AN INVESTIGATION OF CHEMICAL METHODS FOR THE SYNTHESIS OF alpha-AMINO-beta-BUTENOIC ACID (VINYLGLYCINE)

Thesis for the Degree of M. S.

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Approved James L. Fairley

ABSTRACT

The interconversions between homoserine, threonine, and alpha-aminobutyric acid have been demonstrated. The mechanism involved in the conversion of homoserine to threonine has not been established experimentally. However, it has been postulated that homoserine undergoes dehydration to alpha-amino-beta-butenoic acid (vinylglycine) and becomes rehydrated to give rise to threonine. Thus, several attempts have been made to carry out a chemical synthesis of vinylglycine for the purpose of testing this and other theories.

Many unsuccessful attempts were made to prepare alpha-amino-beta, gamma-dibromobutyric acid (I) and alpha-amino-vinylacetonitrile (II). It was proposed to obtain vinylglycine through the debromination of (I) or by the hydrolysis of (II). Futher attempts were made to synthesize vinylglycine through the malonic ester synthesis. This involved the preparation of phthalimide diethylmalonate and reacting with 2-bromo-1-ethanol, 1,2-dibromoethane, and acetaldehyde using pyridine as a catalyst. The dehydration of the primary alcohol resulting from the condensation failed to give phthalimide vinylmalonic ester. The dehydrohalogenation of the resulting oil, alleged phthalimide(beta-bromoethyl)-diethylmalonate, with subsequent hydrolysis and decarboxylation, gave a spot for an unknown amino acid when subjected to paper chromatography. The dehydration of the condensation product, alleged phthalimide(alpha-hydroxyethyl)-diethylmalonate, obtained by reacting acetaldehyde and phthalimide diethylmalonate failed to give a vinyl group. Unsuccessful results

were also obtained when the phthalimide diethylmalonate was replaced by formylamino-diethylmalonate and subjected to the same type reactions given above. Potassium phthalimide diethylmalonate failed to react with alpha-chloro-vinylacetonitrile in the normal way.

VITA

The author was born January 13, 1929. The bachelor of science degree was conferred to him in June, 1950 at A. and T. College, Greensboro, N.C. He was a special research assistant, on a government sponsored project, from 1948-1950. With part-time research, he taught general chemistry from 1950-1951 at A. and T. College.

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INTRODUCTION

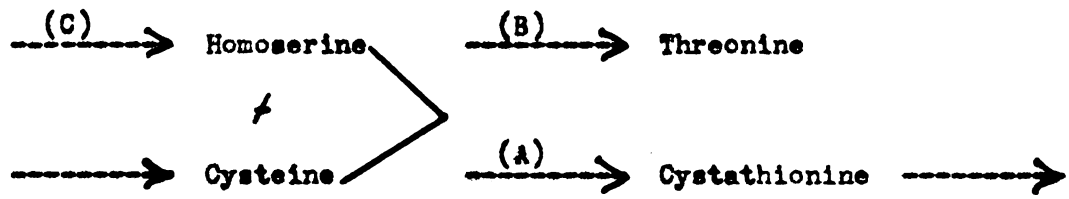
During the past decade scientific investigators have recorded many new observations, learned new facts, and proposed new theories concerning the pathways of biogenesis of the organic constituents of biological systems. Of these various constituents, the important group of alpha-amino acids has received considerable attention. Nevertheless, many uncertainties remain as to the precise nature of the reactions involved in the synthesis and transformations of some of these compounds. Among these uncertainties is the nature of the mechanisms which bring about the interconversions possible among threonine, homoserine and aminobutyric acid. One possibility here is that each of these compounds may be converted to a common intermediate, alpha-amino-beta-butenoic acid (vinylglycine), which then may give rise to each of the other compounds by the addition of water or hydrogen.

A direct test of this possibility has not yet been possible, for the postulated intermediate, vinylglycine, has never been prepared and its properties are accordingly unknown. An investigation of various methods for the chemical synthesis of this compound has therefore been undertaken.

HISTORICAL

A. Amino Acid Metabolism

Experimental results obtained by Horowitz, Teas, and Fling (1, 2), using methionineless and threonineless mutants of Neurospora crassa, gave specific evidence for the following relationship between the biosynthesis of threonine and methionine.



Homocysteine ⟶ Methionine

Buss (3) has shown that there are at least three genetically different mutants in the group blocked at Stage-A which grow when supplied with cystathionine but not when supplied with cysteine. Fling and Horowitz (4) used these mutants to carry out experiments to obtain a futher insight on the conversion of cysteine to cystathionine. The results of a cross-feeding experiment showed that the extracts of any of the three mutants supported the growth of a threonineless mutant, No. 35423 (blocked at Stage B), and of a homoserineless mutant, No. 51504 (blocked at Stage C). The active fractions from extracts of methionineless mutant 9666 will support the growth of homoserineless mutant 51504 in the absence of threonine (only L-homoserine will support normal growth of strain 51504 in the absence of threonine). Chromatographic and biological results provided strong evidence for the presence of L-homoserine in extracts of methionineless mutant 9666 and of a

double mutant 9666-35423. Since extracts of a methionineless mutant of Neurospora crassa contained two substances, one active for threonineless mutant 35423 and one for homoserineless mutant 51504, one may regard this as being very strong evidence to support the theory that methionine can be converted to L-homoserine and threonine under the experimental conditions used with these specific strains of Neurospora crassa. Strain 51504 was employed by Horowitz et al (2) in conducting the following study. Strain 51504 was orginally classified as a threonineless mutant, since threonine was the only amino acid tested upon which it showed any growth. Of twenty-five amino acids tested in concentrations of one mg. per 20 cc. of medium, only DL-threonine supported growth. Futher study of the growth requirements of strain 51504 showed some factor or factors present in casein hydrolysate stimulated growth in the presence of small amounts of threonine, although no response was obtained with casein hydrolysate alone.

Methionine was the only amino acid tested which stimulated growth in the presence of threonine. Attempts to replace the threonine portion of the requirement were carried out by supplying each of twenty-five amino acids in the presence of methionine, none of which replaced threonine. Futher tests were made to determine whether known precursors of methionine are able to fulfill the methionine requirements of the mutant, and the results showed that cystathionine and homocysteine (as the thiolactone), but not cysteine, will support growth of strain 51504 when supplied togather with threonine. Thus, the authors reasoned that the cleavage of cystathionine to yield homocysteine and the methylation of homocysteine proceeds normally

in the mutant, but that the synthesis of cystathionine from cysteine is blocked. This suggested that cystathionine and threonine have a common precursor whose synthesis is blocked in the mutant. To futher substantiate this theory the authors searched for a substance which, when supplied to the organism, would satisy both the methionine and threonine requirements. DL-Homoserine was synthesized and found to be active when tested on strain 51504. The activity of DL-homoserine for strain 51504 is equal to or better than that of a mixture of DL-threonine and DL-methionine, a fact which adds weight to the idea that homoserine is a normal biological precursor of threonine and methionine. Thus, it appears that homoserine acts as a precursor for both methionine and threonine in Neurospora crassa. The authors suggested that the conversion of homoserine to threonine may involve the dehydration of homoserine to the beta-gamma-unsaturated amino acid with subsequent rehydration to threonine. It was also suggested that homoserine may act as a specific amino group, or even hydroxyl, donor to the immediate precursor of threonine.

In similar experiments with twelve amino acid-requiring mutant strains of Bacillus subtilis Teas (5) found that seven required threonine, two required threonine and methionine but could use homoserine instead, and three required threonine and methionine but could not grow on homoserine alone. The indications are that in Bacillus subtilis, as in Neurospora crassa, homoserine is a precursor of both threonine and methionine. Cohen and Hirsch (6) carried out the conversion of L-homoserine into L-threonine by using a suspension of Escherichia coli and threonine synthase. Threonine

was found to be destroyed by threonine deaminase at a rate proportional to the threonine concentration. Futher experiments were conducted by Hirsch and Cohen (7) which gave evidence of L-homoserine as an intermediate in the transformation of L-aspartic acid into L-threonine by E. coli. Escherichia coli, type ML converted L-homoserine to L-threonine, and also transform L-aspartic acid to L-threonine. However, mutant ML 52 can not synthesize L-threonine from L-homoserine, but transforms L-aspartic acid only to L-homoserine. In the unmutated ML E. coli no L-homoserine is detectable since it is converted very rapidly to L-threonine.

Umbarger (8) carried out quantitative growth experiments with threonineless mutants of E. coli. Strain RSS-60 grew rapidly on L-threonine or DL-homoserine but grew slowly on D-threonine, alpha-ketobutyrate, alpha-aminobutyrate, or L-isoleucine. Delluva (9) grew E. coli in glucose-phosphate-NH_4Cl medium with either C^{14}-labeled oxalacetate, aspartate, or formate. The distribution of C^{14} in the threonine and serine indicated that a four-carbon unit was the source of threonine. Kalan and Ceithaml (10) carried out experiments which involved the biosynthesis of methionine in E. coli. Four groups of methionine-requiring mutants of E. coli, strain W, were isolated by the penicillin method, one of which would grow on alpha-amino-butyric acid, homoserine, alanine, valine, isoleucine, as well as methionine, homocysteine, and cystathionine. The results obtained from these experiments are consistent with the hypothesis that a four-carbon unit, amino acid, is a precursor of methionine in E. coli. Cohen, Hirsch, Wiesendanger, and Nisman (11) found that extracts of E. coli converted L-aspartic acid to

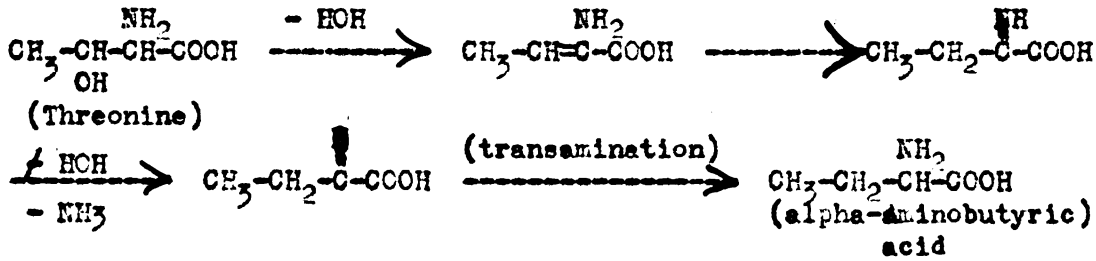
L-threonine. Extracts of E. coli ML 52 possesses a glucose-6-phosphate dehydrogenase which can couple with the system reducing the aspartate to homoserine through triphosphopyridine nucleotide (TPN). Extracts of E. coli B 134 can not reduce aspartyl phosphate to homoserine. Extracts of a third mutant are able to couple dehydrogenation of glucose-6-phosphate with reduction of aspartate, but homoserine is not formed. The sequence for converting aspartate to homoserine is postulated to be: ——→ beta-aspartyl-phosphate ——→ X ——→ L-homoserine. With homoserine as substrate, extracts of E. coli B 134 form small amounts of an alpha-keto acid in addition to threonine. Extracts of E. coli (12) were found to reduce aspartic acid to homoserine in the presence of glutamic acid, triphosphopyridine nucleotide, and adenosinetriphosphate (ATP). Homoserine was transformed to threonine in the presence of ATP and pyridoxal phosphate. Black and Wright (13) carried out an experiment which demonstrated an enzymatic reduction of beta-aspartyl phosphate to homoserine. Watanabe, Konishi and Shimura (14) demonstrated the biosynthesis of threonine from homoserine in aqueous extracts of acetone-dried baker's yeast.

Several experiments have demonstrated the conversion of threonine to aminobutyric acid. Culture filtrates of various bacteria grown in casein hydrolyzate medium were examined by paper chromatography by Wolwod and Proom (15) for the appearance of new ninhydrin spots. It was found that washed suspensions of Staphylococcus aureus cultures produced alpha-aminobutyric acid from threonine possibly by the removal of the hydroxyl group. Lien and Greenberg (16) carried out both in vitro and in vivo experiments

which demonstrated the interconversion of threonine and aminobutyric acid. In the *in vitro* experiments, radioactive threonine was incubated with a suspension of cytoplasmic macro granules of rat liver in a synthetic medium. In the *in vivo* experiments, each rat was injected intraperitoneally with one to 10 mg. of C^{14}-labeled threonine dissolved in 1 cc. of water. The results from both *in vitro* and *in vivo* experiments gave evidence that DL-threonine-2-C^{14} was converted to alpha-aminobutyric acid.

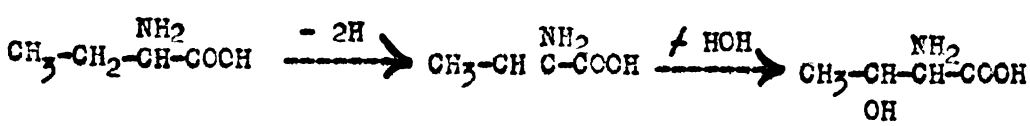
The identification of alpha-aminobutyric acid formed during threonine incubation was based on a positive reaction with ninhydrin, the coincidence of the ninhydrin spot of known alpha-aminobutyric acid with the radioactive material from the peaks as determined by radioautography after two-dimensional chromatography on paper, and the very close coincidence of the radioactive peak and the ninhydrin peak when a portion of the material was rechromatographed with 10 mg. of known alpha-aminobutyric acid on a Dowex column. The tentative identification of the few micrograms of alpha-aminobutyric acid, by Lien and Greenberg was based largely upon chromatographic evidence. In order to verify the previous identification, a large scale enzymatic preparation of alpha-aminobutyric acid was performed by Lien and Greenberg (17) using N^{15}-labeled DL-threonine. The yield of alpha-aminobutyric acid was much higher when threonine was incubated with a rat liver homogenate in KCl-$KHCO_3$ buffer than when the incubation was carried out in the cytoplasmic macro granule system. The alpha-aminobutyric acid was characterized by conducting a micro-Kjeldahl nitrogen determination, an infra-red spectrum, and a chemical degradation. The fact that the transformation proceeds by

the way of alpha-ketobutyric acid suggests a deamination step, with subsequent transamination to form alpha-aminobutyric acid as follows:



In a study of serine and threonine deaminase activities of wild type Neurospora crassa, the formation of alpha-ketobutyric acid from threonine has been demonstrated to take place in cell-free extracts of N. crassa by Reissig (18). Lenti and Grillo (19) found that in quiescent suspensions of E. coli there is active deamination of DL-threonine to alpha-ketobutyric acid.

