[COMMUNICATION FROM THE LABORATORIES OF WINTHROP CHEMICAL COMPANY, INC.]

The Use of Ethyl Acetamidomalonate in the Synthesis of Amino Acids. The Preparation of *dl*-Histidine, *dl*-Phenylalanine and *dl*-Leucine

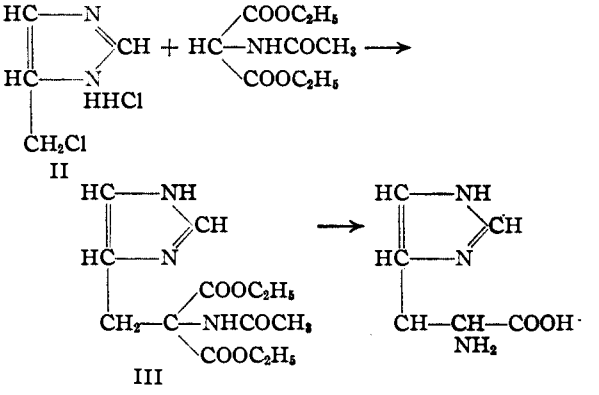
BY N. F. ALBERTSON AND S. ARCHER

The experience of Snyder[1] and ourselves[2] with ethyl acetamidomalonate (I) in the synthesis of tryptophan prompted us to investigate its application as a general synthetic tool in the amino acid field. Although the compound has been known for several years[3] we are aware of only one other instance of its use in amino acid synthesis besides those already cited, namely, in the preparation of "bimethioneine."[4]

The general method employed consisted of condensing an alkyl halide with the ester and degrading the resulting compound to the desired amino acid. It was found that the alkylated acetamidomalonic esters thus far encountered were all readily purified crystalline solids. Generally, it was found advantageous to carry out the saponification, decarboxylation and deacetylation without isolation of any of the intermediates. The fact that acetic acid, a volatile, water-soluble substance, is formed in the hydrolysis facilitated the manipulations somewhat. In our hands deacetylation of the acetamido group proceeded at a much faster rate than the debenzoylation of the corresponding benzamidomalonic ester. For example, the conditions necessary to hydrolyze acetyl-*dl*-tryptophan barely affected the benzoyl derivative of this amino acid.[2]

In order to demonstrate the usefulness of ethyl acetamidomalonate in amino acid synthesis, we wish to describe at this time the condensation with three reactive halides and the subsequent conversion of the compounds thus formed to the corresponding amino acids.

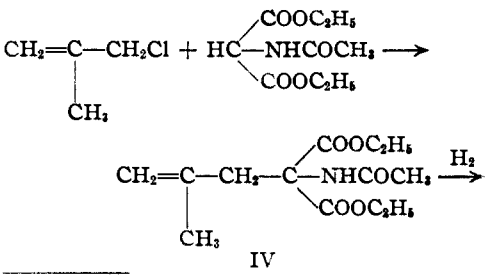
Histidine.—The only recorded syntheses of this amino acid are those of Pyman.[5] In one of these[5a] chloromethylimidazole hydrochloride (II) (prepared in six steps from citric acid) was condensed with two moles of ethyl sodiochloromalonate to give ethyl 2-carbethoxy-2-chloro-3-imidazolepropionate. This ester was saponified and decarboxylated to the α-chloro acid which, upon treatment with ammonia, gave *dl*-histidine. We have found that (II), which may now be prepared in only two steps from fructose,[6] condenses very rapidly with ethyl acetamidomalonate in the presence of two moles of sodium ethylate to yield (III). The latter was obtained as a viscous oil which was induced to crystallize by trituration with water.



Ethyl 2-acetamido-2-carbethoxy-3-imidazolepropionate (III) was converted directly to histidine dihydrochloride by treatment at reflux temperature with concentrated hydrochloric acid. The free amino acid was obtained directly from (III) by the procedure described in the experimental section.

