes the synthesis of glycine, although other "paper" syntheses are mentioned.

$$CNCH_2COOC_2H_5 \xrightarrow{NH_2NH_2\cdot H_2O} CNCH_2CONHNH_2 \xrightarrow[HCl]{NaNO_2}$$

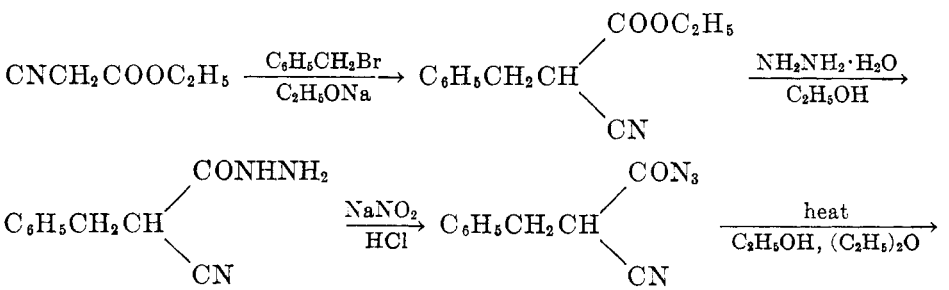
$$CNCH_2CON_3 \xrightarrow[(C_2H_5)_2O]{C_2H_5OH} CNCH_2NHCOOC_2H_5 \xrightarrow[heat]{Ba(OH)_2}$$

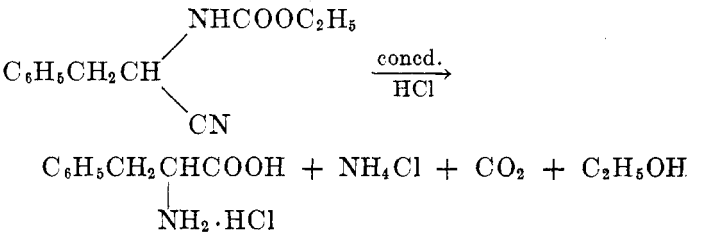
$$\xrightarrow{H_2SO_4} NH_2CH_2COOH$$

Glycine

(b) Leucine, valine, norleucine, tyrosine, and phenylalanine

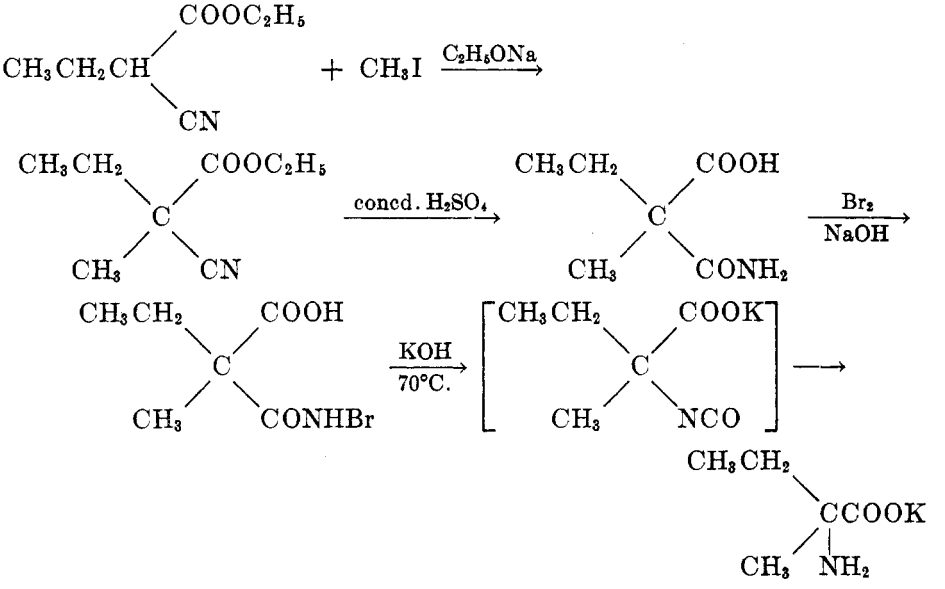
Darapsky *et al.* (88) also used the hydrazide of cyanoacetic ester for the preparation of amino acids. Their work has been substantiated and extended by Gagnon *et al.* (139), who found, however, that the method is inoperative for the preparation of diamino acids.





3. Cyanoacetic ester: Hofmann rearrangement

Li *et al.* (206) made an interesting use of the well-known Hofmann rearrangement to synthesize an α-amino acid (isovaline).