Heyns and Walter (20) demonstrated, by chemical means, the formation of alpha-aminobutyric acid from threonine. Threonine hydrochloride heated a few degrees above the m.p. (140-2) decomposed with gas evolution and darkening, and after a few minutes alpha-aminobutyric acid was formed. Only threonine gave this reaction; under similar conditions serine gave alanine. Wieland and Wirth (21) have conducted similar experiments. Armstrong and Binkley (22) reasoned that alpha-aminobutyric acid may serve as a substitute for methionine and threonine, involving a mechanism as simple as alpha-beta-dehydrogenation followed by readdition of water to the unsaturated amino acid formed.



Diets lacking in methionine and in threonine, but adequate in all other respects, were administered to young white rats, and the ability of alpha-aminobutyric acid to substitute for each of these was tested. The results showed that alpha-aminobutyric acid can neither substitute for threonine nor provide the four-carbon chain for the synthesis of methionine under the conditions of the experiment. Armstrong and Binkley (23) conducted experiments to determine whether young white rats were able to carry out the synthesis of methionine from homoserine, choline, and cystine. As a results of the growth experiments it was concluded that DL-homoserine in the presence of cystine and choline will not substitute for methionine under the conditions specified. Fromageot and Clauser (24) demonstrated the non-reversibility of the conversion of methionine and threonine to alpha-aminobutyric acid in the rat.

Experiments conducted by Fairley (25) demonstrated that either aminobutyric acid, homoserine and threonine could support the growth of certain pyrimidineless strains of N.crassa. Aminobutyric acid and homoserine were about equal in ability to promote growth, while threonine was considerably poorer in this respect. These results indicate that, although the compounds are interconvertible or convertible to a common intermediate, homoserine is not converted to aminobutyric acid through threonine, and strengthens the possibility that vinylglycine may be the central compound in the interconversion reactions.

B. Chemical:

Several theoretical pathways for the chemical synthesis of alpha-amino-beta-butenoic acid (vinylglycine) may be proposed, but, a search for the proper conditions to transform these theoretical pathways into practical applications may prove to be less fruitful. As has been indicated, a search of the literature revealed that vinylglycine has not been reported in any respect. Thus, in attempts to synthesize this compound the preparation and chemical behavior of compounds possessed of molecular structures closely related to that of vinylglycine should be of great significance.

Before one attempts to carry out any type of synthesis, which might lead to the formation of a compound such as vinylglycine, many significant factors are considered. For instance: the stability of the compound, is it most stable as such, as a salt, or some other derivative; what are the significant properties of each intermediate for a specific synthesis; what conditions are necessary to activate the last reaction in the formation of the final product, and will these conditions be sufficiently drastic to promote decomposition or structural changes in the molecule as it is formed; and most of all, is the compound stable under normal conditions, i.e. room temperature, if not, to what conditions must said compound be subjected to effect stabilization, and what approach should be employed to unmask these ideal conditions without effecting the compound in the process of doing so. An absolute answer for these and other questions about a compound which has not been synthesized can not be obtained, but one can make use of a theoretical approach and whatever correlations can be derived by employing the behavior of closely related substances.

Linstead, Noble, and Boorman (26) made a study of methods for the preparation of Δ^3-olefinic acids with unbranched chains, and presumed that the

results are applicable to acids substituted by alkyl groups at the delta-carbon or beyond. Δ^3-n-Butenoic acid, prepared by the triethanolamine base-catalyzed method, was obtained in an optimum yield from malonic acid and an excess of acetaldehyde. The method of Houben (27) gave vinylacetic acid from allyl bromide, and carbon dioxide by the Grignard reaction. This compound yielded a dibromide corresponding to that prepared by Fichter and Sonneborn (28). Using essentially the method of Bruylant (29), Linstead, Noble, and Boorman obtained the best yields of vinylacetic acid, by starting with allyl cyanide. It was found that vinylacetic acid, although easily isomerized, is not an exceptionally unstable substence when kept at room temperature in the pure state. Vinylacetic acid gives the reaction of a carboxyl compound and of an olefin practically independently of each other because there is no conjugation of the unsaturation in the two groups. On boiling with dilute acids or alkalies, vinylacetic acid changes to crotonic acid. Gaseous HBr causes this change even at zero degrees. Concentrated alkalies produces two molecules of acetate by shifting the double bond and splitting the alpha-beta unsaturated compound as it is formed. The change from beta-gamma to alpha-beta unsaturation in a straight chain acid is easy because of the greater lability of the alpha hydrogen as compared with the gamma hydrogen. In one reaction, vinylacetic acid shows a complication between its two reactive groups. It readily changes to butyrolactone because the carboxyl and ethylene linkages can approach each other closely in space (30). This is a very general property of beta-gamma unsaturated acids, and particularly the four-carbon acids.

Ramband (31) made a study of intramolecular transpositions and influences of acids, esters, and nitrile groups on intramolecular transpositions of the allyl type. Double decomposition reactions with alpha-substituted unsaturated compounds of the formula RCH=CH-CH-R' gave "normal products" of the type
X
RCH=CH-CH-R' and "abnormal products" RCH-CH=CH-R' by intramolecular trans-
Y Y
position of the allyl type. Ramband made a study of the influence that the nature of the radicals R and R' has on the orentiation of the reactions giving rise to two classes of products and in particular the effect of CN, COOH, COOR' on reactions in which an allyl transposition is possible. The isomerization is proposed to take place in the sense RCH=CH-CH-R' ⟶ RCH-CH=CH-R' by the migration of a negative group (anionotropy) or by the shift of a hydrogen atom, RCH=CH-CH-R' ⟶ RCH_2-CH=C-R' (prototrophy). A series of reactions were carried out on CH_2 CH-CH-R' where R' is as follows: R' equal CN (I), R' equal COOH (II), R' equal COOMe (III), R' equal COOEt (IV), R' equal COOPr (V), leaving R' intact but substituting the alcoholic "OH" group by another negative group (Cl, Br, AcO and N-Et_2). The direct acetylation of I, II, III, and IV with AcONa went normally and gave satisfactory yields of alpha-acetins which were extremely resistant to isomerization. The acetylation of trans-gamma-hydroxycrotonic acid, HO-CH_2-CH=CH-COOH, gave the expected gamma-acetin, AcO-CH_2-CH=CH-COOH. The action of AcONa on gamma-brominated esters, Br-CH_2-CH=CH-COOR, gave gamma-acetins by a totally normal reaction. The alpha-acetins were saponified normally to the acid by the action of dilute alkalies. The more stable gamma-acetins gave, after heating with KOH for several days, a hydroxy acid with all the characteristics

of gamma-hydroxycrotonic acid. Saponification of the gamma-bromoesters with cold AgOH gave gamma-hydroxy esters. In a chilled solution of KOH and $Ba(OH)_2$ the saponification of the gamma-bromo esters gave gamma-bromocrotonic acid. On boiling, the hydrolysis became complete, the reaction remained normal and gave gamma-hydroxycrotonic acid. The gamma-bromo compounds gave gamma-amines on treatment with anhydrous dimethyl amine, $(CH_3)_2NH$, in dry ether.

Chloronation of $CH_2{=}CH{-}\underset{OH}{CH}{-}CN$, $CH_2{=}CH{-}\underset{OH}{CH}{-}COOMe$, $CH_2{=}CH{-}\underset{OH}{CH}{-}COOEt$, and $CH_2{=}CH{-}\underset{OH}{CH}{-}COOH$, with $SOCl_2$ in the presence of pyridine gave excellent yields of the expected alpha-chloro-derivatives. Gamma-hydroxycrotonic acid was chloronated normally to the known gamma-chlorocrotonic acid. The abnormal reactions by anionotropy according to the scheme $AY + CH_2{=}\underset{X}{CH}{-}CH{-}R \longrightarrow CH_2{-}CH{=}\underset{Y}{CH}{-}R$ were studied by the bromonation of the alpha-alcohols, the transformation of the alpha-bromo-derivatives into acetins and alcohols, and the action of $CaBr_2$ and HCl on the alpha-chloro compounds. The prototropic abnormal reactions following the scheme $CH_2{=}\underset{X}{CH}{-}CH{-}R \longrightarrow CH_3{-}CH{=}\underset{X}{C}{-}R$ were investigated by the action of NaOH on the alpha-chloro compounds, the action of NH_3, Et_2NH, and AcONa on the alpha-chloro esters, the action of strong acids on the alpha-chloro esters and nitriles, the action of bases on alpha-hydroxy acids and esters, the action of acids on the alpha-hydroxy compounds, and the action of PBr_3 on the alpha-hydroxy nitriles. The results have been compared with those obtained in analogous studies where the CN, COOH, and COOR functions were replaced by a hydrocarbon residue or by a hydrogen atom. From a comparison of the tabulated results it is evident that the presence of a group COOH, COOR, or CN, in the alpha-position to

a negative group susceptible to migration, acts as a stabilizer and hinders the alpha,gamma-migration by allyl transposition to a certain extent. It favors the migration of the hydrogen atom attached to the same carbon atom. The gamma-substituted acids, nitriles, and esters do not seem to give any abnormal reactions either by anionatropy or by prototropy.

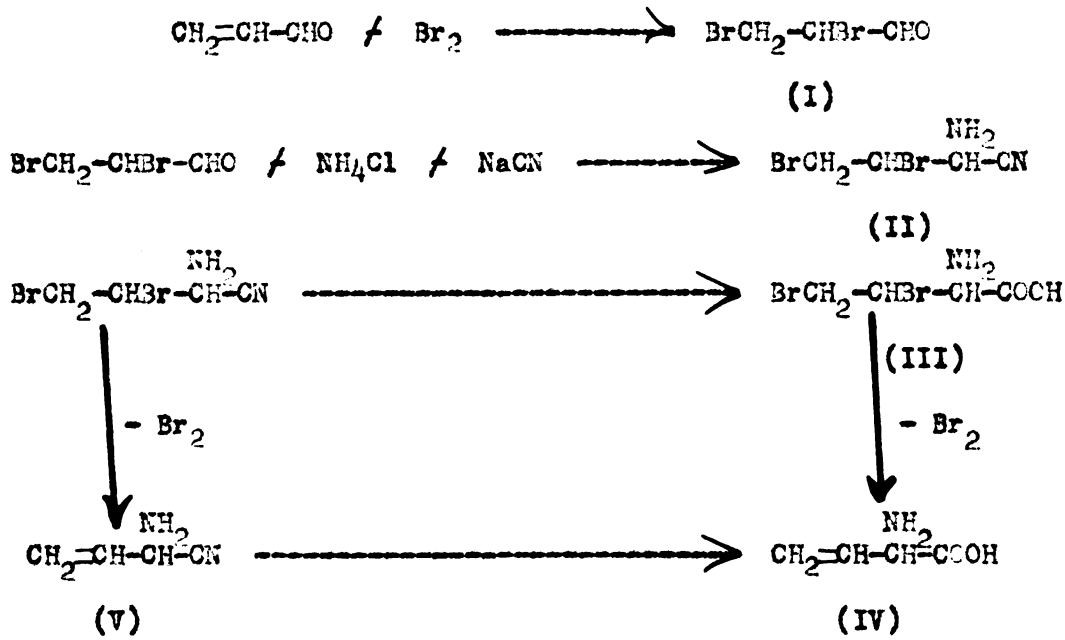
Ramband (32) conducted futher investigations on the preparation and behavior of alpha-hydroxyvinylacetic acid and some of its derivatives. Large quanitites of pure vinylglycolic nitrile, $CH_2=CH-\underset{OH}{CH}-CN$ and from it the corresponding acid and ester have been prepared. These compounds react with $SOCl_2$ and Ac_2O in the normal way without any isomerization or transposition of any kind. The gamma-bromo-crotonates yields amines, and acetins by normal reactions. [redacted] passing dry HCl in cold solutions of vinylglycolic nitrile, $CH_2=CH-\underset{OH}{CH}-CN$, in Me-OH, Et-OH, and pr-OH, boiling the saturated solutions under reflux, pouring the reaction mixture into a large volume of cold water and extracting with Et_2O, gave the esters $CH_2=CH-\underset{OH}{CH}-COOMe$, $CH_2=CH-\underset{OH}{CH}-COOEt$, $CH_2=CH-\underset{OH}{CH}-COOPr$. The hydrolysis of vinylglycolic nitrile by dilute HCl at zero degrees gave a yellow sirup which is laborious to extract with Et_2O and difficult to purify either by distillation or by isolation of the zinc-salt. Treatment of alpha-hydroxypentenonitrile with Ac_2O and AcONa gave alpha-hydroxyvinylacetic acid, $CH_2=CH-\underset{OH}{CH}-COOH$, which absorbs bromine. The dropwise addition of $SOCl_2$ to cold mixtures of the esters of vinylglycolic acid with pyridine gave the alpha-chloro-derivatives. gamma-Hydroxycrotonic acid reacted violently and gave gamma-chlorocrotonic acid, $ClCH_2-CH=CH-COOH$. The saponification of the methyl ester of alpha-chlorovinylacetic acid by

heating with dilute HCl for three hours gave the acid, CH_2 CH-CH-COOH, which absorbs bromine.
Cl

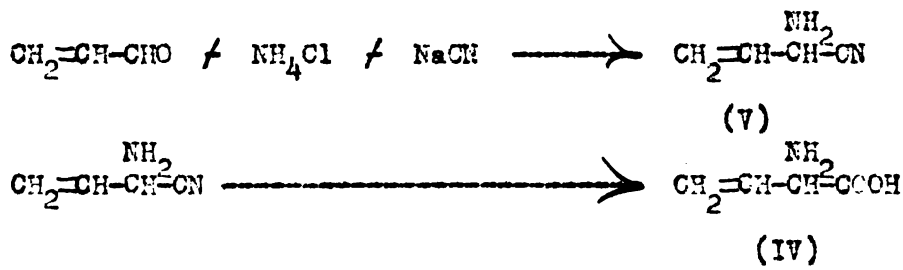
This document is primarily concerned with the investigation of synthetic methods applicable to the synthesis of vinylglycine. Thus, with an intimate evaluation of properties, methods of preparation, and specific reactions of closely related substances, a number of pathways have been proposed and investigated with respect to the above synthesis.

