sodium hydroxide at 20°. For comparison L-asparagine was also titrated. Separate solutions were used for the acid and alkaline curves. Titrations were carried out using the Radiometer TTT1a automatic titrator with a glass electrode (G 202A) and a calomel reference electrode. Corrections for the 3 ml. of water used as solvent were similarly determined. The apparent pKa values derived from these data for β-cyano-L-alanine are pKa$_1$ 1.7; pKa$_2$ 7.4; for L-asparagine, pKa$_1$ 2.1; pKa$_2$ 9.0; reported[14] for L-asparagine pKa$_1$ 2.02; pKa$_2$ 8.80 (0.02M, 25°).

Reaction with ninhydrin. (a) *Ninhydrin in n-butyl alcohol.* Absorption spectra were obtained with the Beckman DU spectrophotometer. Twenty to forty microliters of an aqueous solution of β-aminopropionitrile (0.75 μm),[15] β-cyano-L-alanine (0.43 μm), γ-cyano-α-L-aminobutyric acid (0.31 μm), or leucine (0.2 μm) was added to 5 cc. of a 0.2% solution of ninhydrin in n-butyl alcohol (reagent grade) in test tubes. The latter were covered and immersed in a boiling water bath. After 15 min. the solution corresponding to β-aminopropionitrile was clear green (λ_{max} 315, λ_{max} 655). It was qualitatively unchanged after further heating for 15 min. In contrast, the ninhydrin color (green with blue cast) (λ_{max} 305, λ_{max} 410, λ_{max} 655) obtained with β-cyano-L-alanine changed on further heating to a lavender-gray (λ_{max} 308, λ_{max} 415 and high general absorption at 570–590 mμ) and lost the 655 mμ maximum in the green. The reaction spectrum obtained with γ-cyano-α-L-aminobutyric acid after 15 min. resembled that of leucine (λ_{max} 315, λ_{max} 415, λ_{max} 585).

(b) *Ninhydrin in cellosolve-aqueous buffer.* Twenty to eighty microliters of an aqueous solution of amino compound was added to a mixture containing 4 cc. of sodium citrate buffer, pH 3.25,[9] and 2 cc. of ninhydrin reagent in cellosolve and sodium acetate buffer.[9] The solutions were heated in a bath at 100° for 15 min. β-Aminopropionitrile and β-cyano-L-alanine produced purple colors (λ_{max} 320, λ_{max} 401, λ_{max} 580). The purple solutions obtained with leucine and γ-cyano-α-L-aminobutyric acid showed bands with maxima only at 410 mμ and 580 mμ. The absorption spectra are shown in Fig. 1.

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Synthesis of α-Hydroxymethylamino Acids by Means of a Selective Reduction with Lithium Borohydride

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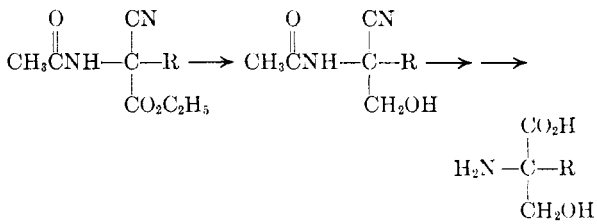
A general synthesis of α-hydroxymethylamino acids is described. A suitably alkylated ethyl acetamidocyanoacetate is treated with lithium borohydride to reduce selectively the ester. After acid hydrolysis, good yields of the desired substituted serines are obtained.

Many selective reductions of functional groups in organic molecules have become possible since the introduction of the metal hydride reducing agents. Lithium borohydride was reported by Nystrom, Chaikin, and Brown[1] to be an effective agent for reducing aldehydes, ketones, and esters to alcohols. It has been found[2] to reduce tertiary amides, but not primary and secondary amides, and has been used[3] for the reduction of the ester of p-toluenesulfonyl peptide esters without affecting the peptide bonds. It has been reported[4] not to reduce nitriles when used for the hydrogenolysis of the halogen of halonitriles. These observations made it seem likely that lithium borohydride could be used for the selective reduction of an ester in a molecule containing also nitrile and acylamino groups. When applied to ethyl acetamidocyanoacetates, this should lead to a general synthesis of α-substituted



R = —H, —CH_3, —$CH_2CH_2CH_3$, —$CH_2C_6H_5$

serines, providing a general method for synthesizing amino acids having an α-hydroxymethyl substituent. α-Hydroxymethylamino acids have heretofore been prepared by partial oxidation of 2-amino-2-alkyl-1,3-propanediols.[5] The method described herein would appear to have a wider applicability, because of the ease of obtaining the required starting materials.

