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%.

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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.

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

(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).

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

solution was acidified with 50 ml. of 3 *N* hydrochloric acid and then heated under reflux for one hour. The hot solution was filtered and the filtrate allowed to remain in the icebox for eighteen hours. The crystalline material was collected on a filter and dried. The yield of *dl*-N-acetylphenylalanine identified by the method of mixed melting points) was 7.1 g. (83%). It melted at 142.5–145°. One crystallization from hot water raised the melting point to 145–146° (lit.,[10,11] 142–143° and 151–152°).

***dl*-N-Acetylleucine.** (a) **Preparation of Diethyl Isobutylacetamidomalonate.**—To 1.22 g. (0.053 atom) of sodium in 75 ml. of absolute ethanol (dried and distilled from magnesium methylate) in a 200-ml., 3-necked, round-bottomed flask with mechanical stirrer, reflux condenser protected by a calcium chloride tube, was added 11.50 g. (0.053 mole) of diethyl acetamidomalonate and 7.8 g. (0.055 mole) of isobutyl bromide. The clear solution was stirred at reflux temperature for fifteen hours.

The hot reaction mixture was filtered and the solid washed with hot absolute ethanol. The filtrate was evaporated under reduced pressure on a steam-bath and the oily residue was caused to crystallize by the addition of 20 ml. of water. The condensation product was collected on a filter. The yield was 6.7 g. (46%). The crude diethyl isobutylacetamidomalonate melted at 78–81°; after recrystallization from water it melted at 82–83°.

Anal. Calcd. for $C_{12}H_{23}O_6N$: C, 57.11; H, 8.48. Found: C, 56.90; H, 8.63.

(b) ***dl*-N-Acetylleucine.**—A mixture of 10.8 g. of crude diethyl isobutylacetamidomalonate and 25 ml. of 20% sodium hydroxide solution was heated under reflux for three hours, allowed to cool, and then acidified with concentrated hydrochloric acid. This acid solution was then allowed to reflux for thirty minutes. The hot solution was filtered and the filtrate cooled. An oil separated and soon solidified. The *dl*-N-acetylleucine showed a melting point of 155–157° (lit.,[12] 161°) and weighed 4.5 g. (64%).

***dl*-N-Acetylnorvaline.** (a) **Preparation of Diethyl *n*-Propylacetamidomalonate.**—To 1.23 g. of sodium in 75 ml. of absolute ethanol (dried and distilled from magnesium methylate) in a 200-ml., 3-necked, round-bottomed flask with mechanical stirrer, reflux condenser protected from atmospheric moisture by a calcium chloride tube, and cork stopper was added 11.5 g. of diethyl acetamidomalonate and then 7.0 g. of *n*-propyl bromide. This solution was heated at reflux temperature with stirring for ten hours.

The hot mixture was filtered and the solid extracted with hot absolute ethanol. The filtrate was evaporated under diminished pressure on a steam cone and the residue treated with 20 ml. of water. The condensation product was separated by filtration and dried in an evacuated desiccator containing calcium chloride. It weighed 9.8 g. (71%) and melted at 91.0–93.5°. Recrystallization from water raised the melting point to 93–93.5°. It was shown not to be diethyl acetamidomalonate by analysis and by the method of mixed melting points.

Anal. Calcd. for $C_{13}H_{21}O_6N$: C, 55.58; H, 8.16. Found: C, 55.60; H, 8.34.

(b) ***dl*-N-Acetylnorvaline.**—A mixture of 7.1 g. of diethyl *n*-propylacetamidomalonate and 25 ml. of 20% sodium hydroxide solution was heated under reflux for two and one-half hours. The cooled saponification mixture was acidified by the addition of 10 ml. of concentrated hydrochloric acid and then heated at reflux temperature for one hour. The hot solution was filtered and the filtrate was allowed to stand in the icebox for twenty-four hours. Crystallization was aided by scratching with a glass rod. The *dl*-N-acetylnorvaline was collected on a filter and dried. It weighed 3.75 g. (86%), and melted at 115–117°. After recrystallization from water it melted at 116–117°.

Anal. Calcd. for $C_7H_{13}O_3N$: C, 52.85; H, 8.23. Found: C, 52.61; H, 8.23.

(10) Johnson and Cahill, *J. Biol. Chem.*, **126**, 37 (1938).
(11) Bergmann and Stern, *Ber.*, **63**, 437 (1930).
(12) Fischer, *ibid.*, **34**, 449 (1901).