Should acrolein lend itself to the general reactions of aldehydes, with minimum condensation and polymerization, it would serve as an excellent starting material for this synthesis. Both the olefinic and aldehyde groups are sensitive to oxidation. Mild oxidation (takes place even in air) gives glyceric acid, CH_2CH-CHOH-COOH, and more vigorous oxidation breaks the chain. The unsaturated nature of acrolein is shown by its great instability and tendency to polymerize. However, according to Moureu, acrolein can be preserved for much longer periods by the addition of small quantities of other substances, themselves easily oxidizable (phenols, hydroquinone, etc.). The olefinic group can be protected by bromination while the aldehyde group is entered into reaction. Thus, it appears that, after the protection of the ethylenic group by bromination, the Strecker synthesis might be applicable to the formation of alpha-amino-beta,gamma-dibromocyanohydrin (II), with subsequent debromonation of the amino nitrile (II) to give the unsaturated compound (V), which could readily be hydrolyzed to alpha-amino-beta-butenoic acid (IV), or the debromonation of the dibromo amino acid (III) would also give (IV). However, the debromination of (III) would probably prove to be

less practical, since the product (IV) has a very good chance of forming the corresponding lactone under the conditions which would be in effect during the debromination. The above course of reactions are illustrated by the following equations:

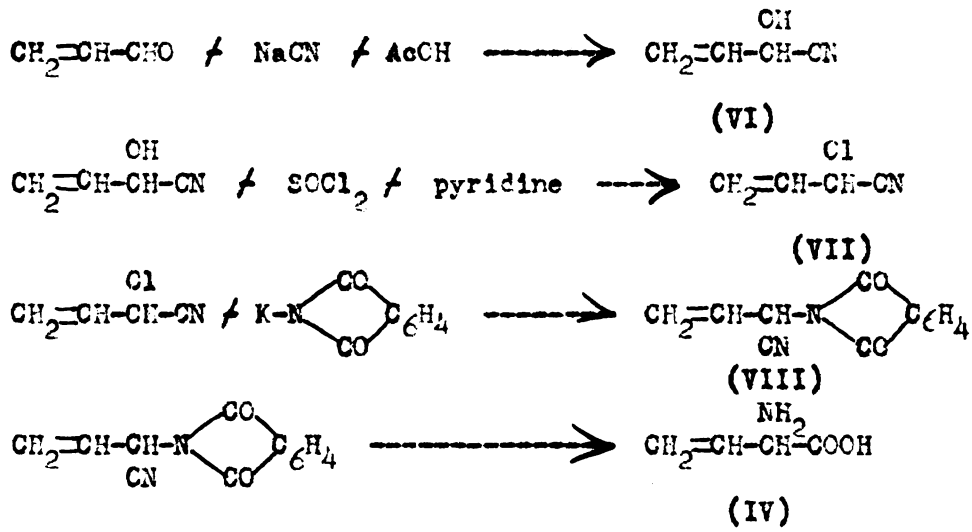


Attempts were made to prepare (V) directly by a modification of the original Strecker synthesis employed by Barker and Skinner (33), Zelinsky and Standnikoff (34, 35). Mild hydrolysis of (V) would give (IV). The reactions are as follows:



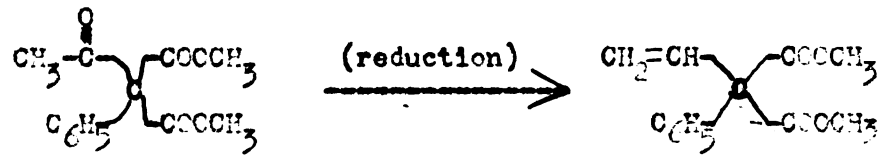
Ramband (31, 32) and Glattefeld (36) prepared vinylglycolic acid (VI) from acrolein. Ramband converted the alcohol group of (VI) to the chloro-

derivative (VII) by reacting it with $SOCl_2$ and pyridine. Many unsuccessful attempts were made to replace the chloro-group also with other groups. One such attempt involved reacting (VII) with NH_3 which would normally give the corresponding alpha-amino derivative, but an abnormal reaction resulted. However, no attempts were made to react (VII) with potassium phthalimide. The high temperature necessary for this reaction to go at a reasonable rate makes it questionable, as to whether (VII) will undergo polymerization rather than react in the normal way. Should this reaction proceed in the normal way, it would give rise to an intermediate (VIII) which could readily be hydrolyzed to give (IV).



Attempts were made by Voorhees (37) to introduce the vinyl group directly into malonic ester through the reaction of vinyl bromide with the sodium derivative of phenylmalonic ester. All attempts in this direction were unsuccessful. Voorhees and Skinner (38) made attempts to prepare vinyl analogs of barbital (veronal) and phenobarbital (luminal) by the introduction of the halogen ethyl group into the mono-alkylated malonic ester,

with subsequent elimination of halogen acid directly or indirectly, and condensation of the resulting compound with urea. They attempted to prepare dimethyl phenyl-vinylmalonate through the following ketone but the reduction did not proceed in the desired manner.



(Dimethyl phenyl-acetylmalonate) (Dimethyl phenyl-vinylmalonate)

Diethyl beta-chloroethyl-ethylmalonate was obtained in 50% yield from the condensation of ethylene chloro-iodide with ethyl malonic ester. The conversion of dimethyl ethylchloroethylmalonate to dimethyl ethyl-vinylmalonate was attempted by refluxing with freshly powered calcium oxide in xylene and with sodium ethylate in ether resulted in the recovery of the unchanged ester. Heating in sealed tubes in ether solution with sodium ethylate resulted in the breakdown to lower boiling esters. When the diethyl ethylchloroethylmalonate was heated with dimethyl amine in absolute ether in a sealed tube diethyl beta-dimethylamine-ethylmalonate was obtained. All of the above methods failed to be applicable for the introduction of a vinyl group in substituted malonic esters.

Cope and McElvain (39) proposed two seemingly possible methods of synthesis of vinylethylmalonic ester. The first of these involved the preparation of the quaternary salt (II) and the decomposition of the corresponding base by distillation into the vinylethylmalonic ester. The preparation of salt (II) was attempted by reacting trimethylamine with ethyl-

(beta-bromoethyl)-malonic ester (I). This reaction, when carried out under conditions which gave a satisfactory rate of reaction, gave tetramethylammonium bromide rather than salt (II). The malonic ester was isolated as the hydrochloride of ethyl(beta-dimethyl-aminoethyl)-malonic ester (III).

$$(CH_3)_3N + BrCH_2-CH_2-\underset{Et}{C}-(COOEt)_2 \longrightarrow (CH_3)_3N-CH_2-CH_2-\underset{Et}{C}-(COOEt)_2$$

(I) (II)

$$CH_3Br + (CH_3)_2N-CH_2-CH_2-\underset{Et}{C}-(COOEt)_2(CH_3)_3N + CH_3Br \longrightarrow (CH_3)_4Br$$

(III)

It was found that 90% of (II) precipitated when the reactants were allowed to stand in ether solution for several weeks. But, due to the poor yield and the time required, the authors abandoned this method as being impractical. The second and successful method of synthesis of vinylethylmalonic ester involved the reaction of 1,2-dibromoethene with sodio-ethylmalonic ester, followed by the reduction of the ethyl(beta-bromovinyl)-malonic ester (IV) with zinc dust and alcohol at 170 degrees to vinylethylmalonic ester (V).

$$BrCH=CHBr + Na^{+}\,{}^{-}C-(Et)(COOEt)_2 \longrightarrow BrCH=CH-C-(Et)(COOEt)_2$$

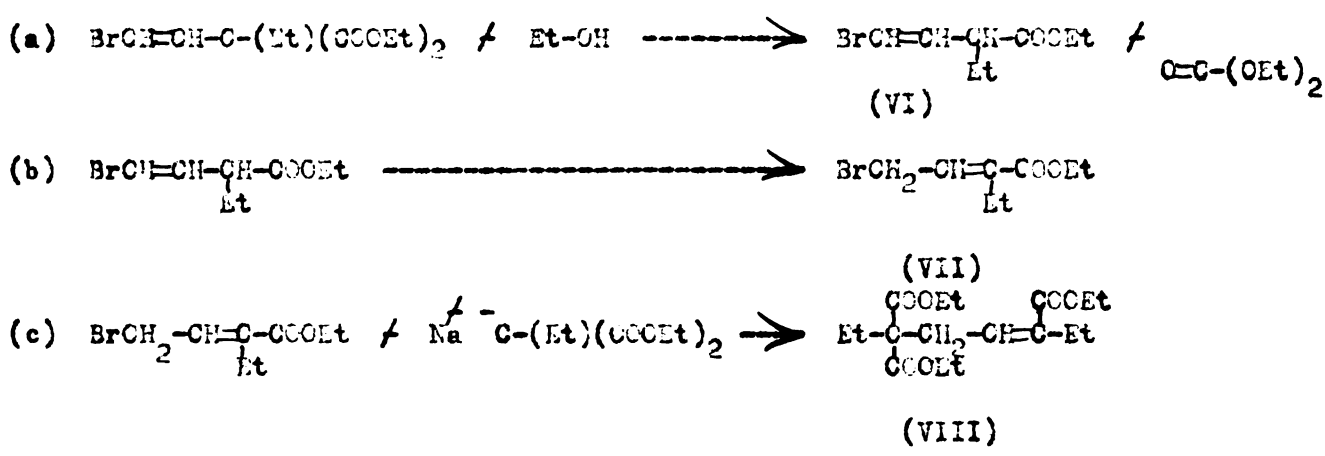
(IV)

$$\longrightarrow CH_2=CH-C-(Et)(COOEt)_2$$

(V)

The above reactions are somewhat misleading in their simplicity for the preparation of ethyl(beta-bromovinyl)-malonic ester (IV). The most favorable conditions of reaction produced along with (IV) an approximately equal quantity of a high-boiling, bromine-free tricarboxylic ester. This compound

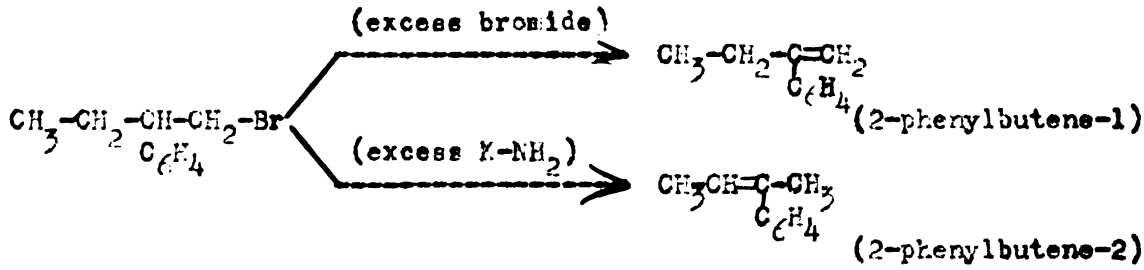
was shown to be 3,6,6-tricarbethoxyoctine-3 (VIII), which was formed from ethyl(beta-bromovinyl)-malonic ester by the following reactions.



The orgin of the alcohol necessary for reaction (a) was unexplainable at first since the reaction between 1,2-dibromoethene and sodio-ethylmalonic ester had been carried out in absolute ether. But, a further insight into the reaction involved in the formation of the sodio-ethylmalonic ester showed that only 77% of the theoretical quantity of hydrogen was evolved, thus 23% of the sodium used had reduced the malonic ester instead of forming the sodium derivative, which provided sufficient alcohol for the reaction to take place in the formation of compound (VIII).

Sorensen and Andersen (40) reacted two moles of sodio-phthalimidmalonic ester with one mole of 1,2-dibromoethane and obtained ethylene-bis(phthalimidmalonic ester). By using an excess of 1,2-dibromoethane (41) and carrying the reaction a step further, they obtained the lactone of beta-oxyethylphthalimidmalonic ester. The lactone was believed to have been formed from phthalimide(beta-bromoethyl)-malonic ester by the loss of ethyl bromide. No attempts were made to isolate and characterize the alleged intermediate in this experiment. Gamma-Bromopropylphthalimidmalonic ester has been prepared

by this method. Hauser (42) et al. carried out reactions involving the dehydrohalogenation of 2-phenyl-1-bromopropane and 2-phenyl-1-bromobutane with potassium amide in liquid ammonia to form largely unrearranged olefins. The reaction or 2-phenyl-1-bromobutane in the presence of less than an equivalent of potassium amide gave 2-phenylbutene-1, while the reaction of this bromide with an excess of potassium amide yielded 2-phenylbutene-2 as shown below:



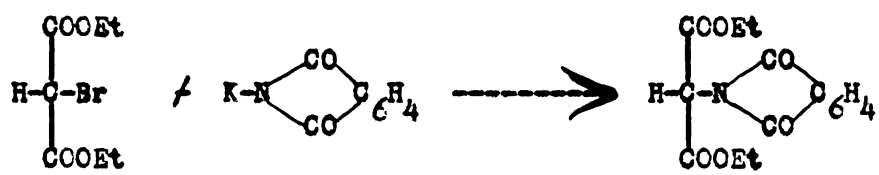
The formation of 2-phenylbutene-2 is explained on the basis of the prototropic change of 2-phenylbutene-1, which was presumably first formed, the change to 2-phenylbutene-2 being brought about by the base.