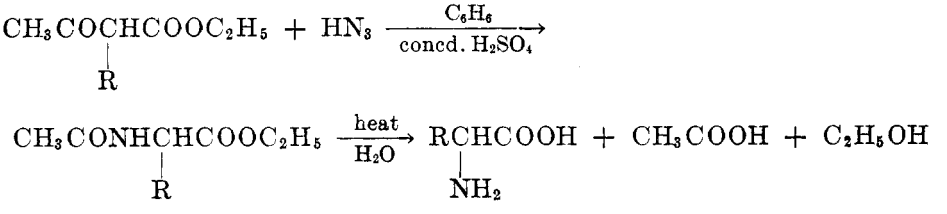


4. Hydrazoic acid: Schmidt reaction

This interesting reaction is essentially a Beckmann–Curtius rearrangement, the net result being the insertion of an imine residue.

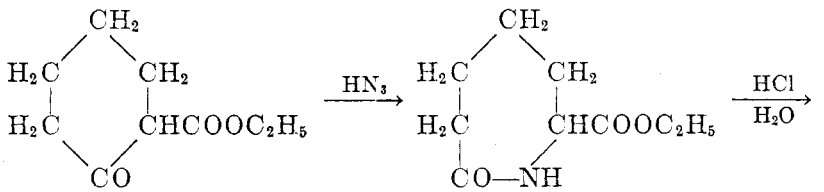
(a) Glycine, phenylalanine, and aspartic acid

Schmidt prepared these amino acids in from 80–98 per cent yields (272) by the action of hydrazoic acid on acetoacetic ester.

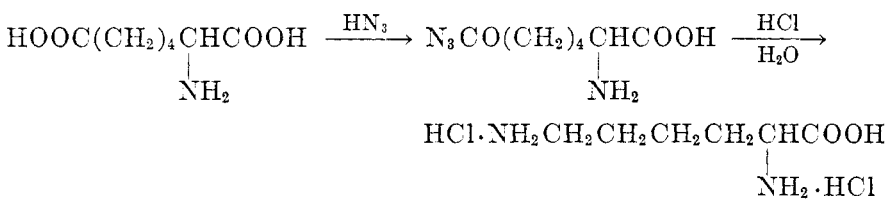


(b) Lysine

Adamson (5) observed that when α-amino dicarboxylic acids are submitted to the Schmidt hydrazoic acid reaction, the carboxyl group remote from the α-NH_2 is replaced by NH_2.

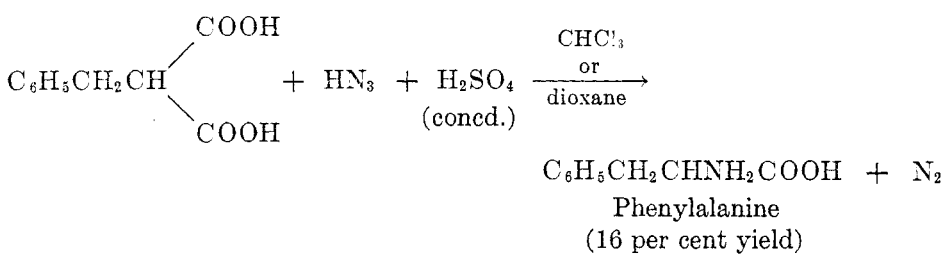


Ethyl cyclohexanone-2-carboxylate



(c) Phenylalanine

Briggs *et al.* (61) used the Schmidt reaction to prepare phenylalanine from benzylmalonic acid.



Phenylalanine
(16 per cent yield)

It should be recalled that hydrazoic acid is toxic and explosive.

G. MISCELLANEOUS

A number of syntheses have been proposed which have been used to prepare only one or two amino acids or which are only applicable to specific amino acids. These will now be briefly considered according to the amino acids to which they have been applied.

1. Alanine and glycine

(a) Glycine from cyanide

Lange (199) and Emmerling (107) prepared small quantities of glycine from hydrogen cyanide and cyanogen, respectively.

$$3HCN \xrightarrow[\text{trace } H_2O]{\text{standing}} NH_2CH(CN) \xrightarrow[\text{dilute HCl}]{\text{heat}}$$

$$NH_2CH_2COOH + CO_2 + 2NH_4Cl$$

Glycine

$$(CN)_2 + 6HI + 2H_2O \xrightarrow{\text{heat}} HI \cdot NH_2CH_2COOH + NH_4I + 2I_2$$

More recently Gluud *et al.* (141) have successfully used cyanoformic acid or ester (141) to make glycine.

$$CNCOOH \xrightarrow[\text{electrolytic}]{H_2} NH_2CH_2COOH$$

$$CNCOOC_2H_5 + (CH_3CO)_2O \xrightarrow[H_2]{Ni} CH_3CONHCH_2COOC_2H_5 \xrightarrow{HCl}$$

$$HCl \cdot NH_2CH_2COOH$$

Alderson (11) has replaced the sulfonic acid group of aminomethylsulfonic acid with —CN and hydrolyzed the resulting product to yield glycine.

$$NH_2CH_2SO_2OH + NaOH + NaCN \xrightarrow{\text{heat}} NH_2CH_2CN \xrightarrow[\text{heat}]{HCl}$$

$$HCl \cdot NH_2CH_2COOH \xrightarrow{C_5H_5N} NH_2CH_2COOH + C_5H_5N \cdot HCl$$

Glycine

Pyridine hydrochloride is soluble in absolute alcohol.