Phenylalanine.—This amino acid has been prepared by a number of investigators using a variety of procedures.[7] The benzylation of ethyl acetamidomalonate in sodium ethylate solution proceeded very smoothly in approximately 90% yield. The resulting intermediate was hydrolyzed directly to the amino acid with hydrobromic acid (65% yield) or by saponifying with sodium hydroxide and continuing the degradation after acidification (67% yield).

Leucine.—One of the procedures for the making of this amino acid involves the preparation of isobutyl iodide and its subsequent conversion to ethyl isobutylbenzamidomalonate. Saponification, decarboxylation and debenzoylation are then required to yield leucine.[8] The preparation of isobutyl iodide may be avoided by proceeding according to equations given



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

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

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

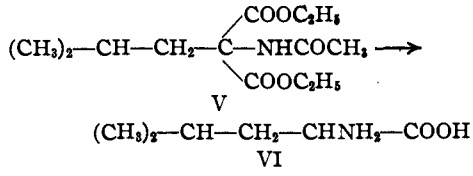
(4) Snyder, *et al.*, THIS JOURNAL, **65**, 2216 (1943). We wish to thank the Referee for calling our attention to the very recent article of Dakin, *J. Biol. Chem.*, **154**, 553 (1943), wherein the condensation of ethyl acetamidomalonate and isobutylene oxide is reported.

(5) (a) Pyman, *J. Chem. Soc.*, **99**, 1395 (1911); (b) **109**, 186 (1916).

(6) Darby, Lewis and Totter, THIS JOURNAL, **64**, 463 (1942); Totter and Darby, *Org. Syn.*, **24**, 69 (1944).

(7) "The Chemistry of Amino Acids and Proteins," Charles C. Thomas Co., Springfield, Ill., 1944, p. 84, contains a summary of previous methods.

(8) *Idem*, p. 50.



Methylallyl chloride reacted smoothly with I to yield IV (80%). The latter is hydrogenated quantitatively at room temperature in the presence of Raney nickel catalyst to give ethyl acetamidoisobutylmalonate (IV). This method of isobutylation is to be preferred since the starting halide is quite inexpensive, is not contaminated with other reactive isomers and cannot undergo rearrangement during the condensation.

The structure of the intermediate V was confirmed by hydrolyzing to the free dibasic acid which on heating evolved carbon dioxide to give acetyl-*dl*-leucine, m. p. 155–157°, identical with an authentic specimen.

Experimental

4-Hydroxymethylimidazole Hydrochloride.—Levulose was converted to 4-hydroxymethylimidazole picrate by the method of Darby.[9] Sucrose may be used in place of the fructose but the yields are lower. A solution of 85.5 g. of sucrose in 200 cc. of water was warmed on a steam-bath for one-half hour with one cubic centimeter of concd. hydrochloric acid. This solution was used in place of 45 g. of fructose and gave 30.2 g. of the picrate melting above 197°.

4-Chloromethylimidazole Hydrochloride.—The method of Pyman[9] gave 85–90% yield of suitable material, m. p. 141.5–143.5°.

Ethyl 2-Acetamido-2-carbethoxy-3-imidazolepropionate (III).—A solution of 5.62 g. of sodium and 26.6 g. of ethyl acetamidomalonate in 200 cc. of dry ethanol was chilled in an ice-bath. To the cold stirred solution 18.6 g. of 4-chloromethylimidazole hydrochloride was added rapidly. Sodium chloride precipitated at once. After standing at room temperature for one-half hour, the mixture was filtered (Filtercel) and the filtrate concentrated to a pale yellow, viscous oil. Trituration with a small amount of water resulted in a rapid exothermic crystallization. After recrystallization from water, 25.8 g. (67%) of the monohydrate of III was obtained, m. p. 96°. Recrystallization from water raised the melting point to 99°. The compound reverted to the glassy, anhydrous state on warming at 80°.