Should a bromide be prepared which could be dehydrohalogenated to give the corresponding olefin and possessed of a structure which would not permit prototropic isomerization, one could introduce a vinyl group into the proper structure which would give rise to alpha-amino-beta-butenoic acid. Thus, it was concluded that phthalimide(beta-bromoethyl)-diethylmalonate (XVIII) would be a suitable compound to test this theory. The synthesis of (XVIII) may be accomplished by starting with diethylmalonate and preparing monobromodiethylmalonate (XV), which gives phthalimide diethylmalonate (XVII) when condensed with potassium phthalimide (XVI). By properly designing and carrying out the reaction it should be possible to condense 1,2-dibromo-

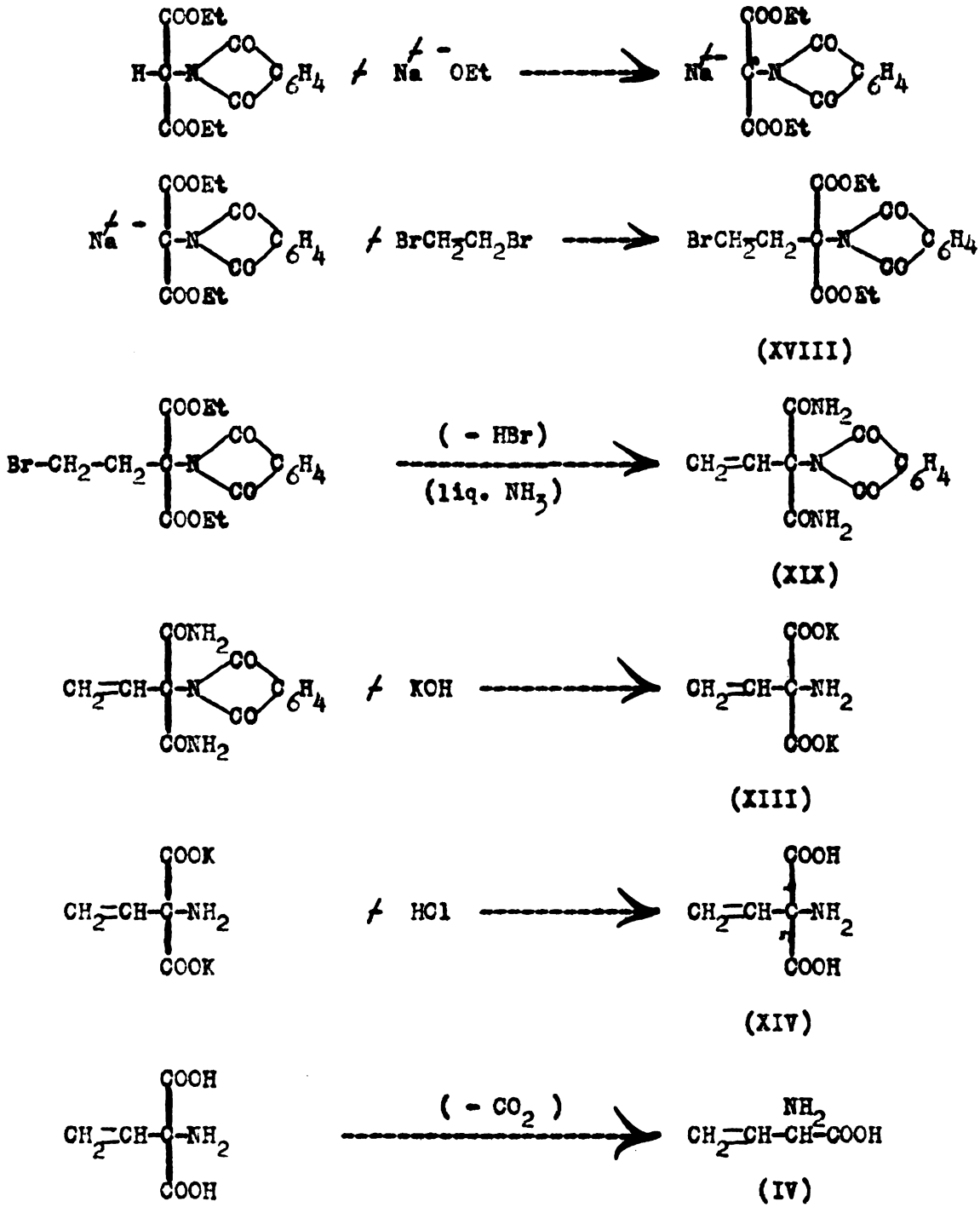
ethane with the sodio-derivative of (XVII) to give rise to (XVIII). However, the stability of (XVIII) may prove to be a major factor. The dehydrohalogenation of (XVIII) with potassium amide in liquid ammonia would give rise to (XIX), which would form (XIII), (XIV), and (IV) upon hydrolysis, acidification, and decarboxylation respectively. It would be necessary to use an excess of potassium amide in the dehydrohalogenation of (XVIII), since the liquid ammonia would react with the ester to liberate ethanol which would subsequently react with the potassium amide forming ammonia and potassium ethylate, leaving no potassium amide to dehydrohalogenate the bromide. The yield of (XIX) is also questionable, since the dehydrohalogenation reaction will be competing with the formation of the corresponding amino compound. The reactions involved are as follows:

(XV)

(XVI)



(XVII)



(XVIII)

(XIX)

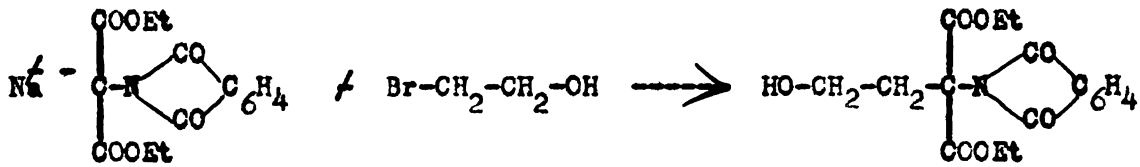
(XIII)

(XIV)

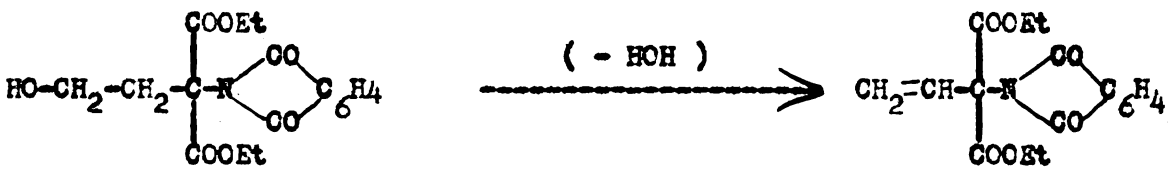
(IV)

It was of interest to further the basic idea involved in the preparation of (XI) and (XV), primary and secondary alcohol functions respectively, by

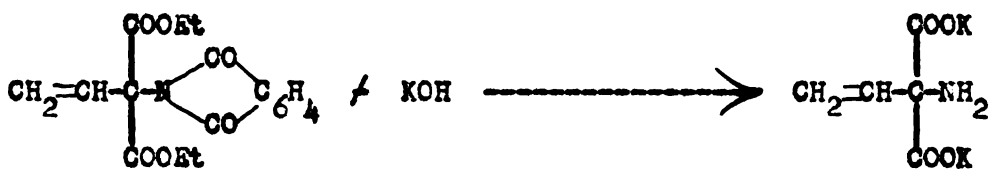
replacing the formylamino group with a phthalimide group, employing essentially the same experimental conditions. The reactions involved in the preparation of the primary alcohol function are as follows:



(XX)

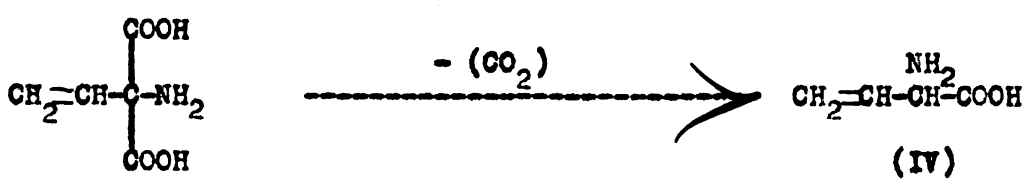


(XXI)



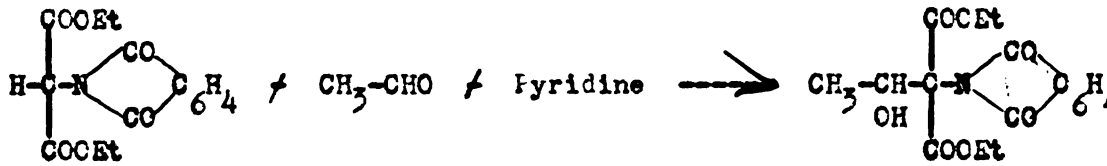
(XIII)

(XIV)

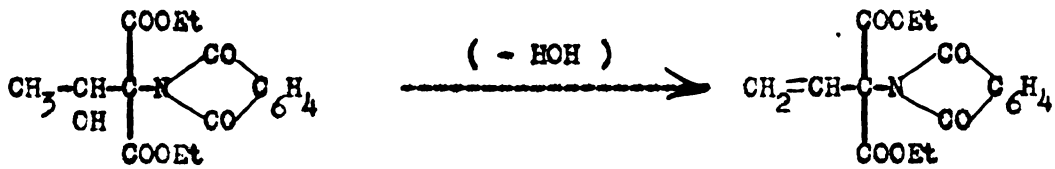


(IV)

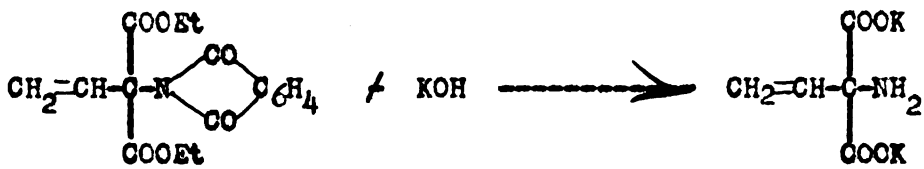
The reactions involved in the preparation of the secondary alcohol function are as follows:



(XXII)

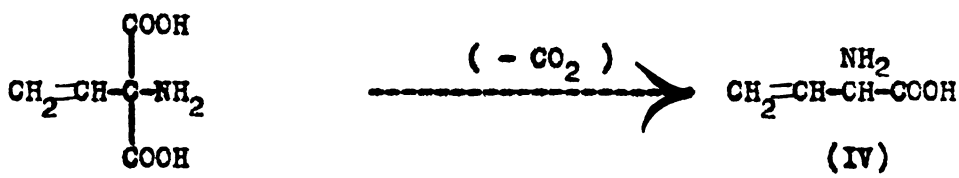


(XXI)



(XIII)

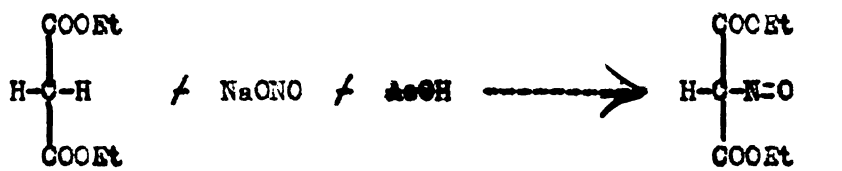
(XIV)



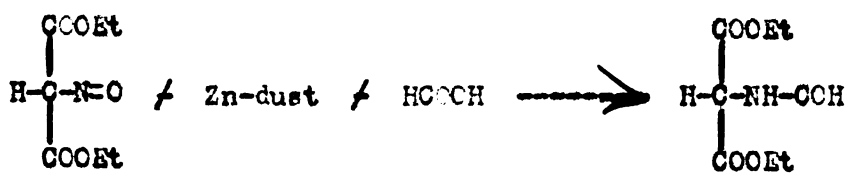
(IV)

Several methods have been employed for the introduction and protection of an amino group on malonic ester in the synthesis of alpha-amino acids. Redemann and Dunn (43) carried out a nitrosation reaction using butyl nitrite