N-Acetylnorleucine. (a) **Preparation of Diethyl *n*-Butylacetamidomalonate.**—A dry, 200-ml., 3-necked, round-bottomed flask, equipped with grease-sealed mechanical stirrer and a Hopkins reflux condenser protected by a calcium chloride-soda lime tube, was swept out with dry nitrogen. Into the flask were introduced 75 ml. of absolute ethanol (dried and distilled from magnesium ethylate), and 1.20 g. (0.052 atom) of sodium. After solution of the sodium was complete, 11.5 g. (0.053 mole) of diethyl acetamidomalonate, 0.2 g. potassium iodide and 8.20 g. (0.06 mole) of *n*-butyl bromide were introduced. Nitrogen was passed through the apparatus at a slow rate continually, except for the periods of addition of the reactants. The reaction mixture was heated and stirred under reflux for twelve hours. The hot reaction mixture was filtered by suction and the solid washed with hot absolute ethanol. The filtrate and washings were evaporated under diminished pressure on a steam cone. The residue was taken up in water containing a little sodium bisulfite and the solution was saturated with salt and extracted six times with ether. Evaporation of the ether left a viscous orange-yellow oil, which solidified in the ice chest but remelted below room temperature.

(b) ***dl*-N-Acetylnorleucine.**—The oil obtained above was refluxed for one hour with 50 ml. of 10% aqueous sodium hydroxide. The cooled solution was acidified with 10 ml. of concentrated hydrochloric acid and refluxed for an hour. The hot solution was treated with Norite and filtered. The cooled filtrate on standing twenty-four hours yielded 3.6 g. of a yellowish-white crystalline solid. Concentration and recooling of the filtrate yielded an additional 0.9 g. of crystals (total yield 4.5 g., 50% based on sodium). It melted at 101–103°. Recrystallization from hot water gave white crystals melting at 104.5–105.5°.

Anal. Calcd. for $C_8H_{15}O_3N$: C, 55.49; H, 8.73. Found: C, 55.66; H, 8.96.

Glutamic Acid Monohydrate.—To a solution of 0.075 g. of sodium in 10 ml. of absolute ethanol in a 200-ml., 3-necked, round-bottomed flask equipped with mechanical stirrer, reflux condenser and dropping funnel, was added 10.85 g. (0.05 mole) of diethyl acetamidomalonate. This solution was stirred for two hours at room temperature while 9.6 g. (0.067 mole) of a 60% solution of methyl acrylate in methanol was added dropwise. Stirring was continued at reflux temperature for nine hours.

The reaction mixture was evaporated under reduced pressure on a steam-bath and the residue was heated to reflux with 18 ml. of 6 *N* hydrochloric acid. Eighteen cubic centimeters of concentrated hydrochloric acid was added in seven portions at regular intervals during the first seventy minutes of the hydrolysis, and heating was continued for six hours.

The cooled hydrolysis mixture was filtered and the filtrate treated with Norite. The light yellow filtrate was evaporated on a steam-bath under reduced pressure. The sirupy residue was dissolved in 7 ml. of hot water and 3 ml. of concentrated ammonium hydroxide was added. The solution was cooled by tap water and was seeded with *dl*-glutamic acid monohydrate. Another 3 ml. of concentrated ammonium hydroxide was added with further cooling by tap water. The solution was then cooled in ice. After five minutes the white solid was collected on a filter. The yield of *dl*-glutamic acid monohydrate after crystallization from fifty per cent. ethanol was 4.6 g. (56%). Concentration of the mother liquor yielded an additional 0.7 g. (total yield, 64%).

Anal. Calcd. for $C_5H_9O_4N$: N, 8.48; Found: (Van Slyke) N, 8.38, 8.45, 8.45.

Summary

A useful modification of the Sörensen method of synthesis of amino acids is described. Diethyl acetamidomalonate is alkylated with benzyl chloride, butyl bromide, isobutyl bromide and propyl bromide and the products are converted

to the acetyl derivatives of *dl*-phenylalanine, *dl*-norleucine, *dl*-leucine and *dl*-norvaline. Attempts to use the secondary halides, *s*-butyl bromide and isopropyl bromide, in similar alkylations were unsuccessful. *dl*-Glutamic acid is prepared from diethyl acetamidomalonate and methyl acrylate.

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3,12-Dihydroxy-7-ketocholanic Acid[1]

BY WILLARD M. HOEHN AND JACOB LINSK

Various investigators[2] have studied the partial oxidation of cholic acid. The results published to date have indicated varying degrees of success. This paper presents two methods which have been found to give good yields and which may be readily applied to large quantities, for the isolation of pure 3,12-dihydroxy-7-ketocholanic acid. Information is presented also to explain the melting points assigned to this acid by the investigators.

In an effort to ascertain the purity of the ethyl 3,12-dihydroxy-7-ketocholanate, a mixture was prepared with ethyl cholate. The melting point was observed to be higher than that for either compound. Crystallization of a mixture of various quantities of these esters from methanol gave a complex which contained one molecule of each compound. The melting point of the complex was approximately 10° higher than that of the ethyl 3,12-dihydroxy-7-ketocholanate. Among the derivatives prepared were ethyl 3-benzoxy-7-keto-12-hydroxycholanate, 3,12-diformoxy-7-ketocholanic acid[3] and a monobromo derivative of ethyl 3,12-diacetoxy-7-ketocholanate. The bromo derivative was recovered after treatment with dry pyridine or collidine at reflux temperatures. Further work is being carried out with this bromo compound which is tentatively considered to have the bromine at C_6.