Anal. Calcd. for $C_{13}H_{19}O_5N_3$: C, 52.52; H, 6.44; N, 14.23. Calcd. for $C_{13}H_{19}O_5N_3 \cdot H_2O$: C, 49.52; H, 6.71; N, 13.33. Found: C, 50.03; H, 7.17; N, 13.69.

***dl*-Histidine Dihydrochloride.**—A solution of 14.7 g. of III in 200 ml. of concd. hydrochloric acid was refluxed for six hours and then concentrated *in vacuo* to a pale yellow solid. This was triturated with ethanol and filtered to give 6.25 g. of white crystals of the crude dihydrochloride. It was taken up in a small quantity of concd. hydrochloric acid and alcohol then added. On cooling, the pure amino acid dihydrochloride, m. p. 231–235° (dec.), was obtained, 5.4 g. (48%).

***dl*-Histidine.**—The free amino acid may be obtained directly from III by the following procedure. A solution of 16.7 g. of ethyl 2-acetamido-2-carbethoxy-3-imidazolepropionate in 85 cc. of water containing 5 cc. of concd. sulfuric acid was refluxed for ten hours. At the end of this time the solution was cooled, treated with a slight excess of barium hydroxide solution and filtered. The filtrate was saturated with carbon dioxide, filtered, and concentrated until crystals appeared. These were redissolved by the addition of hot water, an equal volume of alcohol added and the solution allowed to cool. The *dl*-histidine which separated melted at 271–272° (dec.) and weighed 3.67 g. (45%). It gave a positive Knoop test for histidine. Concentration of the mother liquors afforded an additional quantity of slightly less pure material.

Ethyl 2-Acetamido-2-carbethoxy-4-methyl-4-pentenoate (IV).—To a stirred, boiling solution of 10.9 g. of I and 1.15 g. of sodium in 100 cc. of absolute ethanol there was added 5.9 g. of β-methylallyl chloride. Refluxing was continued for six hours longer and the solution concentrated *in vacuo*. The pale yellow residue was taken up in a mixture of 25 cc. of water and 25 cc. of chloroform. The aqueous layer was thoroughly extracted with chloroform. The combined organic extracts were concentrated to give 11.6 g. of an almost colorless crystalline solid. Crystallization from benzene–ligroin yielded 10.7 g. (79%) of IV. An analytical sample, crystallized from water, melted at 92–93°.

Anal. Calcd. for $C_{13}H_{21}O_5N$: C, 57.55; H, 7.80; N, 5.16. Found: C, 57.81; H, 8.11; N, 5.06.

Ethyl 2-Acetamido-2-carbethoxy-4-methylpentanoate (V).—A solution of 10.7 g. of IV in 100 cc. of ethanol was reduced at 40 lb. at room temperature in the presence of a small quantity of Raney nickel. The theoretical quantity of hydrogen was absorbed in twenty minutes. A quantitative yield of the dihydro derivative, m. p. 84°, was obtained on evaporation of the solvent. Crystallization from water did not raise the melting point.

Anal. Calcd. for $C_{13}H_{23}O_5N$: C, 57.20; H, 8.47; N, 5.13. Found: C, 57.32; H, 7.58; N, 5.08.

Acetamidoisobutylmalonic Acid.—A solution of 35 cc. of 10% sodium hydroxide and 20 cc. of ethanol containing 5.46 g. of V was boiled for fifteen minutes and then partially concentrated. The solution was decolorized, cooled and acidified to give 3.41 g. (77%) of the free *dl*-acid, m. p. 121–122.5° (dec.).

Anal. Calcd. for $C_9H_{15}O_5N$: N, 6.45. Found: N, 6.07.

When heated the acid lost carbon dioxide to give acetyl-*dl*-leucine, m. p. 155–157°, undepressed when mixed with a known specimen.