(b) Glycine and alanine by metathesis

Schroeter (276, 277) used what amounts to double decomposition between an acid amide (*p*-toluenesulfonamide) and an acid chloride to synthesize alanine and glycine. This is a most suggestive paper, and the process described should avoid many of the by-products formed by direct amination of α-halogen acids (*cf.* Section III,A).

$$p\text{-}CH_3C_6H_4SO_2NH_2 + CH_3CHBrCOOH \xrightarrow{2N\ NaOH}$$

$$p\text{-}CH_3C_6H_4SO_2NHCH(CH_3)COOH \xrightarrow[\text{heat}]{CH_3COCl \text{ or } ClCH_2COCl}$$

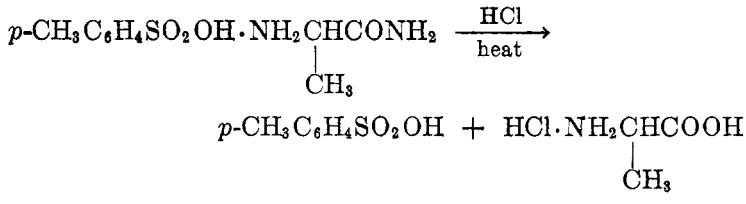
$$p\text{-}CH_3C_6H_4SO_2Cl + CH_3CHNH_2COOH$$

Alanine

Freudenberg and Huber (137) prepared alanine from lactic acid along somewhat similar lines.

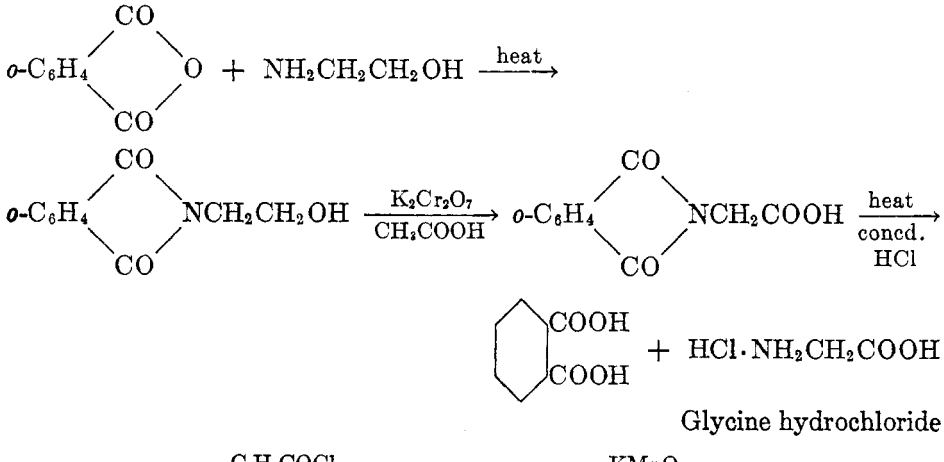
$$p\text{-}CH_3C_6H_4SO_2Cl + CH_3CHOHCOOC_2H_5 \longrightarrow$$

$$p\text{-}CH_3C_6H_4SO_2OCH(CH_3)COOC_2H_5 \xrightarrow[C_2H_5OH]{NH_3}$$

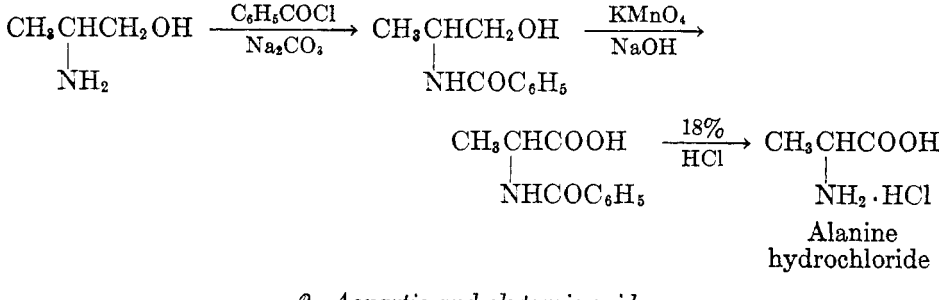


(c) Glycine and alanine by oxidation of amino alcohols

Billman and Parker (36, 37) have made use of the commercially available amino alcohols for the preparation of the simpler amino acids. The yields are excellent.