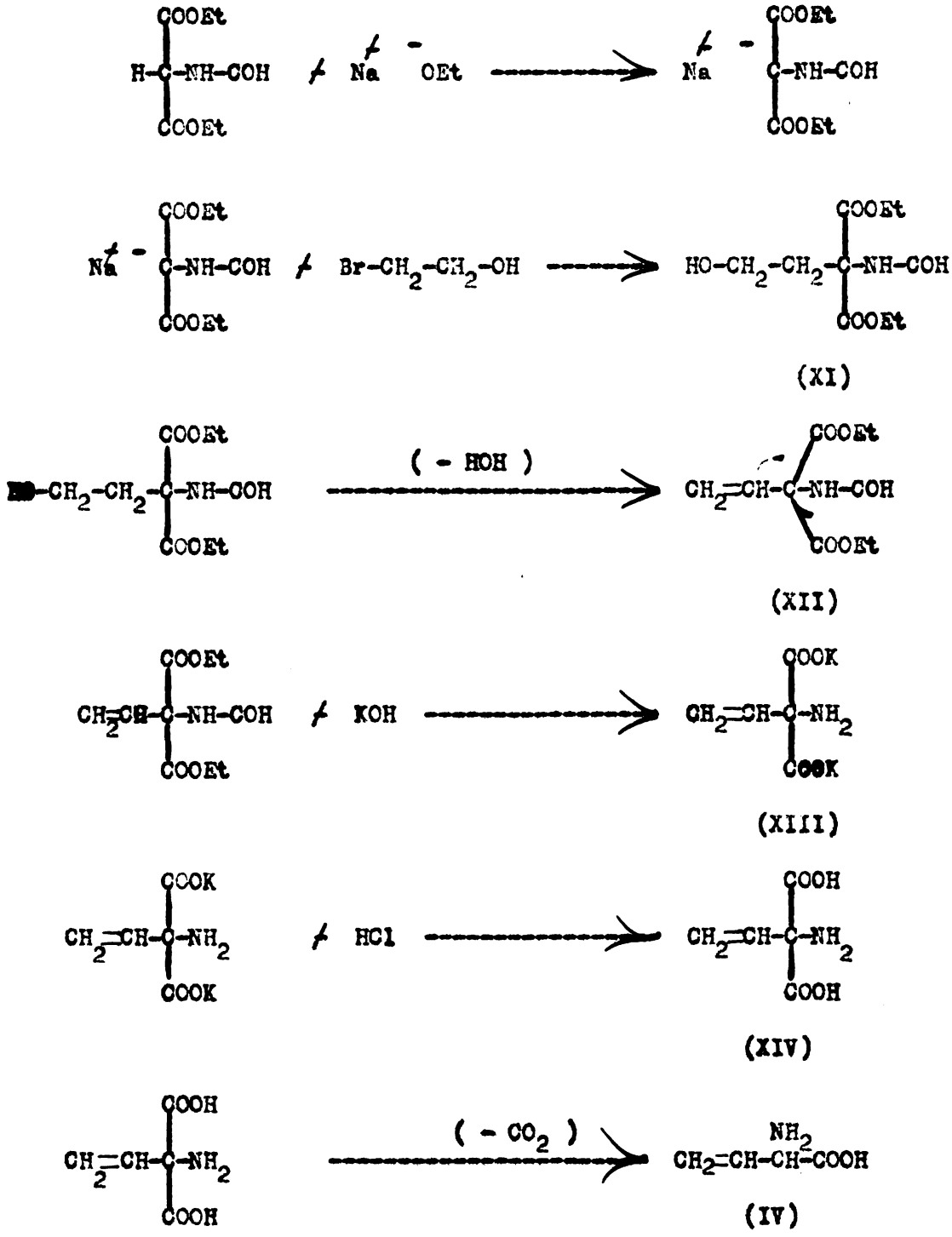
and malonic ester, with subsequent preparation of the acetylaminomalonic ester (44). The acetylation of the amino group gives a relatively poor yield by this method. Galat (45) used sodium nitrite and acetic acid in preparing nitrosomalonic ester. Subsequent reduction of this compound, by the procedure of Conrad and Schulze (46), will give formylaminomalonic ester which may be employed as an intermediate in the preparation of alpha-amino acids, Galat (45) has employed formylaminomalonic ester as an intermediate in the synthesis of amino acids. By using this intermediate one can enjoy several advantages; higher yields, reduction of nitrosomalonic ester (IX) to formylaminomalonic ester (X) without having to prepare the N-derivative in a separate step. Thus, it was felt that this intermediate (X) may be useful in the preparation of formylamino(beta-hydroxyethyl)-diethylmalonate (XI). Subsequent dehydration of (XI) would give rise to formylamino-vinyl-diethylmalonate (XII), providing no structural rearrangements occured. Hydrolysis of (XII), base catalyzed, would give the salt of amino-vinylmalonic acid (XIII). By liberating the free acid (XIV), decarboxylation could be accomplished without too many difficulties forming (IV) as follows:



(IX)



(X)



(XI)

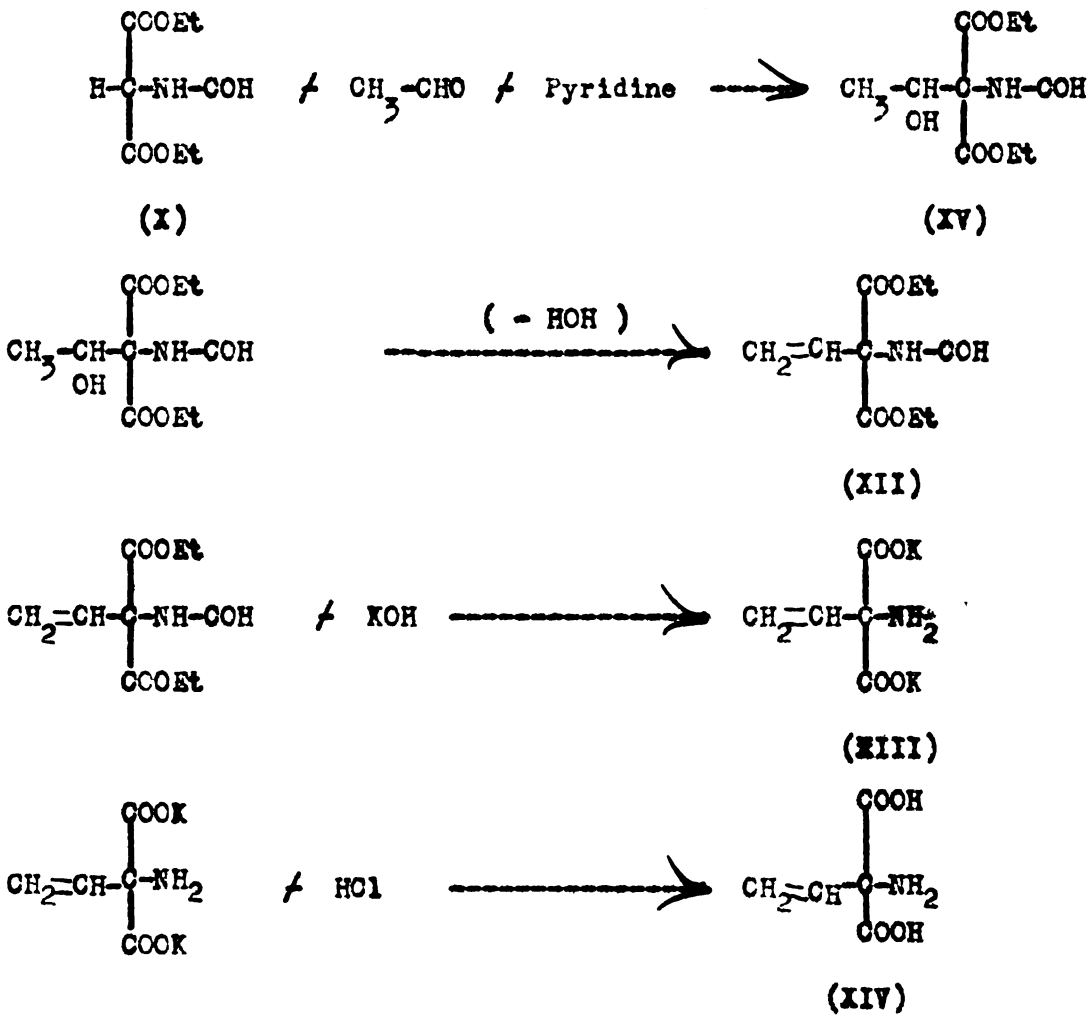
(XII)

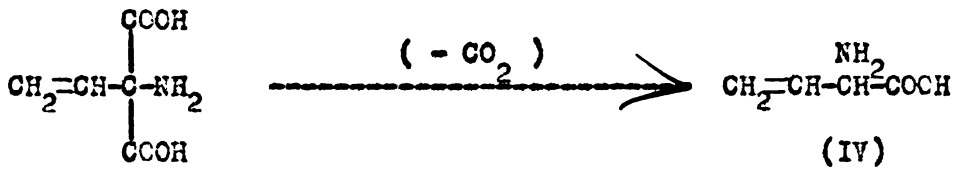
(XIII)

(XIV)

(IV)

The major problem which will be confronted in this synthesis involves the dehydration of the primary alcohol function without effecting the remainder of the molecule. Further attempts were made to prepare a similar compound which would undergo dehydration with greater ease. Thus, the dehydration of formylamino-alpha-hydroxyethyl-diethylmalonate (XV), containing a secondary alcohol function adjacent to a tertiary carbon atom, would give rise to the vinyl compound (XII), providing no molecular rearrangement occured. Subsequent hydrolysis, acidification, and decarboxylation of (XII) would give rise to (IV) going through (XIII) and (XIV). The reactions involved are as follows:





(IV)

The limiting factors in the above synthesis may involve the extent and rate which the aldol condensation between acetaldehyde and (X) will go in the formation of (XV). Malonic acid readily condenses with aldehydes in the presence of organic bases, but the methyl, ethyl, and propyl esters would be expected to effect the condensation to different degrees, as well as the substituted group.

EXPERIMENTAL

ACROLEIN SYNTHESIS:

Experiment-1-a:

A 2 l. two-necked round-bottom flask was equipped with a stirrer and a dropping funnel so arranged that the stem almost touched the stirring rod. Into the flask was placed 150 cc. of carbon tetrachloride, 66.6 cc. (1 mole) of freshly distilled acrolein stabilized with hydroquinone. The flask was placed in an ice-water bath, the stirrer started and 53 cc. (1.03 moles) of bromine was added to the dropping funnel. A small stream of bromine was allowed to flow until the addition was complete. The ice-water bath was replaced by a water-bath and the dropping funnel replaced by a condenser. The mixture was refluxed for one hour, leaving a pale yellow solution. The carbon tetrachloride was removed by distillation under reduced pressure. The product, 1,2-dibromopropionaldehyde, was distilled over at 86°/18mm. giving a pale yellow fuming liquid.

Ammonium chloride(59.0 g.) was dissolved in 185 cc. of water, cooled to 0°, and combined with the 1,2-dibromopropionaldehyde obtained above (0.79 mole). The flask was provided with a mechanical stirrer and placed in an ice-water bath. The stirrer was started and a solution containing 49.0 g. of NaCN dissolved in 140 cc. of water, previously cooled to 10°, was added a few milliliters at a time so that the temperature did not exceed 60° at any time. The mixture was stirred one hour after the addition of the NaCN solution had been completed, then the ice-water bath was removed and the stirring continued two hours at room temperature. A reddish sirup had

began to settle out at this point. The flask was stoppered and allowed to sit at room temperature twelve hours. The reaction mixture was strongly acidified with hydrochloric acid and evaporated under reduced pressure to about 350 cc., then an equal volume of concentrated hydrochloric acid was added to the concentrate and refluxed for one hour. The mixture was then evaporated almost to dryness in a round-bottom flask with constant mechanical stirring, at about 110°, then heated to 120° for a period of thirty minutes, leaving a dark viscous mass. This mass was extracted with a mixture of methanol-ethyl ether (10:1) using 200 cc. portions, leaving a large amount of salts. The extracts were combined, filtered, and evaporated to dryness, giving a brown, gummy, semi-crystalline residue. The residue was extracted with several 25 cc. portions of hot water, leaving a black resinous mass.

The hot water extract was heated to 100° and treated with powdered basic lead carbonate, in small portions, until effervescence ceased. The mixture was cooled to about 9° and filtered. The red filtrate was treated with H_2S to remove the lead. The lead sulfide was filtered off under a slight vacuum, then filtered under the influence of gravity. The red filtrate was evaporated on the steam-bath until crystals began to form, then transferred to a beaker and allowed to cool. The crude crystals were filtered off and the filtrate was again evaporated on the steam-bath as before, cooled, filtered, and the crude crystals combined. The residue was washed three times with absolute alcohol and the crystals collected by centrifugation. The filtrate was evaporated again giving more crystals, which were washed with absolute alcohol and combined with the above. The crystals were dissolved in the minimun

volume of water, treated with Norite, heated to boiling, allowed to stand for ten minutes, and filtered. The filtrate was evaporated on the steam-bath until crystals began to form, cooled throughly and filtered. The crystals were washed with absolute alcohol three times using 10 cc. protions. The filtrate was evaporated to about two-thirds of its volume, allowed to cool and more crystals formed. The white crystalline compound was very soluble in water, very slightly soluble in absolute alcohol, and insoluble in acetone and ether. The compound possessed both nitrogen and bromine.

Anal. found: N, 15.3% (macro-Kjeldahl)

calcd. for $C_4H_7O_2NBr_2$: N, 5.36%

The crystals were dissolved in dilute hydrochloric acid and evaporated to almost dryness. The residue was extracted with ten 25 cc. portions of methanol-ethyl ether (10:1) solution. The residue obtained upon evaporation of the extract was dissolved in 300 cc. of water, heated to 100°, and treated with basic lead carbonate as before, cooled throughly and treated with H_2S and filtered. The filtrate was evaporated on the steam-bath until crystals began to form, cooled, and the crystals filtered off. The nitrogen content of the purified crystals was 14.1%, less than the original by 1.2 %.

Experiment-1-b:

A 2 l. two-necked round-bottom flask was provided with an efficient mechanical stirrer, and a long stem dropping funnel with the stem about three or four centimeters from the bottom of the flask. The equipped flask was placed in an ice-dry ice-bath. Into the flask was placed 250 cc. of CCl_4 and 230 cc. (3.45 moles) of acrolein stabilized with hydroquinone

(freshly distilled). 190 cc. (3.58 moles) of bromine was placed in the dropping funnel. The stirrer was started and a small stream of bromine was allowed to flow into the mixture until the addition was complete. The cold-bath was removed and the mixture refluxed for ten minutes. The reaction mixture was then concentrated under reduced pressure until free of carbon tetrachloride. The product, 1,2-dibromopropinnaldehyde, was distilled over at 86°/18mm. giving a pale yellow liquid. 378.0 g. (1.75 moles) of dibromoacrolein were added to a flask equipped with an efficient mechanical stirrer and placed in an ice-dry ice-bath. The stirrer was started and the dibromoacrolein cooled to -5°, then a solution containing 187.25 g. (3.5 moles) of NH_4Cl dissolved in 550 cc. of water, cooled to 0, was added. The mixture was stirred vigorously for 15 minutes and a solution, previously cooled to -15, containing 171.4 g. (3.5 moles) of NaCN dissolved in 450 cc. of water was added at a rate so that the temperature remained below 25° (about two hours). The stirring was continued for three hours at room temperature, then the flask was tightly stoppered and allowed to stand for 14 hours. The reaction mixture was strongly acidified with HCl and evaporated to about 850 cc. An equal volume of concentrated HCl was added and the mixture refluxed for three hours, allowed to stand 12 hours and evaporated almost to dryness. The residue was extracted with 3.5 l. of ethyl ether-methanol (1:4) in 200 cc. portions to remove the hydrochloride of the dibromo-amino acid.

