soid 700 Å. long, with an axial ratio of 18 to 1. A disk-shaped (oblate) ellipsoidal model is also compatible with all the observed data, but appears inherently less probable.

4. Some of the non-clottable protein of Fraction I also consists of elongated molecules of the order of 600 Å. long.

BOSTON 15, MASS. RECEIVED MAY 7, 1947

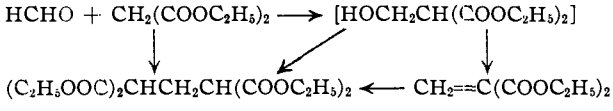
[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

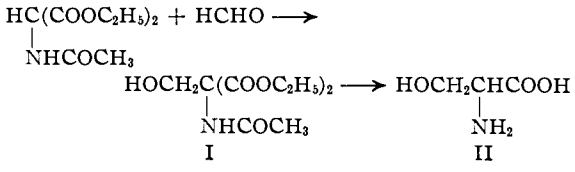
A New Synthesis of *dl*-Serine[1]

BY JOHN A. KING[2]

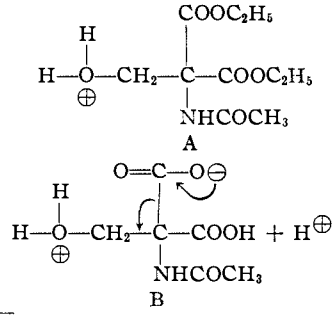
The amino acid *dl*-serine has been synthesized by four general methods: (1) a Strecker type of synthesis on glycolaldehyde[3] or ethoxyacetaldehyde[4,5,6]; (2) a Claisen-type formylation of ethyl hippurate with subsequent reduction and hydrolysis[7,8]; (3) an alkylation of the sodium enolate of ethyl phthalimidomalonate with chloromethyl ether followed by hydrolysis[9,10]; and (4) from acrylic esters by conversion to the α-bromo-β-alkoxy (or -hydroxy) esters followed by saponification, amination and hydrolysis.[11-14] None of these methods is especially convenient, the starting materials for some of them are difficult, laborious or expensive to prepare, and the over-all conversion to pure serine is not particularly good by any of them. The relative inaccessibility of serine prompted a search for a convenient and economical synthesis that would give a good yield of product without the use of high-pressure amination or drastic acid hydrolysis of an ether linkage. The present paper reports such a synthesis.

In the Knoevenagel condensation of formaldehyde with ethyl malonate in the presence of catalytic amounts of diethylamine the product isolated is the bis compound, ethyl α,α′-dicarbethoxyglutarate,[15] but when a trace of caustic soda is used as the condensation catalyst it has been claimed[16] that the bimolecular condensation product, methylolmalonic ester, is the initial reaction product. Apparently, however, this aldol-like primary condensation product has never been isolated from the reaction because of its tendency to undergo dehydration to methylene malonic ester or further condensation to the bis compound. Should formaldehyde similarly condense with the now readily available ethyl acetamidomalonate[17,18,19] the product, ethyl α-acetamido-α-carbethoxy-β-hydroxypropionate (I), would be incapable of intramolecular dehydration and might be expected to produce serine (II) on acid hydrolysis. Formaldehyde and ethyl acetamidomalonate were found to condense to give a quantitative yield of I. However, concentrated hydrochloric acid hydrolysis of I caused complete destruction of the molecule and gave no serine; the nitrogen came out as ammonium chloride and the rest of the molecule was converted to pyruvic acid. The same results were obtained with 1 *N* hydrochloric acid.





This behavior appears more rational after consideration of the electronic structure of the molecule. The oxygen of the hydroxyl group in the molecule is inherently nucleophilic and, in the presence of strong mineral acid, is susceptible to proton attack, forming the oxonium ion A. Under the reaction conditions employed the ester link-



(1) Presented before the Division of Organic Chemistry of the American Chemical Society at the 111th Meeting, Atlantic City, April 14, 1947.

(2) Present address: Warner Institute for Therapeutic Research, 113 West 18th Street, New York, N. Y.

(3) Fischer and Leuchs, *Ber.*, **35**, 3788 (1902).

(4) Leuchs and Geiger, *ibid.*, **39**, 2644 (1906).

(5) Dunn, Redemann and Smith, *J. Biol. Chem.*, **104**, 511 (1934).

(6) Redemann and Icke, *J. Org. Chem.*, **8**, 159 (1943).