Glycine hydrochloride

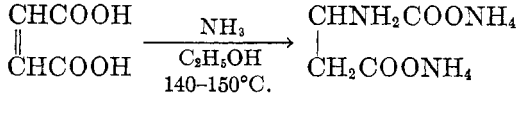


Alanine hydrochloride

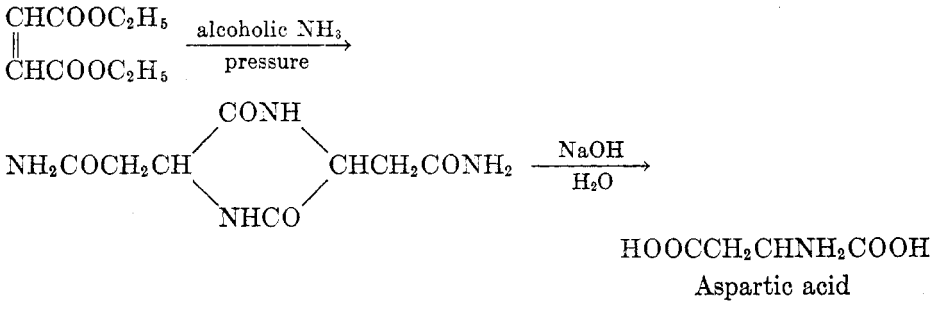
2. Aspartic and glutamic acids

(a) Aspartic acid from maleic or fumaric acid

Engel (108) synthesized ammonium aspartate by the addition of ammonia to maleic or fumaric acid. The reaction is catalyzed by mercuric salts (110) and ammonium chloride (319).



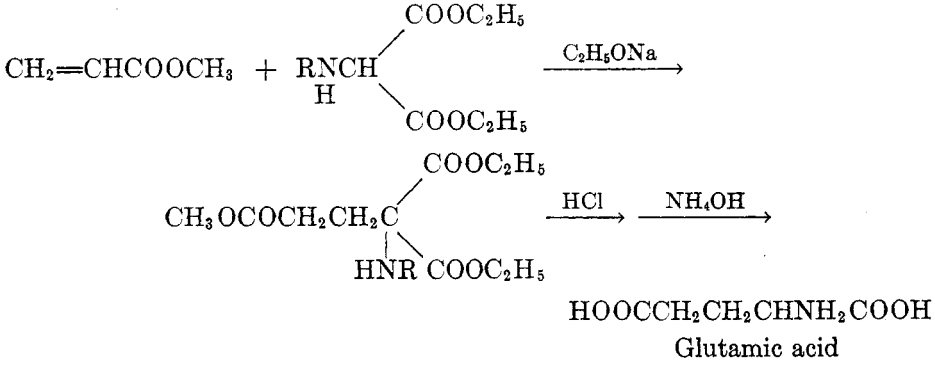
Dunn and Fox (96), using diethyl fumarate, obtained the diketopiperazine of aspartic acid amide as the intermediary product.



Wolff (339) as early as 1850 also claimed to have synthesized aspartic acid by heating ammonium maleate. The identification of aspartic acid given in Wolff's paper does not appear to be conclusive.

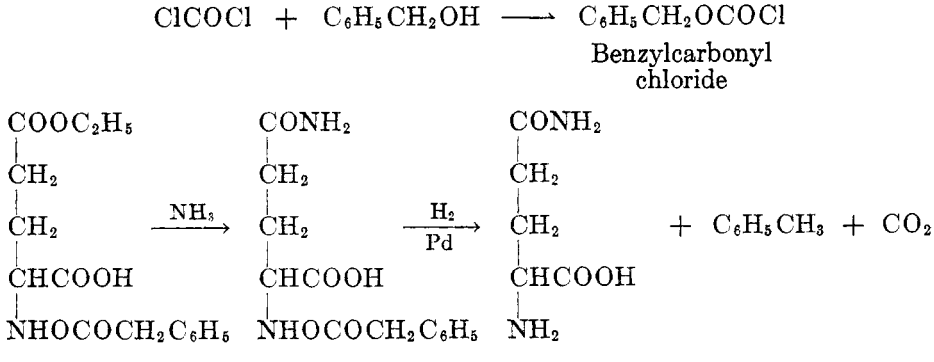
(b) Glutamic acid by Michael condensation

Marvel and Stoddard (224) and Snyder *et al.* (289) have synthesized glutamic acid from methyl acrylate and phthalimidomalonic ester or acetamidomalonic ester, respectively. This appears to be a Michael condensation (*cf.* 289) rather than an alkylation reaction.