The extract was evaporated to approximately one-half its volume and a volume of water equal to the volume of solvent removed was added. This process was continued until most of the organic solvent had been removed. The water

solution of the material was treated with Norite, brought to the boiling point, allowed to stand for 15 minutes, and filtered. The filtrate was heated to 100° and treated with basic lead carbonate until effervescence ceased. The mixture was throughly cooled and filtered. The filtrate was treated with H_2S and filtered. The filtrate was evaporated on a steam-bath to about 450 cc., and four volumes of absolute methanol was slowly added to the hot filtrate, giving rise to a gelatinous precipitate upon cooling. The mixture was filtered and the filtrate evaporated on the steam-bath until crystals began to form, cooled, and the liquid decanted. The precipitate was washed with cold absolute alcohol until most of the red color had been removed. The evaporation of the filtrate with subsequent cooling was continued as long as crystals could be obtained which could be washed free of the reddish-brown material with methanol. Care was taken after the second evaporation of the filtrate to prevent a very impure product from precipitating. The precipitates were dissolved in the minimum volume of hot water and filtered into five volumes of absolute alcohol. Crystallization of a white substance occured upon cooling. The material was recrystallized three times and subjected to a macro-Kjeldahl nitrogen determination.

Anal. found: N, 12.34%

calcd. for $C_4H_7O_2NBr_2$: N, 5.36%

Experiment-1-c:

A 2 l. two-necked round-bottomed flask was equipped with an efficient mechanical stirrer and a long stem dropping funnel so arranged that the stem was extended well below the surface of the liquid. Into the flask

were placed 400 cc. of CCl_4 and 112.12 g. (2 moles) of acrolein stabilized with hydroquinone (the acrolein used in this experiment was not distilled, since former experiments gave evidence of considerable polymerization even when specific precautions were taken to prevent excess heating during the distillation). 106 cc. (2.06 moles) of bromine were placed in the dropping funnel. The stirrer was started and a small stream of bromine was allowed to flow until the addition was complete. The reaction mixture was concentrated under reduced pressure, using a water-bath at 40°, until the CCl_4 was removed. The product, 1,2-dibromoacrolein, was distilled over at 86°/13mm.

118 g. (2 moles) of NH_4Cl and 98 g. (2 moles) of NaCN were dissolved in the smallest volume of water and cooled to -10°, then placed in a flask provided with an efficient mechanical stirrer. The stirrer was started and 216 g. (1 mole) of dibromoacrolein and sufficient methanol to get it into solution were added. The temperature remained below 0° for a few minutes, then it rose sharply to a very high temperature with subsequent formation of a turbid system, followed within a few minutes with the formation of a dark heavy sirup which settled out on standing. The reaction mixture was allowed to stand at room temperature for one hour before the product was extracted with ethyl ether. The ether extract was dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure at room temperature to remove the ether, leaving a dark heavy sirup.

The sirup was divided into two equal parts and subjected to both acid and base hydrolysis. The hydrolysate from the base-catalyzed hydrolysis was made acid with HCl and both reaction mixtures were worked up using the same procedure employed in experiment-1-b. A dark resinous material

resulted from this operation which failed to give a pure product as a result of several crystallizations.

Anal. found: N, 13.2%

calcd. for $C_4H_7O_2NBr_2$: N, 5.36%

Experiment-2-a:

A 2 l. two-necked round-bottom flask was equipped with a mechanical stirrer and a thermometer. Into the flask were placed 1350 cc. of anhydrous ethyl ether, 66.6 cc. (1 mole) of freshly distilled acrolein, and 120 g. of glacial acetic acid. The flask was placed in an ice-bath, the stirrer was started and allowed to continue throughout the reaction. 98.0 g. (2 moles) of NaCN and 117.6 g. (2.2 moles) of NH_4Cl were mixed well by grinding to a powder. This mixture of NaCN and NH_4Cl was added to the content of the flask over a period of two hours. A few milliliters of water (10 to 15 cc.) were added to initiate the reaction. The temperature was kept between 4° to 6° during the addition. The mixture was permitted to sit over night in the ice-bath without reviving the bath. The stirring was continued the next morning at room temperature for three hours, giving a total reaction time of 24 hours. By this time the solution possessed an intense yellow color.

The yellow ether solution was decanted from the solid material, which was extracted with two 250 cc. portions of ethyl ether and then with acetone until most of the yellow color was removed. The extracts were combined, filtered, and dried over anhydrous magnesium sulfate. The filtrate was evaporated on the steam-bath until the ether was completely removed. A dark resinous mass resulted which did not add bromine or decolorize a

solution of $KMnO_4$.

Experiment-2-b:

A 2 l. two-necked round-bottom flask was equipped with a mechanical stirrer and a thermometer. To the flask was added 117.6 g. (2.2 moles) of NH_4Cl dissolved in 500 cc. of water, 45.0 g. of glacial acetic acid, 66.6 cc. (1 mole) of acrolein stabilized with hydroquinone, and sufficient methanol (100 cc.) to dissolve the acrolein. The flask was placed in a dry ice-methanol-bath, the stirrer was started, and the mixture cooled to -20°. 98.0 g. (2 moles) of NaCN was dissolved in 400 cc. of water and added to the cold mixture with vigorous stirring. The NaCN solution was added at such a rate that the temperature was maintained between 20° below zero and -15° (required about 35 minutes). At the end of the NaCN addition the flask was removed from the dry ice-methanol-bath until the temperature of the reaction mixture reached -2, then it was placed in an ice-water bath. The mixture was stirred vigorously for six hours, with a maximum temperature of 2°, then two more hours at 5°, then allowed to stand at room temperature for three hours. The product was extracted with ethyl ether and dried over anhydrous magnesium sulfate, then filtered. The filtrate was concentrated under reduced pressure until it was free of ether, using a water-bath just warm enough to prevent cooling as the ether evaporated. A sirupy yellow substance resulted, however, a resinous substance resulted within an hour which did not add bromine or decolorize a solution of $KMnO_4$.

Experiment-3:

A 5 l. two-necked round-bottom flask was equipped with an efficient mechanical stirrer and a dropping funnel. Into the flask were placed 347.2 g. (6.2 moles) of acrolein stabilized with hydroquinone, 3.2 l. of dry ethyl ether, and 513 g. of glacial acetic acid. The stirrer was started, then 430 g. (8.7 moles) of powdeded NaCN was placed in the dropping funnel and suspended in dry ethyl ether and added over a period of two hours. A few milliliters of water (8to 10 cc.) were added to initiate the reaction. The stirring was continued for nine hours at room temperature after the addition of the NaCN had been completed. The reaction mixture was filtered under a slight reduced pressure. The salts were washed twice with 800 cc. portions of dry ethyl ether. The straw-yellow ether solution was filtered again (gravity). The filtrate was concentrated under reduced pressure to remove the ether and other volatile solvents. The temperature of the water-bath did not exceed 60° during the porcess of concentration. The resulting yellow oil was subjected to a much lower pressure by employing a high-vacuum pump. Vinylglycolic nitrile boils at 65°/120mm.,but it was learned from former experiments that a great deal of polymerization occured under these conditions, therefore the crude compound was used. 466.0 g. of crude vinylglycolic nitrile were obtained from the 347.2 g. of acrolein.

A one-liter two-necked round-bottom flask was equipped with a thermometer, mechanical stirrer, and two dropping funnels so arranged that the tips were very close to the stirring rod. The flask was placed in an ice-salt water-bath. To the flask was added 166 g. (2 moles) of crude vinyl-glycolic nitrile which was diluted in two volumes of dry ethyl ether.

238.0 g. (2 moles) of $SOCl_2$ and 158.2 g. (2 moles) of pyridine were added to the two dropping funnels. The stirrer was started and a temperature of -3° were obtained before the addition of $SOCl_2$ and pyridine was started. There was always a slight excess of $SOCl_2$ in the reaction flask. By the end of this addition the temperature of the reacting mixture had reached 24°. Every drop of pyridine produced a turbidity which turned dark rapidly and acquired a sirupy characteristic. The mixture was allowed to stand for one hour in the cold bath with stirring, then treated cautiously with 200 cc. of water and extracted with ethyl ether, dried over anhydrous anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure, using a water-bath at a temperature not greater than 50°, to remove the ether. 80.0 cc. of a dark brown liquid, crude alpha-chloro derivative of vinylglycolic nitrile, resulted which was not distilled to prevent further polymerization.

The crude compound obtained above (80.0 g., 0.79 mole) was combined with 277.6 g. (1.5 moles) of potassium phthalimide and heated on the steam-bath for six hours, then heated for one hour in an oil-bath at 150°. The reaction mixture was cooled and treated with 200 cc. of water. A water insoluble dark resinous mass remained in the vessel which was insoluble in alcohol, acetone and ether, and was therefore abandoned.

MALONIC ESTER SYNTHESIS:

Experiment-4:

Formylaminomalonic ester.- To a mixture of 152.0 cc. (1 mole) of freshly distilled ethyl malonate and 170.0 cc. (3.0 moles) of glacial acetic acid was added a solution of 190.0 g. (2.75 moles) of sodium nitrite in 275 cc. of water. The mixture was stirred during the addition of nitrite and maintained below 20°. After the addition had been completed, the mixture was stirred for an additional four and one-half hours at room temperature. The product, nitroso-malonic ester, was extracted with chloroform and the solvent removed *in vacuo* on a water-bath. The residue, a yellow oil amounting to about 185.0 g., was dissolved in 300 cc. of technical formic acid (90%), and the mixture was transferred into a three-necked flask provided with a thermometer, a stirrer, and a reflux condenser. A small amount of technical zinc-dust was added and the mixture was stirred and heated until the reaction started. There was an induction period for several minutes after which the reaction proceeded vigorously, unless only a small amount of zinc-dust was present at this stage. 150.0 g. of zinc-dust was then added through the condenser at such a rate that the temperature was maintained between 75-80° without external heating. After the addition of zinc-dust had been completed (twenty to thirty minutes), the mixture was filtered hot, the filter-cake of zinc-formate throughly washed with formic acid and the filtrate evaporated *in vacuo* on a water-bath. The residue, which was an oil containing a small quantity of zinc-formate, was fractionally distilled *in vacuo* and the fraction boiling between 130 and 132° at 2-3 mm. of mercury was collected. It solidified

into a white crystalline mass which had a melting point of 43-49°; the yield was 104.0 g. (51.2%).

Note: One may find difficulties in fractionating the residue left after the volatile solvents have been removed, however, this may be overcome by employing the following method. The residue to be fractionated is placed in a pyrex pound-bottom flask (not over 500 cc.) and joined to a Claisen distillation head which is connected to an adaptor designed for vacuum distillation. The system appears to be most efficient when the reciving flask is not larger than 250 cc. The apparatus is connected to an aspirator which will reduce the pressure to 12-15 mm. of mercury. The temperature of the oil-bath is gradually increased until it has reached 140°. The aspirator is allowed to operate at maximum efficiency with the oil-bath at 140° until all substances which will distill under these conditions have been removed (total time was three to four hours). The reaction flask is removed from the hot bath, allowed to cool, and the vacuum is released. The second fraction is collected by connecting the apparatus to a high-vacuum pump (properly connected to an acetone-dry ice trap) and allowing it to evacuate the system to its maximum ability, then the temperature of the oil-bath is slowly increased until the fraction immediately below the one desired to be collected is completely stripped off, that is, this fraction is taken up to 129° at 2-3 mm. The reaction flask is cooled before the vacuum is released. The third fraction will be that of the range desired, 130 to 132° at 2-3 mm. It is very important that the pump have evacuated the system to its maximum ability before heating of the reacting flask is started, then the proper adjustments of pressure and temperature are made. The fact that a very dark material in the reaction

flask appears should not be interpted that the compound desired has decomposed, the dark material is mostly due to zinc formate decomposition, etc.

Sodio-formylaminomalonic ester.- 5.66 g. (0.25 mole) of Na were dissolved in 472 cc. of absolute ethanol, to which 50.0 g. (0.25 mole) of formylaminomalonic ester were slowly added with stirring. One-liter of benzene was added to the mixture and distillation was continued until one-liter of benzene-ethanol azetrope distillate had been obtained, then more benzene was added and the distillation continued in this way until the residue was completely free of ethanol, leaving the sodio-formylaminomalonic ester suspended in 250 cc. of benzene.

To this suspension was added 40.0 g. (0.32 mole) of ethylene bromohydrin and a few crystals of NaI. The flask was provided with a condenser equipped with a $CaCl_2$ tube to prevent moisture from entering and allowed to react at room temperature for 89 hours. A pale yellow solution resulted and a large amount of salt settled out. The reaction mixture was filtered, and the salts washed with anhydrous ethyl ether. The filtrate was concentrated under reduced pressure, in a nitrogen atmosphere, to remove the benzene and unreacted ethylenebromohydrin, leaving a pale yellow oil. 30.5 g. of the pale yellow oil were dissolved in seven volumes of benzene and refluxed over 22.1 g. of P_2O_5 for four hours. The flask was provided with a condenser equipped with a $CaCl_2$ tube to prevent moisture from entering. The reaction mixture was fractionated under reduced pressure. The first fraction consisted of benzene and a small amount of water, and the second fraction consisted of other volatile substances, leaving a solid residue. Neither one of the fractions

or residue would add bromine or decolorize $KMnO_4$ solution. Thus, a more accurate fractionation was not conducted.

Experiment-5:

An ethereal solution of acetaldehyde was prepared by heating an excess of paracetaldehyde with a few drops of sulfuric acid (1.5 cc. of conc. H_2SO_4 and 1.5 cc. of water) in a water-bath. The evolved acetaldehyde was passed upward through a short inclined condenser to remove any paracetaldehyde. It was dried by passing through a tube containing $CaCl_2$ and collected in 80 cc. of dry ethyl ether cooled by an external bath of ice-bath.