The oxidation of non-crystalline ethyl 3-hydroxy-7-keto-12-acetoxycholanate produces a crystalline ethyl 3,7-diketo-12-acetoxycholanate (m. p. 164–165°; $[\alpha]^{25}D$ + 38° (dioxane)). Hydrolysis of this ester gave the corresponding acid (m. p. 168–169°; $[\alpha]^{25}D$ − 13° (dioxane)). The structure of the compound was confirmed by reducing it to the known 12-hydroxycholanic acid.[4] Oxidation gave the 12-ketocholanic acid which was directly compared with a sample prepared from desoxycholic acid. Methyl 12-hydroxycholanate prepared from the corresponding hydroxy acid was also compared with an authentic sample.

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Experimental[5]

Ethyl 3,12-Dihydroxy-7-ketocholanate: (a) Bromine Oxidation of Cholic Acid.—In a three-necked flask 500 g. of cholic acid was dissolved in 3.0 liters of water containing 70 g. of sodium hydroxide and to this solution was added 275 g. of sodium bicarbonate. The solution (approximately 3.5 liters) was maintained at a temperature of −5 to −2° by means of an ice–salt-bath and with vigorous stirring a solution of 200 g. of technical bromine in 50 cc. of chloroform was added over a period of two hours. Stirring was continued for two hours longer and the mixture allowed to stand two days, when it was diluted to 6 liters with water and acidified to congo red with hydrochloric acid. The material which precipitated in a lump was washed free of mineral acid by kneading under a stream of cold tap water. The oxidized material was spread evenly on a glass tray and dried to constant weight. The solid was dissolved in 1.3 liters of standard denatured 2B alcohol to which 50 g. of sulfuric acid had been added. The solution was allowed to stand at room temperature overnight and filtered. The esterification mixture was taken into benzene and washed with water, dilute sodium bicarbonate solution and finally with water. The benzene solution was dried by distillation of the benzene and the solvent completely removed at 20 to 25 mm. pressure while the resinous mass was heated in a bath of boiling water. The residue was dissolved in 1 liter of methanol and on standing overnight the crystalline ester which formed was filtered by suction and washed with 50 cc. of methanol. The ethyl ester (m. p. 152–157°), after air drying, weighed 287 g. Recrystallization from 600 cc. of methanol gave 216 g. of ethyl 3,12-dihydroxy-7-ketocholanate (2c) (m. p. 158–160°; $[\alpha]^{25}D$ + 2 ± 2° (dioxane)). A second crop of ethyl ester was obtained on concentration of the mother liquor.

(b) **Chromic Acid Oxidation of Ethyl Cholate.**—To a solution of 88 g. of ethyl cholate in 500 cc. of 70% acetic acid solution, cooled to −5°, was added 85 cc. of *N* chromic acid solution over a period of one and a half hours. The mixture was stirred during the addition of the oxidant and after another half hour of stirring the reaction mixture was poured into water and extracted with benzene. The benzene layer was washed with water, dilute hydrochloric acid and again with water. The benzene was removed,

(1) Reported in part at the April, 1944, meeting of the American Chemical Society at Cleveland, Ohio.

(2) For instance: (a) Kaziro and Shimado, *Z. physiol. Chem.*, **249**, 220 (1937); (b) Charonnat and Horeau, U. S. Patent 2,244,328, June 3, 1941; (c) Haslewood, *Biochem. J.*, **109**, 107 (1943); (d) Gallagher and Long, *J. Biol. Chem.*, **147**, 133 (1943); (e) Hoehn, Schmidt and Hughes, *ibid.*, **156**, 59 (1944). (f) An article by Haslewood (*Biochem. J.*, **38**, 108 (1944)) has been brought to our attention since this paper was submitted. Haslewood described the 3,12-dihydroxy-7-ketocholanic acid (m. p. 196–197°), the 3,7-diketo-12-hydroxycholanic acid (m. p. 165–166°), the ethyl 3-benzoxy-12-hydroxy-7-ketocholanate (m. p. 138–139°) and ethyl 3-benzoxy-7,12-diketocholanate (m. p. 168–169°).

(3) The authors are grateful to Dr. R. B. Moffett for the preparation of this compound: *cf.* "Preparation of Phenyl Ketones from Bile Acids," Moffett and Hoehn, presented at Org. Div. A. C. S. April, 1904.

(4) (a) Wieland and Schlichting, *Z. physiol. Chem.*, **150**, 270 (1925); (b) Wieland and Kapitel, *ibid.*, **212**, 276 (1932); (c) Barnett and Reichstein, *Helv. chim. acta*, **21**, 926 (1938).

(5) All specific rotations observed by J. Stickley; neutral and saponification equivalents determined by J. Stickley.