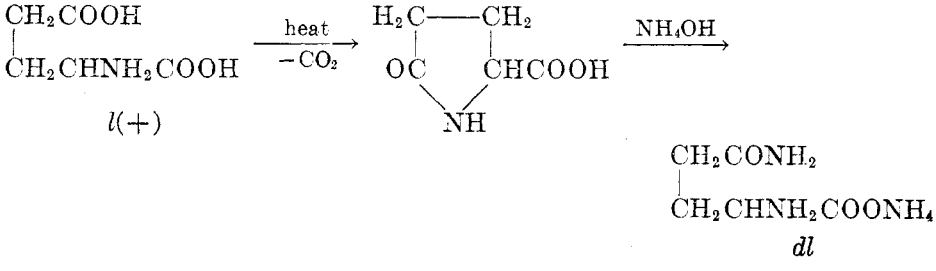


(c) Glutamine and asparagine

Bergmann *et al.* (34, 35, 235) synthesized asparagine and glutamine from the corresponding carbobenzoxy amino acids.



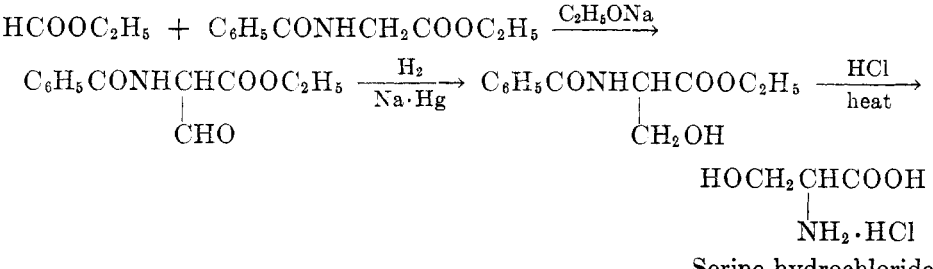
Lichtenstein (207) has synthesized glutamine from glutamic acid *via* pyrrolidonecarboxylic acid. Considerable quantities of ammonium pyrrolidonecarboxylate are also formed.



3. Serine and threonine

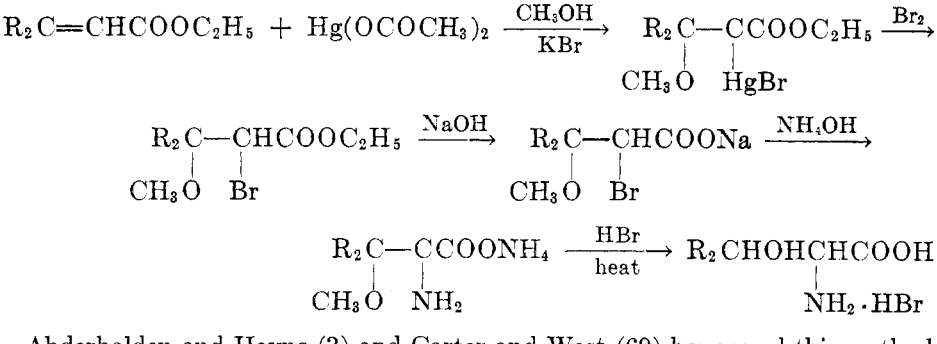
(a) Serine from ethyl hippurate

As long ago as 1904, Erlenmeyer and Stoop (115) synthesized serine from ethyl formate and hippuric acid ester.

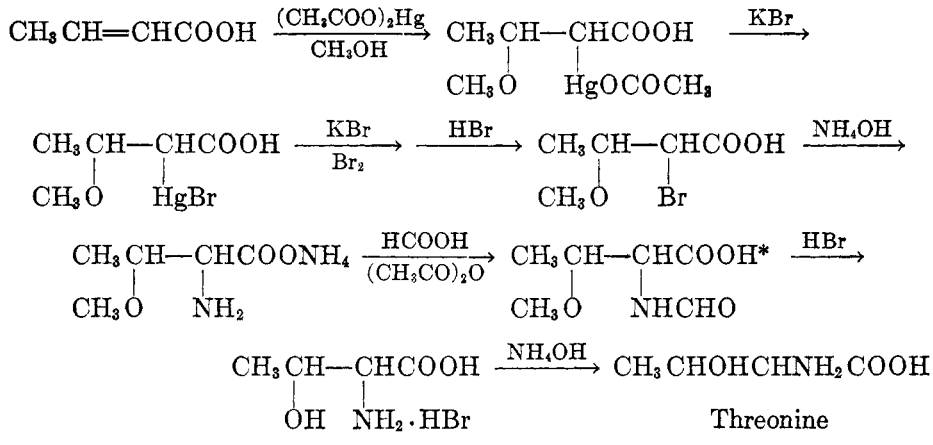


(b) Serine and threonine by the addition of mercury salts to α,β-olefincarboxylic esters

Schrauth and Geller (275) found that when mercuric salts in alcohol were allowed to react with α,β-olefincarboxylic esters, Hg^{++} was introduced into the α-position and the alkyl group of the alcohol in the β-position.