(7) Erlenmeyer, *Ber.*, **35**, 3769 (1902).

(8) Erlenmeyer and Stoop, *Ann.*, **337**, 236 (1904).

(9) Mitra, *J. Indian Chem. Soc.*, **7**, 799 (1930).

(10) Maeda, Terumi and Suzuki, *Bull. Inst. Phys. Chem. Research* (Tokyo), **17**, 267 (1938); *C. A.*, **34**, 6931 (1940).

(11) Schiltz and Carter, *J. Biol. Chem.*, **116**, 793 (1936).

(12) Carter and West, *Organic Syntheses*, **20**, 81 (1940).

(13) Wood and du Vigneaud, *J. Biol. Chem.*, **134**, 413 (1940).

(14) Mattocks and Hartung, *ibid.*, **165**, 501 (1946).

(15) Knoevenagel, *Ber.*, **27**, 2346 (1894).

(16) D'Alelio, U. S. Patent 2,330,033; *cf.* Welch, *J. Chem. Soc.*, 257 (1930), who believes that the primary reaction product is monomethylolmalonic ester when amine catalysts are used.

(17) Locquin, *Bull. soc. chim.*, [4] **49**, 42 (1931).

(18) Snyder and Smith, THIS JOURNAL, **66**, 350 (1944).

(19) Albertson, Archer and Suter, *ibid.*, **66**, 500 (1944); **67**, 36 (1945).

ages undergo hydrolysis to the diacid which now, by virtue of the electronic displacements in its ionized state (B), can both expel a water molecule and lose carbon dioxide[20] to yield α-acetamidoacrylic acid. However, it is already known[21,22] that α-acylaminoacrylic acids are decomposed to pyruvic acid and amines by even gentle acid or alkaline treatment.

$$CH_2{=}C(NHCOR)COOH \xrightarrow{H_2O} CH_3COCOOH + RCONH_2 \xrightarrow{H_2O,\ HCl} NH_4Cl$$

Because it seemed probable that any acid strong enough to hydrolyze the carbethoxyl groups of I would also be strong enough to initiate the above-outlined degradation, recourse was had to alkaline hydrolysis of I. When the material was treated with exactly one equivalent of normal caustic it underwent rapid saponification at room temperature to give the half ester half sodium salt of I, from which crystalline α-acetamido-α-carbethoxy-β-hydroxypropionic acid (III) could be isolated in 54% yield. This acid lost carbon dioxide when heated just above its melting point and gave a quantitative yield of ethyl α-acetamido-β-hydroxypropionate (IV). The latter, on hydrochlo-

$$I \longrightarrow \underset{\underset{NHCOCH_3}{|}}{HOCH_2C(COOH)COOC_2H_5} \longrightarrow \underset{\underset{NHCOCH_3}{|}}{HOCH_2CHCOOC_2H_5} \longrightarrow II$$

III IV

ric acid hydrolysis, gave serine (II), identified as its methyl ester hydrochloride, m. p. 134° (dec.).

The melting point of serine methyl ester hydrochloride was reported as 114° by Fischer and Suzuki[23] and apparently has subsequently not been questioned. In the present work this substance has been prepared from *dl*-serine synthesized by the procedure herein described, from *dl*-serine prepared by the procedure of Carter and West,[12] and from a commercial sample of *dl*-serine that was purchased.[24] In each case the ester hydrochloride, after suitable recrystallization from methanol–ether, melted at 134° either alone or when mixed with either of the other two samples. It is thought possible that the melting point reported by Fischer was a typographical error and that it should have been 134° instead of 114°.

The above-described partial hydrolysis of the diester I is somewhat tedious, so the ester was treated with slightly more than two equivalents of caustic to form the disodium salt of the malonic acid from which the acid itself, α-acetamido-α-carboxy-β-hydroxypropionic acid (V), could be obtained. The latter, on being heated in weakly acidic aqueous solution lost carbon dioxide to

$$I \longrightarrow \underset{\underset{NHCOCH_3}{|}}{HOCH_2C(COOH_2)} \longrightarrow \underset{\underset{NHCOCH_3}{|}}{HOCH_2CHCOOH} \longrightarrow II$$

V VI

give N-acetylserine[25] (VI) which was not isolated but was hydrolyzed directly to serine.