***dl*-Leucine.**—A solution of 2.73 g. of V in 9 cc. of 48% hydrobromic acid was refluxed for seven and one-half hours and then decolorized. Concentrated ammonium hydroxide was added to the filtrate to *p*H 6 and the solution chilled overnight. The amino acid was collected by filtration, washed and dried; wt. 1.14 g. (87%), m. p. 278–283° (dec.).

Benzoylation by the Schotten–Baumann procedure gave benzoyl-*dl*-leucine, m. p. 138–141°.[10]

Ethyl 2-Acetamido-2-carbethoxy-3-phenylpropionate (VI).—When an equivalent quantity of benzyl chloride was substituted for β-methylallyl chloride in the preparation of IV and refluxing continued for two hours rather than six, the product was isolated in 90% yield, m. p. 100–104°. On recrystallization from water the m. p. was raised to 104–104.2°.

Anal. Calcd. for $C_{16}H_{21}O_5N$: C, 62.53; H, 6.90; N, 4.56. Found: C, 62.35; H, 7.29; N, 4.73.

***dl*-Phenylalanine.**—When the above ester was degraded by the procedure described under *dl*-leucine, phenylalanine was isolated in 65% of the theoretical yield. It was also prepared by saponification of VI with dilute sodium hydroxide to give, after acidification, the free dibasic acid. Deacetylation and decarboxylation were effected by refluxing for five hours with dilute sulfuric acid (*cf.* preparation of histidine) and adjusting the *p*H to the isoelectric point (*p*H 6) with ammonium hydroxide. The yield of the amino acid, m. p. 257° (dec.), was 67%.

A portion was benzoylated according to Fischer[11] to yield N-benzoylphenylalanine, m. p. 183–184°.

(9) Pyman, *J. Chem. Soc.*, **99**, 668 (1911).

(10) Fischer, *Ber.*, **33**, 2313 (1900), reports a m. p. of 137–141°.

(11) Fischer and Mannegrat, *Ber.*, **33**, 2383 (1900), report 187–188°.

Summary

1. The usefulness of ethyl acetamidomalonate the synthesis of amino acids is pointed out.
2. A four-step synthesis of *dl*-histidine and its dihydrochloride is described. The over-all yield based on fructose is 16%.
3. A three-step synthesis of *dl*-leucine is described. The over-all yield is 69%.
4. A two-step synthesis of phenylalanine is described. The yield is 60%.

WINTHROP CHEMICAL COMPANY, INC.
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[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Synthetic Amino Acids. Syntheses from Acetamidomalonic Ester[1]

BY H. R. SNYDER, JOSEPH F. SHEKLETON[2] AND CAMERON D. LEWIS

One of the most useful general methods of synthesis of amino acids is the Sörensen process, in which phthalimidomalonic ester is alkylated and the condensation product is subjected to hydrolysis and decarboxylation.[3] The general procedure has been improved by Redemann and Dunn,[4] who substituted benzamidomalonic ester for the phthalimido derivative. A third variation, involving the alkylation of acetamidomalonic ester, has been used for the synthesis of ω,ω'-bimethionine,[5] of tryptophan[6,7] and of γ-hydroxyisoleucine[8]; the alkylating agent in the first instance was a chloride, in the second a quaternary ammonium salt, and in the third an alkene oxide. This method is illustrated by the following scheme.

$$[CH_3CONHC(CO_2C_2H_5)_2]^-Na^+ \longrightarrow RC(CO_2C_2H_5)_2NHCOCH_3 \longrightarrow RCH(NHCOCH_3)CO_2H \longrightarrow RCH(NH_2)CO_2H$$

This process appears to be the most direct route to the acetyl derivatives of racemic amino acids, and the greater availability of acetamidomalonic ester, as compared to the phthalimido and benzamido esters, suggests that its use might constitute a further improvement in the Sörensen method. There now has been occasion to apply the process to the synthesis of the acetyl derivatives of phenylalanine, leucine, norleucine and norvaline, from acetamidomalonic ester and benzyl chloride, isobutyl bromide, butyl bromide and propyl bromide, respectively. Attempts to prepare derivatives of isoleucine and valine, by alkylation with *s*-butyl bromide and isopropyl bromide, respectively, were unsuccessful. Evidently secondary halides are of little use in any of the variations of the Sörensen method.