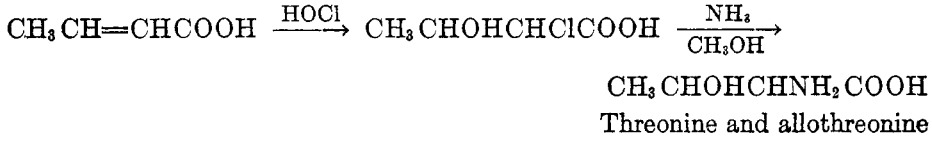
Abderhalden and Heyns (3) and Carter and West (69) have used this method to prepare threonine, while Carter (268) and Botvinnick *et al.* (52) employed it to make serine and β-hydroxynorvaline, respectively.



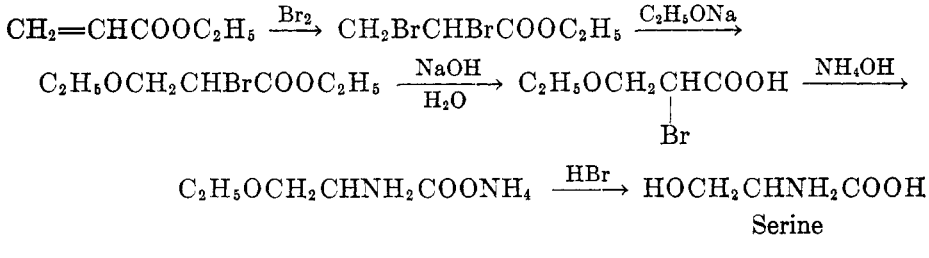
* This step is used to separate *dl-O*-methylformylthreonine from the relatively more soluble *dl-O*-methylformylallothreonine (331).

(c) Serine and threonine by halogenation of olefinic acids

Burch (65) synthesized a mixture of threonine and allothreonine by the action of hypochlorous acid on crotonic acid.



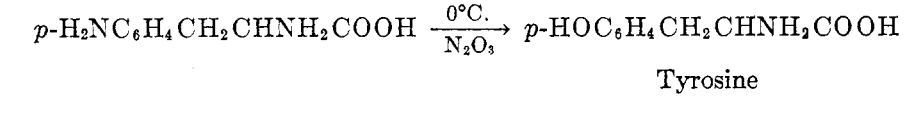
Wood and du Vigneaud (341) prepared serine by bromination of acrylic ester.



4. Tyrosine, halogenated tyrosines, and thyroxine

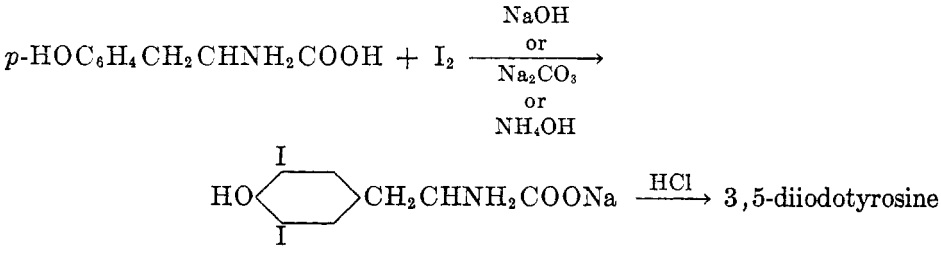
(a) Tyrosine

Erlenmeyer and Lipp (114) prepared tyrosine by the following reaction, which is of theoretical interest only.



(b) Iodogorgoic acid, dibromotyrosine, and dichlorotyrosine

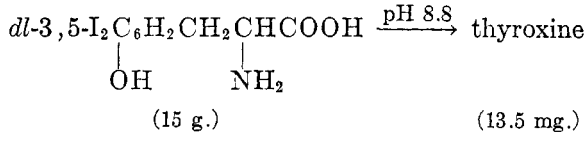
Wheeler and Jameson (334) synthesized diiodotyrosine in good yields by the addition of iodine to tyrosine in alkaline solution (*cf.* 266).