This last-discussed route from the malonic ester I to *dl*-serine (II), *via* the malonic acid V and the acetyl amino acid VI, was employed for the preparation of *dl*-serine in 65% over-all yield based on ethyl acetamidomalonate.

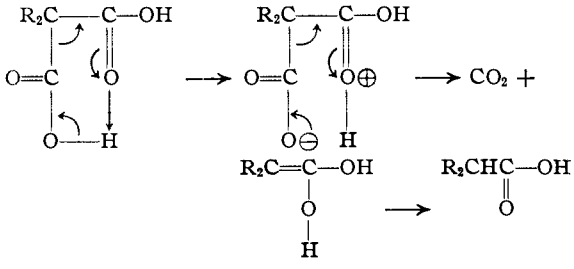
A similar attempt was made to utilize ethyl acetamidocyanoacetate[26] (VII) in a synthesis of serine. This ester condensed with formaldehyde

$$\underset{\underset{NHCOCH_3}{|}}{\underset{|}{\overset{CN}{|}}HCCOOC_2H_5} + HCHO \rightleftharpoons \underset{\underset{NHCOCH_3}{|}}{\underset{|}{\overset{CN}{|}}HOCH_2CCOOC_2H_5}$$

VII VIII

to give a quantitative yield of ethyl α-acetamido-α-cyano-β-hydroxypropionate (VIII) which for many months was a non-crystalline non-distillable resin. As was now expected, on the basis of the behavior of I, the hydroxy ester VIII was converted to pyruvic acid and ammonium chloride by acid hydrolysis. The isolation and characterization of pyruvic acid from the acid hydrolysis is proof that VIII actually has the structure indicated, that condensation did not occur on the nitrogen atom, and that the uncrystallizable resin was not merely a mechanical mixture of VII and formaldehyde. In the case of VIII, however, alkaline hydrolysis was also unsuccessful; the only nitrogenous product isolated was glycine, identified as its methyl ester hydrochloride. Since the only difference between I and VIII consists in the replacement of a carbethoxy group in the former by a cyano group in the latter, the difference in behavior in the two can be attributable only to the

(20) The author's conception of the decarboxylation of this and other malonic acids discussed in the present paper is somewhat similar to that suggested by Westheimer and Jones, THIS JOURNAL, **63**, 3283 (1941), for the decarboxylation of β keto acids. Very briefly, and graphically expressed, it is:



(21) Bergmann and Grafe, *Z. physiol. Chem.*, **187**, 187 (1930).

(22) King and McMillan, completed but unpublished work on α-acylaminoacrylic acids and the pseudoxazolones derived from them by azlactonization.

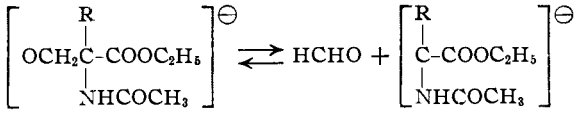
(23) Fischer and Suzuki, *Ber.*, **38**, 4193 (1905).

(24) Purchased from Merck and Co.

(25) N-Acetylserine was prepared by Synge, *Biochem. J.*, **33**, 1924 (1939), but was not analyzed because it was "a resin, soluble in water and alcohol, slightly soluble in ethyl acetate and acetone, and insoluble in other organic solvents. No attempt was made to characterize the compound further, as all attempts to make it crystallize were unsuccessful."

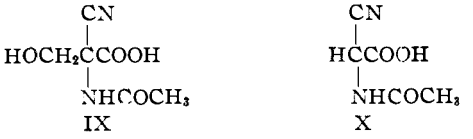
(26) (a) Albertson and Tullar, THIS JOURNAL, **67**, 502 (1945); (b) Tullar, U. S. Patent 2,393,723.

cyano group. In the case of both I and VIII one of the actions of a base will be to extract a proton from the molecule and give rise to an anion, as the



initial step in reverse aldolization. The probable reason for reversal in the case of the cyano compound is the greater resonance stabilization of the cyanoacetate enolate anion, as compared with the malonate enolate anion, with a concomitant lowering of the activation energy of the transition state in the cleavage. Therefore, in the presence of aqueous base the molecule VIII merely undergoes a reverse aldol condensation, reforming VII which is converted, in the normal manner,[27] to glycine. It should be clearly understood that the difference between I and VIII is solely one of degree and not one of kind; in the malonate displacements apparently are insufficient to cause any appreciable reversal, whereas in the cyanoacetate the critical threshold for reversal has obviously been passed.