(1) This is the eighth of a series of communications on synthetic amino acids and their derivatives; for the seventh, see THIS JOURNAL, 67, 38 (1945).

(2) Present address: General Aniline and Film Corporation, Easton, Pennsylvania.

(3) Sörensen, *Z. physiol. Chem.*, 44, 448 (1905); *Compt. rend. trav. lab. Carlsberg*, 6, 1 (1903); 6, 187 (1905); *Bull. soc. chim.*, [3] 33, 1042 (1905); [3] 33, 1052 (1905).

(4) Redemann and Dunn, *J. Biol. Chem.*, 130, 341 (1939); see also Painter, THIS JOURNAL, 62, 232 (1940).

(5) Snyder, Howe, Cannon and Nyman, THIS JOURNAL, 65, 2211 (1943).

(6) Snyder and Smith, *ibid.*, 66, 350 (1944).

(7) Albertson, Archer and Suter, *ibid.*, 66, 500 (1944).

(8) Dakin, *J. Biol. Chem.*, 154, 549 (1944).

The preparation of glutamic acid[9] from ethyl phthalimidomalonate and an acrylic ester differs from other amino acid syntheses in that the malonic ester derivative is used as a component of a Michael condensation rather than in an alkylation. Substitution of acetamidomalonic ester for the phthalimido derivative in this preparation also leads to the amino acid in excellent yields. The reactions are shown in the following scheme.

$$CH_2{=}CHCO_2CH_3 + CH_3CONHCH(CO_2C_2H_5)_2 \xrightarrow{NaOC_2H_5} CH_3CONHC(CO_2C_2H_5)_2CH_2CH_2CO_2CH_3 \xrightarrow[-CO_2]{H_2O} HO_2CCH(NH_2)CH_2CH_2CO_2H$$

None of the intermediates was isolated.

In the present work the yields (*ca.* 60%) of acetylphenylalanine, acetylnorvaline and glutamic acid were approximately the same as those obtained by the use of the other aminomalonic ester derivatives. The acetyl derivatives of leucine and norleucine were obtained in yields of only 30–50%. The experimental conditions required in the hydrolysis of the alkylated acetamidomalonic esters are less strenuous than those employed on the benzoyl and phthalyl derivatives.

Experimental

***dl*-N-Acetylphenylalanine. (a) Preparation of Diethyl Benzylacetamidomalonate.**—A solution of 1.15 g. (0.05 atom) of sodium in 75 ml. of absolute ethanol (dried and distilled from magnesium methylate) was prepared in a 200-ml., 3-necked, round-bottomed flask equipped with mechanical stirrer and reflux condenser protected from atmospheric moisture by a calcium chloride tube. To this was added 10.85 g. (0.05 mole) of acetamidomalonic ester and then 6.3 g. (0.05 mole) of benzyl chloride. The yellow solution was stirred under reflux for twelve hours.

The hot reaction mixture was filtered and the precipitate washed with hot absolute ethanol. The combined filtrates were evaporated under diminished pressure on a steam-bath and the residue was cooled and washed onto a filter. The solid weighed 12.7 g. (82%) and melted at 91–94°. After two crystallizations from water the melting point was 106°.

Anal. Calcd. for $C_{16}H_{21}O_5N$: C, 62.53; H, 6.89. Found: C, 62.73; H, 7.16.

(b) *dl*-N-Acetylphenylalanine.—In a 200-ml., round-bottomed flask a mixture of 60 ml. of 10% sodium hydroxide solution and 12.7 g. of the crude condensation product was heated under reflux for four hours. The cooled

(9) Marvel and Stoddard, *J. Org. Chem.*, 3, 198 (1938).