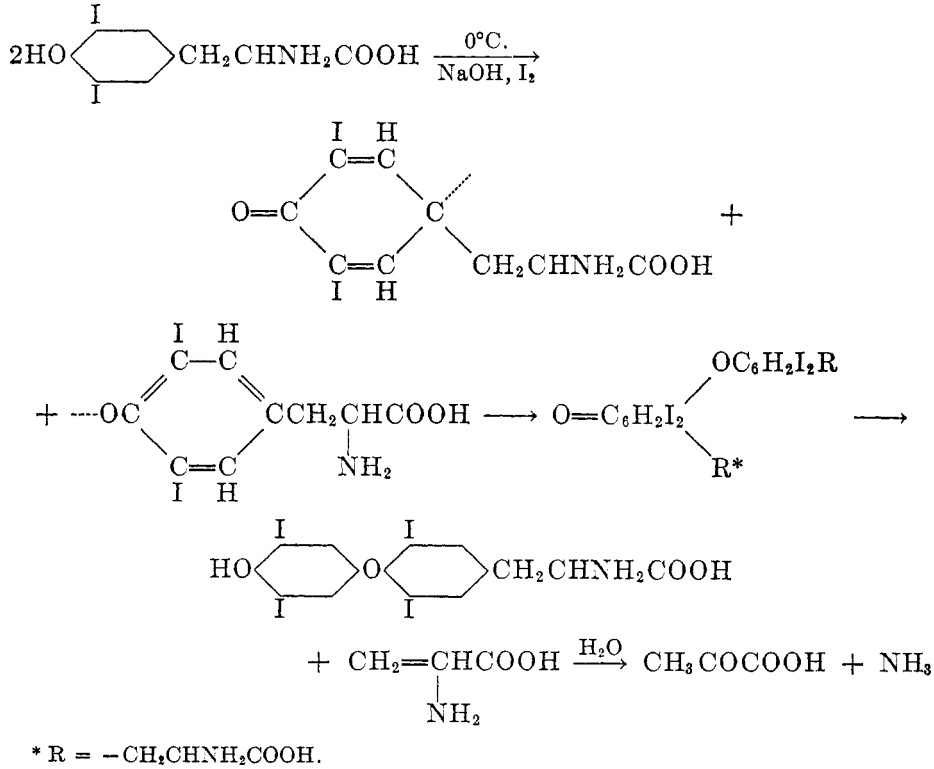
Zeynek (343) prepared 3,5-dibromo- and 3,5-dichloro-tyrosines by the halogenation of tyrosine in hot glacial acetic acid.

(c) Thyroxine from diiodotyrosine

v. Mutzenbecher (234), Block (40), and others have found that small yields of thyroxine are formed from iodogorgoic acid in mildly alkaline solution (pH 8.8) at 37°C.



This reaction has been explained by Johnson (171; *cf.* 153a) as follows:

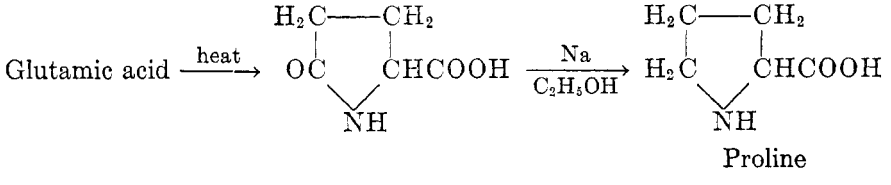


* R = $-CH_2CHNH_2COOH$.

5. Proline

(a) Proline from pyrrolidonecarboxylic acid

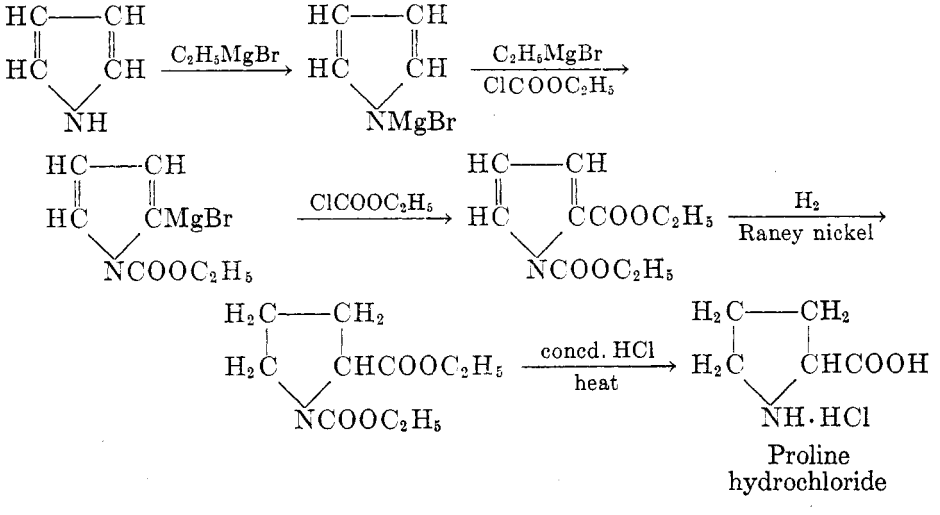
Fischer and Boehner (126) obtained proline in less than 4 per cent yield by the reduction of pyrrolidonecarboxylic acid.