In an experiment in which VIII was treated with one equivalent of base and then carefully acidified, in the hope of obtaining α-acetamido-α-cyano-β-hydroxypropionic acid (IX) the product actually obtained was a non-crystalline resin having the correct nitrogen analysis for acetamidocyanoacetic acid (X). Thus, the cyano compound



IX X

VIII cannot be used as an intermediate in a serine synthesis of the type herein discussed.

Experimental Part[28,29]

Ethyl α-Acetamido-α-carbethoxy-β-hydroxypropionate, I.—To a thick mush of ethyl acetamidomalonate (43.4 g., 0.20 mole) and water (25 cc.) there was added in one portion a solution of formalin (17.0 g. of 37% formaldehyde in water, 6.3 g. of formaldehyde, 0.21 mole) which had been just neutralized to litmus with 1 *N* sodium hydroxide. Then 0.5 cc. of 1 *N* sodium hydroxide was added as a catalyst and the mixture was allowed to stand two hours. All the solid was in solution after about thirty minutes. The clear solution was then distilled under vacuum azeotropically with ethanol and benzene until all the water and ethanol had been removed, then the dry residual oil was taken up in benzene (300 cc.), filtered from a small amount of white solid (sodium salts derived from the catalyst) and crystallized by the slow addition to the warm solution of Skellysolve B (1 liter). The first crop of colorless crystals weighed 43.3 g. and the second crop weighed 5.2 g., for a total of 48.5 g. (98.5% yield) of product which melted at 65–65.5°. If the filtered benzene solution is taken to dryness and the residue is triturated with Skellysolve B there is obtained a quantitative yield of colorless crystalline condensation product, m. p. about 62–64°.

Anal. Calcd. for $C_{10}H_{17}NO_6$: C, 48.58; H, 6.88; N, 5.66. Found: C, 48.38; H, 6.80; N, 5.68.

Acid Hydrolysis of I.—The above ester (12.3 g., 0.050 mole) was dissolved in concentrated hydrochloric acid (100 cc.) and the mixture was refluxed two hours then taken to dryness under vacuum. The residue was dried by azeotropic distillation with benzene then the dry residue was leached with boiling ethanol and filtered. The filter cake of white crystalline ammonium chloride weighed 2.10 g. (80% of the nitrogen present in the starting material); it gave the usual tests for ammonium and chloride ions.

A similar run was made, using smaller amounts of ester and acid, but in the same proportions; a few cc. of the solution was treated with sodium acetate and semicarbazide hydrochloride, yielding the semicarbazone of pyruvic acid, m. p. 202–203° (dec.), undepressed when mixed with an authentic sample.[30]

Exactly the same results were obtained when 1 *N* hydrochloric acid was used.

α-Acetamido-α-carbethoxy-β-hydroxypropionic Acid, III.—Ethyl α-acetamido-α-carbethoxy-β-hydroxypropionate (12.35 g., 0.050 mole) was dissolved in 43.5 cc. of 1.148 *N* sodium hydroxide at room temperature. The saponification was rapid and slightly exothermic. A small amount of gelatinous precipitate was removed by filtration, the filtrate was acidified with 4.5 cc. of concentrated hydrochloric acid (0.20 cc. more than 0.050 mole), and then evaporated to a semi-crystalline sirup in a vacuum desiccator over phosphorus pentoxide and solid potassium hydroxide. The sirupy residue was triturated with absolute alcohol (100 cc.) and most of the sodium chloride was removed by filtration. The filtrate was evaporated to a thick sirup in a stream of filtered air, taken up in dry alcohol and diluted with an equal volume of dry ether to precipitate the rest of the sodium chloride which was removed by filtration. The filtrate was again evaporated to a thick sirup in a stream of filtered air and partially crystallized on standing overnight at room temperature. The white crystalline solid weighed 5.9 g. (54% yield) and melted at 112–113° (dec.).

Anal. Calcd. for $C_8H_{13}NO_6$: N, 6.39. Found: N, 6.42.

Ethyl α-Acetamido-β-hydroxypropionate, IV.—Ethyl α-acetamido-α-carboxy-β-hydroxypropionate (2.19 g., 0.010 mole) was placed in a small test-tube and heated in an oil-bath at 125° until no more evolution of gas occurred. The cooled residue was taken up in absolute alcohol, ten volumes of dry ether now added to precipitate a very small amount of gelatinous material which was removed by filtration. The filtrate was evaporated in a stream of filtered air, then the residue was allowed to stand at room temperature under 0.10 mm. pressure for several hours. The pale yellow oil weighed 1.75 g. (quantitative yield).