To this solution were added 49.0 g. (0.24 mole) of formylaminomalonic ester and 49.0 g. of pyridine. The mixture was kept for two days at 0° and then one day at room temperature. The ether and pyridine were removed by distillation under reduced pressure, using a water-bath at 30°, leaving an almost white residue. The product was crystallized several times by dissolving in the minimum volume of hot benzene and adding high-boiling ligroin (d, 0.72-0.74) until a permanent cloudyness occured, then placed in the freezing-compartment of a refrigerator for crystallization. The yield was 26.0 g. of a compound which possessed a sharp melting point of 49-50°.

Anal.	found:	C, 47.96; H, 6.30; N, 6.77
		47.88 6.27 6.73
	calcd. for $C_{10}H_{17}O_6N$:	C, 48.55; H, 6.93; N, 5.66

A 250 cc. round-bottom flask was equipped with a condenser provided with a $CaCl_2$ tube. Into the flask was placed 5.0 g. of P_2O_5 and 2.0 g. of alleged alpha-hydroxyethylformylaminomalonic ester dissolved in 100 cc. of ethyl propionate, and refluxed for three hours. The liquid was decanted

from the solid material and fractionated under reduced pressure. The fraction containing the product gave a positive Bayer's test for unsaturation. No attempts were made to carry out the next series of reactions to arrive at alpha-amino-beta-butenoic acid or even characterize the substance which gave the above test, since only traces of the material were present.

Experiment-6-a:

Ethyl monobromomalonate.- A 5 l. three-necked round-bottom flask was fitted with a stirrer, a reflux condenser, and a separatory funnel with the stem extended far enough to be below the liquid surface. The condenser was equipped with a tube leading into a container to trap most of the HBr gas which would be evolved during the reaction. In the flask were placed 1120 g. (7 moles) of freshly distilled diethyl malonate and 1050 cc. of CCl_4. 371 cc. (7.21 moles) of bromine was placed in the separatory funnel. The stirrer was started, and a small volume of bromine was run into the solution. The flask was heated by means of a heating-mantel until the reaction started. Then the remaining volume of bromine was added gradually at such a rate as to keep the liquid boiling gently. It was then refluxed until no more HBr gas was evolved (about 1.5 hours). The mixture was cooled and washed five times with 350 cc. portions of 5% Na_2CO_3 solution. It was then distilled under reduced pressure, fractions being taken up to 130°/40 mm. and at 130-150°/40 mm. The lower boiling fraction was redistilled. The fractions boiling at 130-150°/40 mm. were combined and redistilled under reduced pressure. The product boils at 132-136°/33 mm. (121-125°/16 mm.), which amounted to about 1230 g. (75-77% yield).

Potassium phthalimide.- 200 g. of phthalimide were dissolved in 4 l. of boiling hot absolute methanol by heating in a 5 l.round-bottom flask on the steam-bath. To this hot solution 76 g. of KOH, dissolved in 300 cc. of 75% methanol, was added slowly with stirring. The final solution was cooled at once and the potassium phthalimide which precipitated was filtered off. To this filtrate was added another 200 g. of phthalimide and dissolved by heating on the steam-bath, and immediately upon effecting this solution, 76 g. of KOH dissolved in 300 cc. of 75% methanol were added slowly with stirring. The solution was cooled, and the potassium phthalimide filtered off. The two portions of potassium phthalimide were combined and washed with acetone to remove any non-reacted phthalimide. This procedure gave 85-88% yield of the product on the basis of the 400 g. of phthalimide used.

Phthalimide diethylmalonate.- 550 g. (2.97 moles) of potassium phthalimide and 440 g. (1.42 moles) of monobromomalonic ester were combined, well mixed, and brought to 140° over a period of twenty minutes. The yellow mass was heated for one hour at a temperature between 145-150°, with constant stirring. While the content of the flask was hot it was poured into a large beaker and dissolved in 2.5 l. of boiling-hot 50% alcohol, crystals appeared upon cooling. The crystalline mass was washed with 2 l. of water. The product was pulverized in a large morter and added to a beaker containing water, then filtered under suction. The product was washed three more times with water and filtered under suction, and drying was accomplished by allowing the product to remain under suction. 518 g. (92% yield) of phthalimide diethylmalonate was obtained The compound possesses a faint yellow color, but almost gave a colorless solution

in benzene and alcohol. This degree of purity was obtained from the compound by dissolving in warm methanol and slowly adding water until the proper conditions for crystallization was reached.

Sodio-phthalimide diethylmalonate.- 23.0 g. (1 mole) of Na were dissolved in 900 cc. of absolute ethanol, towhich were added 303.13 g. (1 mole) of phthalimide diethylmalonate and 1.5 l. of benzene. The mixture was distilled until one liter of benzene-ethanol azetrope was collected. This was continued until all traces of ethanol had been removed, and finally all of the benzene was distilled off leaving a dry yellow substance, sodio-phthalimide diethylmalonate. A 2 l. round-bottom flask was provided with a condenser equipped with a $CaCl_2$ tube. To the flask were added 138.2 g. (0.38 mole) of sodio-phthalimide diethylmalonate, 921.2 g. (4.9 moles) of 1,2-dibromoethane, and one liter of benzene, then heated on the steam-bath for 75 hours. The reaction mixture was cooled, and filtered under suction. The residue was washed twice with 100 cc. portions of dry ethyl ether. A futher investigation of this residue revealed that it was largely unreacted sodio-phthalimide diethylmalonate with a small quantity of inorganic bromide. The filtrate was concentrated under reduced pressure, using a water-bath, until the ether, benzene, and unreacted 1,2-dibromoethane was removed, leaving a dark residue.

Upon crystallization of this residue phthalimide diethylmalonate was recovered, and a very small amount of an organic bromide. Thus, the reaction proceeded only to a very slight extent when benzene was employed as a solvent.

Experiment-6-b:

This experiment is a reproduction of experiment-6-a in the preparation of the sodio-phthalimide diethylmalonate.

Phthalimide(beta-bromoethyl)-diethylmalonate.- To a 5 l. round-bottom flask equipped with a condenser provided with a $CaCl_2$ tube were combined 327.0 g. (1 mole) of sodio-phthalimide diethylmalonate, 517 cc. (6 moles) of 1,2-dibromoethane, and 2 l. of high-boiling ligroin. The mixture was well mixed and heated on the steam-bath, with occasional stirring, for 24 hours, then cooled and filtered. The lumpy residue was ground to a powder, using a mortar and pestle and returning to the flask and covered with 5 moles of 1,2-dibromoethane and the filtrate. Heating on the steam-bath was continued for 96 hours more, then filtered while hot. The residue was washed twice with 200 cc. portions of dry ethyl ether. Crystals began to form as the filtrate cooled. The mixture was filtered giving residue-1. The filtrate was concentrated under reduced pressure, using a water-bath between 75-80°, to about 450 cc. Upon cooling more crystals settled out, which were filtered off under suction giving residue-2.

Residues 1 and 2 were combined and washed with two 200 cc. portions of dry ethyl ether and dried under suction, leaving 13.5 g. The resulting residue was found to be unreacted sodio-phthalimide diethylmalonate. The filtrate was concentrated under reduced pressure, using a water-bath at 90-95°, leaving a heavy reddish oil weighing 229.5 g. The oil was distilled under reduced pressure after unsuccessful attempts were made to crystallize it. An overall fraction was distilled over, using an oil-bath, up to

210°/1-3 mm. leaving 25 g. of a black tar residue. The distillate was fractionated giving the following fractions.

fractions	B.P. (C.)	weight (gms.)
I	-148°/1-3 mm.	21.5
II	154-162°/1-3 mm.	26.1
III	166-169°/1-3 mm.	113.7
IV	"black tar residue"	60.0

Fractions I, II, and III were clear oils which crystallized on standing. Fraction III was crystallized from benzene and high-boiling ligroin, which gave 56.5 g. of a white compound, m.p. 75° (uncorrected), which was bromine-free. The compound was soluble in ethyl ether, dimethyl ketone, alcohol, benzene, insoluble in water, and high-boiling ligroin.

Anal. founds C, 59.40; 59.43 H, 5.01; 4.96 N, 5.31;; 5.39 "No Bromine"

The heavy oil, alleged phthalimide(beta-bromoethyl)-diethylmalonate, contained bromine and nitrogen but was not characterized quantitatively. Fractions I, II, and III did not contain bromine. Thus, the compound decomposed when subjected to the conditions used in the fractionation.

Experiment-6-c:

The sodio-phthalimide diethylmalonate was prepared by the same procedure employed in experiment-6-a.

To the flask were added 138.2 g. (0.38 mole) of sodio-phthalimide diethylmalonate and 1 Kg. (5.3 moles) of 1.2-dibromoethane, then heated on the steam-bath, with occasional stirring, for 122 hours. A large amount

of salt settled out and the liquid possessed a deep red color. The content of the flask was cooled throughly, filtered under suction and gravity respectively. The filtrate was concentrated under reduced pressure, using a water-bath at 85°, leaving 100 g. of a heavy red-brown oil which contained nitrogen and bromine. The heavy oil, alleged phthalimide(beta-bromoethyl)-diethyl-malonate, failed to crystallize from a number of solvent systems.

Liquid NH_3 was prepared by passing anhydrous NH_3 gas into a Dewar flask (the container was provided with a $CaCl_2$ to keep out moisture), using dry ice-acetone as the cooling agent. 3.74 g. (0.096 mole) of clean K and a few small pieces of rusty wire gauze were added to 200 cc. of liquid NH_3 and allowed to stand, with occasional shaking, until dissolved (about 1.5 hours). 5.0 g. (0.012 mole) of the heavy oil were dissolved in 50 cc. of anhydrous ethyl ether and added rapidly, with shaking, to the solution of $K-NH_2$ (0.096 mole) in liquid ammonia. The reaction flask was provided with a $CaCl_2$ tube and allowed to react in the Dewar flask for 18 hours. The reaction mixture was poured into an evaporating dish and allowed to remain at room temperature until all of the liquid ammonia had evaporated leaving a yellowish powder. Containers which will permit the concentration of ammonia gas to build up should be avoided during this evaporation, since a concentration of 15 to 20% of NH_3 in air is explosive. This powder was treated with water cautiously, using a medicine dropper, until certain that all excess $K-NH_2$ had reacted, then enough water was added to dissolve the amide and filtered under suction, giving a straw-colored filtrate and a residue which was washed with cold water. The white residue did not add bromine

or decolorize $KMnO_4$ solution. The straw-colored filtrate was combined with an equal volume of concentrated KOH solution and gently refluxed until ammonia was no longer evolved (about 3 hours). The reaction mixture was cooled to 10° and neutralized with 6 N sulfuric acid, previously cooled to 0°. The acid was added dropwise with the container in an ice-bath so that the temperature could be maintained below 30°. A white substance, water insoluble, settled out. The material was filtered off under suction and washed twice with 15 cc. portions of cold water, The white substance was phthalic acid. The filtrate was adjusted to pH 7 more accurately at this point and concentrated under reduced pressure using a water-bath at 45°, (it was found that the corresponding malonic acid would undergo decarboxylation at about 60° in acid media), to 20 cc. The salts which separated out were filtered off under suction, giving a straw-colored filtrate, The neutral straw-colored filtrate, alleged amino-vinylmalonic acid solution, was made acid with one drop of 6 N hydrochloric acid and placed in a water-bath at 60-65° until CO_2 was no longer evolved, as shown by a white precipitate of $BaCO_3$ when the gas was passed into a saturated $Ba(OH)_2$ solution. The resulting solution was cooled to room temperature and neutralized to pH 7, then cooled to 0°. More salts separated out which was filtered under suction. The filtrate added bromine and decolorized a $KMnO_4$ solution at room temperautre within one minute producing a brown precipitate, and gave a positive ninhydrin test. The filtrate which gave the above tests, a solution of alleged alpha-amino-beta-butenoic acid, was concentrated to about one-half its volume and placed in the icebox. This stimulated the precipitation of more salt. This process of

concentrating the filtrate to remove a few milliliters of solvent and cooling was continued until the crystals which separated out gave a slight positive test for unsaturation with $KMnO_4$ solution.

The resulting filtrate, homoserine, and threonine were subjected to paper chromatography using 6" X 22" strips of Whatman # 1 paper.

Experiment-7:

Sodio-phthalimide diethylmalonate was prepared by the same procedure employed in experiment-6-a.

A 500 cc. round-bottom flask was equipped with a condenser provided with a $CaCl_2$ tube. To the flask was added 163.5 g. (0.5 mole) of sodio-phthalimide diethylmalonate, 100 g. (0.8 mole) of 2-bromo-1-ethanol, and a few crystals of NaBr. The mixture was heated on the steam-bath 49 hours, with occasional shaking. The reaction mixture was cooled and extracted with four 500 cc. portions of dry ethyl ether and filtered. The filtrate was concentrated under reduced pressure to remove the ether, then the temperature of the water-bath was increased to 50° and the concentration continued until the unreacted 2-bromo-1-ethanol had been removed. The residue, 2.7 g. of an oil, was dissolved in 150 cc. of benzene and added to a 250 cc. round-bottom flask, equipped with a condenser and $CaCl_2$ tube, containing 10.0 g. of P_2O_5 and slowly refluxed three hours. The reaction mixture was cooled, filtered, and the residue extracted with two 100 cc. portions of dry ethyl ether. The filtrate and ether extracts were combined and concentrated under reduced pressure until all benzene and ether had been removed. The residue, alleged phthalimide-vinyl-diethylmalonate, did not add bromine or decolorize $KMnO_4$ solution.

Experiment-8:

An ethereal solution of excess acetaldehyde was prepared by the same method employed in experiment-5.