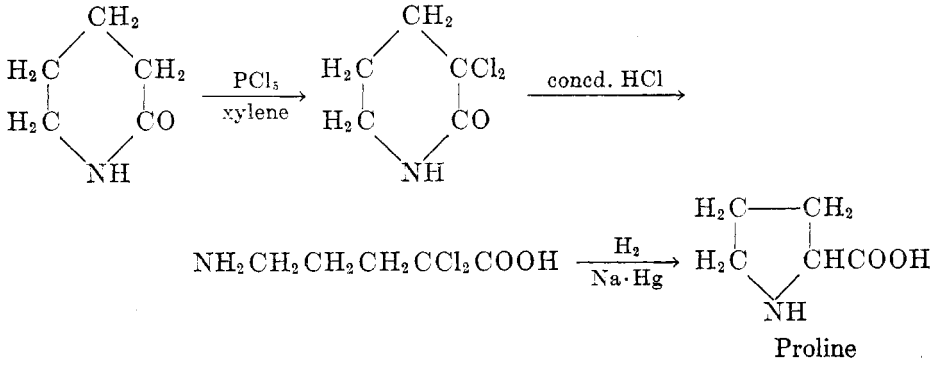
(b) Proline from pyrrole

Putokin (252) obtained proline by the reduction of pyrrolecarboxylic acid with hydrogen, using platinum or palladium oxide catalysts.

Signaigo and Adkins (282) made use of the Grignard reaction to synthesize proline from pyrrole.



Heymons (160) prepared proline from α-piperidone as follows:



6. Arginine and citrulline

(a) Arginine from ornithine

Sørensen (295, 296) in his studies on the structure of amino acids synthesized arginine from α-benzoylornithine and cyanamide.

(b) Citrulline from arginine or ornithine

Kurtz (196) prepared citrulline from ornithine copper by condensation with urea.

$$NH_2(CH_2)_3CHNH_2COOH + Cu_2O \longrightarrow$$

$$RCu \xrightarrow{NH_2CONH_2} \xrightarrow{H_2S} \underset{\text{Citrulline}}{NH_2CONHCH_2CH_2CH_2CHNH_2COOH}$$

Fox (135) isolated citrulline after boiling arginine hydrochloride with 5.68 *N* sodium hydroxide.

7. Cysteine and methionine

(a) Cysteine from serine

Erlenmeyer (115) synthesized cysteine from serine by melting with phosphorus pentasulfide, while Fischer (127) made it from serine in small yields as follows:

$$\underset{|\atop NH_2\cdot HCl}{CH_2OHCHCOOH} \xrightarrow{PCl_5} \underset{|\atop NH_2\cdot HCl}{ClCH_2CHCOOH} \xrightarrow{Ba(SH)_2} \underset{\underset{\text{Cysteine}}{|\atop NH_2}}{HSCH_2CHCOOH}$$

(b) Methionine from ethyl chloroethylhippurate

Tarver and Schmidt (305) used ethyl γ-chloro-α-benzoylaminobutyrate as the starting material for the synthesis of methionine containing radioactive sulfur.

$$C_6H_5CH_2SH + \underset{|\atop NH_2COC_6H_5}{ClCH_2CH_2CHCOOC_2H_5} \xrightarrow[\text{Na}\atop\text{heat}]{CH_3OH} \xrightarrow{NaOH} \xrightarrow{20\%\ HCl}$$

$$\underset{|\atop NH_2\cdot HCl}{C_6H_5CH_2SCH_2CH_2CHCOOH} \xrightarrow[\text{liquid } NH_3\atop CH_3I]{Na} \underset{\text{Methionine}}{CH_3SCH_2CH_2CHNH_2COOH}$$

8. β-Alanine

Although β-alanine has not been found as a protein constituent, it is widespread in nature as a portion of carnosine, pantothenic acid, etc. It is readily synthesized by the reduction of cyanoacetic esters (259, 276, 332).

$$CNCH_2COOC_2H_5 \xrightarrow{H_2} NH_2CH_2CH_2COOC_2H_5 \xrightarrow{H_2O} \underset{\beta\text{-Alanine}}{NH_2CH_2CH_2COOH}$$

H. GENERAL

Benedict (29) found that aniline can replace pyridine in the removal of hydrochloric acid from amino acid hydrochlorides.

Although acylation plus fractionation of the acylated amino acids with brucine, strychnine, etc., is usually employed in the resolution of amino acids (153, 204, etc.), Behrens *et al.* (27, 28) have resolved *dl*-phenylalanine and *dl*-alanine by means of enzymes. Duschinsky (100), however, found that it is possible to prepare *d*- and *l*-histidine from synthetic *dl*-histidine by fractional crystallization of the hydrochlorides. The reader is referred to the original paper for the details of this unique resolution in the amino acid field.

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