Anal. Calcd. for $C_7H_{13}NO_4$: N, 8.00. Found: N, 8.19.

Serine by Hydrolysis of IV.—About a gram of the above ester was dissolved in 15 cc. of concentrated hydrochloric acid and the mixture was refluxed for six hours. The dark solution was taken to a sirup under vacuum on the steam cone, then taken up in water and charcoaled. The aqueous solution of serine hydrochloride was treated with Amberlite IR-4-B A. G. exchange resin and then evaporated to a thick sirup on the steam cone. Trituration of the sirup with absolute alcohol converted it to solid serine which, after washing with absolute alcohol and dry ether, was white, powdery and amorphous; wt., 0.22 g. (about 37% yield). The material was suspended in dry methanol (10 cc.) and the suspension was boiled as dry hydrogen chloride was passed in until all the suspended solid had dissolved and then five minutes longer. The solvent was removed under vacuum on the steam cone and the residue was recrystallized twice from dry methanol–dry ether to give long white crystals of serine methyl ester hydrochlo-

(27) Albertson, THIS JOURNAL, **68**, 450 (1946).

(28) Melting points are uncorrected.

(29) Analyses reported were carried out under the direction of Mr. Morris E. Auerbach in the Analytical Laboratories of this Institute.

(30) Backer, *Rec. trav. chim.*, **31**, 27 (1912).

ride, m. p. 134° (dec.) alone or when mixed with an authentic sample.

Anal. Calcd. for $C_4H_9NO_3 \cdot HCl$: N, 9.00; Cl (ionic), 22.83. Found: N, 8.88; Cl (ionic), 22.75.

α-Acetamido-α-carboxy-β-hydroxypropionic Acid, V.—The diester I (12.35 g., 0.050 mole) was dissolved in a solution of 4.20 g. of C. P. sodium hydroxide (0.103 mole) in water (40 cc.) and the solution, which warmed somewhat during the saponification, was allowed to stand overnight. A small amount of flocculent material was removed by filtration and the filtrate was acidified with concentrated hydrochloric acid. The acidic solution was distilled azeotropically with benzene under vacuum and below room temperature, then the residue was again similarly distilled with benzene and absolute alcohol. The dry residue was then slurried with absolute alcohol and the sodium chloride was removed by filtration. Two volumes of dry ether was added to the filtrate to complete the precipitation of sodium chloride, which was again removed by filtration. The solvent was removed from the filtrate at room temperature. After several days the material partially crystallized in large thick spears, m. p. 88–89°, which weighed about a gram. After recrystallization from benzene the material melted at 92–93°.

Anal. Calcd. for $C_6H_9NO_6$: N, 7.33. Found: N, 6.92.

Serine by Hydrolysis of V.—The malonic ester I (2.47 g., 0.010 mole) was saponified with 20 cc. of 1.21 *N* sodium hydroxide (0.0242 mole) by allowing the solution to stand overnight. There was then added to the alkaline solution 4.0 cc. of glacial acetic acid (making the resultant solution about 10% in acetic acid), and the solution was evaporated to a thick sirup on the steam cone. It was then taken up in concentrated hydrochloric acid (10 cc.) and the solution was refluxed one hour, taken to dryness under vacuum, freed of hydrochloric acid and converted, in the usual manner, to serine methyl ester hydrochloride, m. p. and mixed m. p. 134°.

***dl*-Serine.**—One mole (217 g.) of ethyl acetamidomalonate was condensed with formaldehyde as described above. The aqueous solution was treated with 2.18 moles of aqueous caustic and the malonic acid V was decarboxylated with acetic acid (3.32 moles) as described above. The concentrated aqueous acetic acid solution of VI was refluxed one hour with concentrated hydrochloric acid (600 cc.) to remove the acetyl group and the mixture was taken to dryness. The serine was removed from sodium chloride and sodium acetate by leaching with hot absolute ethanol which converted the serine to serine ethyl ester hydrochloride. The alcoholic salt-free leachings were taken to dryness and the residue was refluxed one hour with concentrated hydrochloric acid (500 cc.) to give a dark-colored solution of serine hydrochloride from which colorless crystalline *dl*-serine (68 g., 65% over-all yield) was obtained by the usual procedure. The product contained 13.29% nitrogen (theoretical for serine, 13.33%) and assayed 99–100% by both perchloric acid titration and by periodate oxidation.