To this solution were added 76.3 g. (0.25 mole) of phthalimide diethylmalonate, and pyridine. The mixture was kept for three days with a maximum temperature of 15° and finally one day at room temperature. The reaction mixture was subjected to a vacuum distillation, using a water-bath at 55°, to remove the ether and pyridine leaving a dark oil and some solid material. The mixture was filtered and the residue (70.5 g.) was characterized, after three crystallizations, on the basis of the nitrogen content derermined by the macro-Kjeldahl method. The dark oil failed to give a crystalline product from various solvent systems and decomposed upon distillation attempts at 1-3 mm. of mercury. 2.0 g. of the oil was dissolved in 100 cc. of benzene and refluxed over 3.0 g. of P_2O_5 for three hours. Thr reaction mixture was cooled and the solution decanted from the residue, which was washed with two 50 cc. portions of dry ethyl ether. The benzene solution and ether extracts were combined, filtered, and concentrated under reduced pressure until the residue was free of benzene and ether. The residue failed to add bromine or decolorize $KMnO_4$ solution.

DISCUSSION AND RESULTS

In experiment-1-a 1.2-dibromopropionaldehyde was prepared by the bromination of acrolein in CCl_4. During the removal of the CCl_4 the reaction mixture often reacted vigorously giving a dark brown brittle polymer. This behavior may be explained as follows; the CCl_4 being removed by distillation contained unreacted bromine which probably took part in a substitution reaction liberating HBr in the system, catalyzing the polymerization of the aldehyde. Hydrogen bromide gas will promote the polymerization of the aldehyde (acrolein) even at zero degrees. This was avoided by starting with acrolein which possessed the minimum degree of polymerization and would add the theoretical amount of bromine. The dibromoacrolein was subjected to the conditions employed by Baker and Skinner (33) in their modification of the original Strecker synthesis. A white crystalline compound was obtained which contained both nitrogen and bromine. The percentage nitrogen found was 15.3%, while the calculated for alpha-amino-beta,gamma-dibromocyanohydrin was 5.36%. The compound was found to be mostly ammonium salts. Further crystallizations failed to give a pure compound. It is felt that the aminocyanohydrin was only formed to a very slight extent.

The lower temperature employed in experiment-1-b did not improve the product any. Experiment-1-c employed the Zelinsky and Stadnikoff (34, 35) modification of the original Strecker (47) synthesis to 1,2-dibromopropionaldehyde, giving a dark heavy sirup. Both acid and base hydrolysis of this material, with subsequent purification, gave a dark heavy oil which formed a dark resinous substance within one hour at room temperature. Experiment-2-a

employed the Zelinsky and Stadnikoff modification of the original Strecker synthesis to non-brominated acrolein, freshly distilled. A reddish-brown resinous material was obtained at the stage where the aminocyanohydrin should have been obtained. Experiment-2-b was a repeat of experiment-2-a, except that a much lower temperature was used, The lower temperature of reaction did not prevent the polymerization of the product. In experiment-3 vinylglycolic nitrile was prepared and characterized by the method of Glatterfeld and Hoen (36) and the corresponding alpha-chlorocyanohydrin was prepared by the method of Rarband (32). Attempts were made to condense this chloro-derivative of vinylglycolic nitrile with potassium phthalimide, which was prepared by the method of Hale and Britton (48). The temperature necessary to promote a reasonable rate of reaction was sufficient to polymerize the chloro-derivative, giving rise to a solid dark mass. The reaction would not go using high-boiling ligroin or benzene as solvents.

The method of Galat (45) was utilized in experiment-4 to prepare formyl-amino-diethylmalonate, from which the sodio-derivative was prepared, and reacted with 2-bromo-1-ethanol. No unsaturated compound was formed when the dehydration of the product was attempted using P_2O_5 and benzene. An aldol condensation reaction was attempted in experiment-5 between formyl-amino-diethylmalonate and acetaldehyde, using pyridine as a catalyst. Analytical results for carbon, hydrogen, and nitrogen determinations indicated that the compound was almost totally unreacted ester. However, an unsaturated compound, alleged formylamino-vinylmalonic ester, was obtained by refluxing an ethyl propionate solution of the compound over P_2O_5. The quantity of

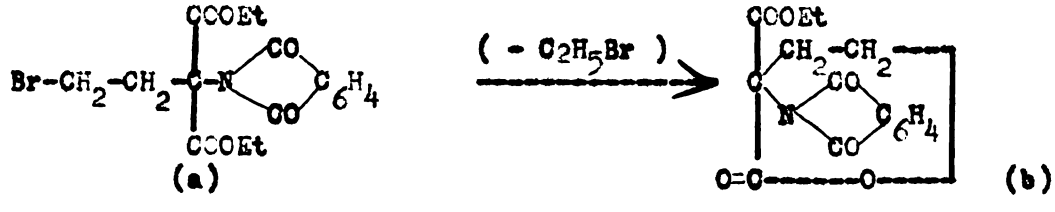
unsaturated material was insufficient to characterize. Thus, it can not be concluded that the positive Bayer's test was only due to the alleged compound. Experiment-6-a involves the preparation of ethyl monobromomalonate by a method submitted by Palmer and McWherter (49), potassium phthalimide by the method of Hale and Britton (48), and phthalimide diethylmalonate by Gabriel's (50) method, from which the sodio-derivative was prepared. The condensation of the sodio-derivative and 1.2-dibromoethane would not go using benzene as a solvent, but in experiment-6-b the condensation was carried out using high-boiling ligroin as a solvent, giving rise to a heavy reddish oil. After the oil failed to give a crystalline product it was fractionated at 1-3 mm. of mercury, leaving a black tarry residue. Fraction I was mostly high-boiling ligroin, while II and III were the same, as shown by m.p's. after crystallization from benzene and ligroin. The crystalline product melted at 73 (uncorrected).

Anal. found: C, 59.40; H, 5.01; N, 5.31; "No Bromine"
59.43 4.96 5.39 "No Ash"

calcd. for $C_{17}H_{18}O_6NBr$:

C, 49.50; H, 4.40; N, 3.39; Br, 19.39

It is quite evident that the "bromine-containing oil" undergoes decomposition when distilled at 1-3 mm. of mercury to yield a bromine-free compound. The decomposition is believed to have proceeded in the following order.



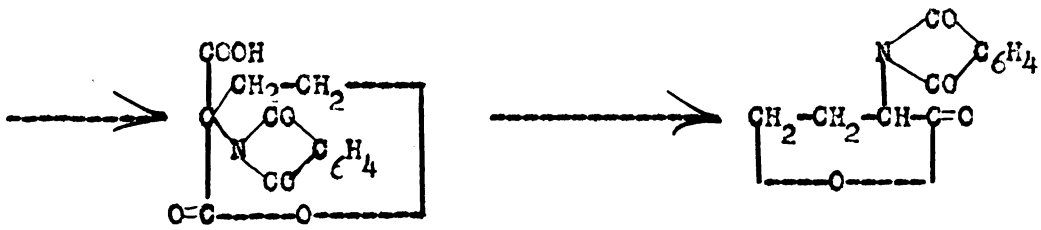
(a) (b)

(a)

% C— 49.51
H— 4.40
N— 3.40
Br— 19.50

(b)

% C— 59.38
H— 4.32
N— 4.63



(c)

% O— 56.70
H— 3.30
N— 5.08

(d)

% C— 61.80
H— 3.90
N— 6.00

One may conclude that, on the basis of the per cent nitrogen found, the crystalline product is a mixture of compounds (c) and (d).

The condensation of sodio-phthalimide diethylmalonate and 1,2-dibromoethane was carried out in experiment-6-e in the absence of high-boiling ligroin, allowing one of the reactants to act as a solvent. The product was a heavy reddish-brown oil, containing both nitrogen and bromine. The oil, alleged phthalimide(beta-bromoethyl)-diethylmalonate, was subjected to the proper conditions for dehydrohalogenation using potassium amide in liquid ammonia. An unsaturated compound was obtained which added bromine and decolorized $KMnO_4$ solution. The final product, alleged alpha-amino-beta-butenoic acid, added bromine, decolorized $KMnO_4$ solution, and gave a positive ninhydrin test. No attempts were made to remove the material from the solution (5 cc.) at this point. The following results were obtained when this material was chromatographed along with homoserine and threonine

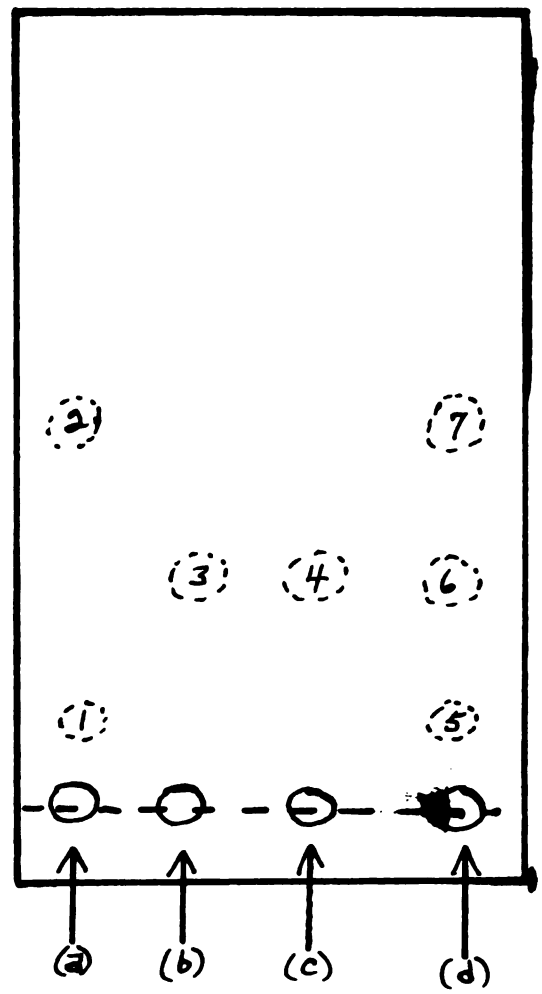
using 6" X 22" strips of Whatman # 1 paper in n-BuOH-formic acid-water (77-10-13 per cent respectively) solvent system.

(a) Unknown (alleged vinylglycine)

(b) Homoserine

(c) Homoserine + Threonine

(d) Unknown + Homoserine + Threonine



Application (a) gave rise to two spots, 1 and 2. Spot 2 has a Rf value of 0.42, which corresponds somewhat to the Rf value of 0.46 listed for glycine in n-BuOH-formic acid-water (600-50-50-respectively) solvent

system (51), while spot 1 does not correspond to any value listed for amino acids. Homoserine and threonine were not resolved under the conditions employed here, as shown by application (c), giving only spot 4 which corresponds to spot 3 from application (b). Application (d) gave rise to spots 5, 6, and 7. Spot 7 corresponds to spot 2 or glycine, while spot 6 corresponds to spots 3 and 4, homoserine and threonine. Spot 5 is relatively close to spot 1 in Rf value, that is, it may be concluded from this particular situation that spots 1 and 5 are due to the same substance. Spots 2 and 7 or glycine would be expected if traces of phthalimide diethylmalonate were present in the sample of alleged phthalimide-vinylmalonic amide undergoing hydrolysis. No attempts were made to elut spots 1 and 5, or even better, to apply larger quantities of the unknown to a heavier paper and elute for characterization purposes. However, attempts were made to crystallize the material (4.5 cc.) from alcohol and water, which resulted in a total non-recovery of any material which added bromine or decolorized $KMnO_4$ solution.

The replacement of the formylaminomalonic ester function by the phthalimide function to obtain the beta-hydroxyethyl-derivative (primary alcohol) in experiment-7 and the alpha-hydroxyethyl-derivative (secondary alcohol) in experiment-8 gave little or no achievements, under the conditions employed, in so far as the introduction of a vinyl group into a malonic ester derivative.

SUMMARY

The following is a summary based on the conditions specified in the procedure employed for each reaction.

1. Various modifications of the original Strecker synthesis failed to yield a stable dibromo-alpha-aminocyanohydrin, starting with 1,2-dibromopropionaldehyde. A white crystalline compound, containing bromine and approximately three times the calculated per cent of nitrogen, was obtained by carrying the synthesis through without isolating the alpha-aminocyanohydrin.

2. Resinous materials were obtained on attempts to prepare the alpha-aminocyanohydrin derivative from acrolein.

3. Unsuccessful attempts were made in condensing potassium phthalimide and alpha-chloro-vinylacetonitrile.

4. The preparation of formylaminomalonic ester and the corresponding sodio-derivative was conducted. Treatment of the sodio-derivative with 2-bromo-1-ethanol and subsequent refluxing over P_2O_5 failed to give a vinyl-derivative of malonic ester. However, an aldol condensation reaction involving acetaldehyde, formylaminomelonic ester, and pyridine as a catalyst, with subsequent refluxing in benzene over P_2O_5 gave a very small quantity of a substance which added bromine. Further investigations along this line were abandoned since it was labeled as being impractical on the basis of the yield and the rate of the reaction.

5. The condensation of sodio-phthalimide diethylmalonate with 1,2-dibromoethane gave an oil, alleged phthalimide(beta-bromoethyl)-diethylmalonate, which decomposed rapidly when distilled at 1-3 mm. of mercury giving a

bromine-free compound. When the oil was carried through a series of reactions, after dehydrohalogenation with potassium amide in liquid ammonia, to the stage where alpha-amino-beta-butenoic acid should have been present, two spots were obtained when the material was subjected to paper chromatography. One spot corresponded to glycine, while the other spot did not correspond to any amino acid.

6. The replacement of the formylamino function by the phthalimide function in the corresponding derivative of diethylmalonate failed to aid in the introduction of a vinyl group when the primary and secondary alcohol functions were introduced and subsequently subjected to refluxing in benzene over P_2O_5.

7. All experiments which involved acrolein as the starting material gave resinous materials regardless of the conditions used. The stability of acrolein is not great enough to be subjected to the conditions necessary for the reactions to go. Thus, it is felt that alpha-amino-beta-butenoic acid (vinylglycine) can not be obtained by the pathways proposed using acrolein as the starting material.

All attempts made to introduce a vinyl group on formylaminomalonic ester and phthalimide diethylmalonate failed. However, the results obtained in this area of reactions were sufficiently favorable to establish that the vinyl derivative, of the above mentioned structures, is probably a practical synthesis under a set of conditions different from those specified in this document.

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