Ethyl α-Acetamido-α-cyano-β-hydroxypropionate, VIII.—To a mixture of ethyl acetamidocyanoacetate (34.0 g., 0.20 mole), formalin (17 g. of 37% formaldehyde in water, 6.3 g. of formaldehyde, 0.21 mole) which had been just neutralized to litmus with 1 *N* sodium hydroxide and water (50 cc.) there was added 0.5 cc. of 1 *N* sodium hydroxide as a catalyst and the mixture was allowed to stand one hour. The water was then removed from the reaction mixture by continuous codistillation with chloroform. A small amount of white solid (sodium salts derived from the catalyst) was removed by filtration and the solvent was removed from the chloroform solution to leave 40.0 g. (quantitative yield) of a viscous pale yellow oil. This material could not be crystallized from any of the usual solvents or solvent mixtures and it decomposed on attempted distillation at either 0.10 mm. or at 10^{-5} mm.

Anal. Calcd. for $C_8H_{12}N_2O_4$: N, 14.00. Found: N, 13.69.

After standing in the laboratory for some time a sample of the material spontaneously crystallized; it melted at 88–89° after recrystallization from chloroform–benzene.

Acid Hydrolysis of VIII.—A mixture of ten grams of the cyano ester VIII and 100 cc. of concentrated hydrochloric acid was refluxed two hours. A portion of the hydrolysis mixture was saturated with sodium chloride and extracted eight times with equal volumes of ether. The solvent was removed from the combined ethereal extracts to leave a small amount of yellow oil which readily gave the semicarbazone of pyruvic acid, identified by comparison with an authentic sample.

Another portion of the acid hydrolysate was taken to dryness under vacuum and the residue was dried by azeotropic distillation with benzene. The dry residue was suspended in dry methanol and the suspension was refluxed as dry hydrogen chloride was passed in for thirty minutes. The chilled mixture was filtered and the filter cake was washed several times with dry methanol and then with dry ether. It gave the usual tests for ammonium and chloride ions.

Alkaline Hydrolysis of VIII.—The ester VIII (20 g., 0.10 mole) was refluxed sixteen hours with 180 g. of 16% aqueous sodium hydroxide. This treatment converts the $\rangle C(CN)COOC_2H_5$ group to $\rangle CHCOONa$.[27] The solution was acidified, taken to dryness under vacuum, dried by distillation with benzene and the residue was leached with hot absolute alcohol. The solvent was removed from the alcoholic leachings and the residue was refluxed one hour with concentrated hydrochloric acid to hydrolyze any acetamido linkage still present. The acid hydrolysis mixture was worked up for serine in the usual manner but the product, after conversion to its methyl ester hydrochloride, melted at 169–170° alone or when mixed with an authentic sample of glycine methyl ester hydrochloride.

Acetamidocyanoacetic Acid, X.—The ester VIII (10.0 g., 0.050 mole) was treated with 41.5 cc. of 1.21 *N* sodium hydroxide (0.050 mole) and the mixture was allowed to stand overnight. A very small amount of material was removed by filtration, the filtrate was chilled and then cautiously acidified with 5 cc. of cold concentrated hydrochloric acid. The acidified solution was concentrated to dryness under vacuum at room temperature and then dried by vacuum distillation with benzene at room temperature. The dry residue was leached with cold absolute alcohol and the solvent was removed from the leachings to give a resin that could not be crystallized from any of the usual solvents. A little of the resin was dissolved in absolute alcohol and lyophilized to yield a fluffy tan mass of indefinite melting point.

Anal. Calcd. for $C_5H_6N_2O_3$: N, 19.72. Found: N, 19.83.

Summary

The amino acid *dl*-serine has been prepared in 65% over-all yield *via* the sequence: ethyl acetamidomalonate, ethyl α-acetamido-α-carbethoxy-β-hydroxypropionate, α-acetamido-α-carboxy-β-hydroxypropionic acid, N-acetylserine, serine. Several other products derived from various of these intermediates and from related compounds have been isolated and characterized.

RENSSELAER, NEW YORK RECEIVED MARCH 8, 1947