The validity of this assumption was established by a synthesis of serine itself from ethyl acetamidocyanoacetate by reduction with lithium borohydride in refluxing tetrahydrofuran, followed by acid hy-

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(3) J. L. Bailey, *Biochem. J.*, **60**, 170 (1955).

(4) L. Friedman, Abstracts of Papers of American Chemical Society 122nd Meeting, September 1952, p. 46M.

(5) J. H. Billman and E. E. Parker, *J. Am. Chem. Soc.*, **67**, 1069 (1945).

drolysis. Berlinguet[6] has described a synthesis of serine from ethyl acetamidocyanoacetate by reduction of the ester with sodium borohydride in aqueous alcohol at room temperature. He was not able under those conditions to obtain complete reduction of the ester, which caused his serine to be contaminated with glycine. Reduction with lithium borohydride in hot tetrahydrofuran overcame this difficulty completely, and glycine was not found in the product. When the lithium borohydride reduction was carried out at room temperature, reduction was not complete.

Ethyl acetamidocyanoacetate was alkylated with methyl iodide, *n*-propyl bromide, and benzyl chloride by the precedure of Albertson,[7] and the three acetamidocyano esters converted to the corresponding α-methylserine (2-amino-3-hydroxy-2-methylpropionic acid), α-*n*-propylserine (2-amino-3-hydroxy-2-propylpropionic acid) and α-benzylserine (2-amino-2-benzyl-3-hydroxypropionic acid). α-Methylserine has been synthesized previously, but by a different method.[5] The amino acids all showed the expected behavior on paper chromatography, migrating faster than serine and slower than the corresponding simple amino acids without the hydroxymethyl group.

It was of interest to study the periodate cleavage of this type of amino acid. α-*n*-Propylserine was found to be readily oxidized by sodium periodate, consuming 1.8 moles of periodate per mole of amino acid. This was close to the expected result, since serine and threonine consume two moles of periodate per mole of amino acid. Thus, the α-substituent did not interfere seriously with the periodate oxidation.

EXPERIMENTAL[8]

Ethyl 2-acetamido-2-cyano-3-phenylpropionate was prepared by alkylation of ethyl acetamidocyanoacetate with benzyl chloride and sodium ethoxide by the procedure used by Albertson[7] for similar compounds. A similar preparation has been described.[9] The product was recrystallized from 95% ethanol, and was obtained in 50% yield; m.p. 130–132° (lit.[9] m.p. 132°).

Lithium borohydride reduction. Synthesis of serine. A mixture of 3.4 g. (0.02 mole) of ethyl acetamidocyanoacetate, 0.42 g. (0.02 mole) of lithium borohydride[10] and 50 ml. of dry tetrahydrofuran was refluxed for 2 hr. and then diluted with methanol, acidified with hydrochloric acid, and evaporated to dryness. Twice more the residue was dissolved in methanol, heated to boiling and evaporated to remove boric acid as methyl borate. The residue was hydrolyzed by refluxing for 8 hr. with 6*N* hydrochloric acid. The hydrochloric acid was removed by repeated addition of water and evaporation, and the remaining amino acid desalted on Dowex-2 anion exchange resin.[11] Paper chromatography in phenol-water and in systems A and B described below revealed serine, contaminated with small amounts of other ninhydrin-positive substances. Identity of the serine was supported by periodate oxidation. Glycine was not present in the product. When the lithium borohydride reduction was carried out in tetrahydrofuran at room temperature for 2 hr., the reduction was incomplete, and glycine was present in the product.

α-Methylserine. By the above procedure, ethyl methyl acetamidocyanoacetate[7] (3.6 g.) was reduced with lithium borohydride (0.42 g.), and the product was hydrolyzed and desalted. The amino acid was recrystallized from water-ethanol to give 2.0 g. (84%) of α-methylserine, prisms, m.p. 253° dec. (lit.[5] m.p. 243°).

Anal. Calcd. for $C_4H_9NO_3$: C, 40.33; H, 7.62; N, 11.76. Found: C, 40.39; H, 7.52; N, 12.03.

α-n-Propylserine. Ethyl *n*-propyl acetamidocyanoacetate[7] (2.1 g.) was reduced, hydrolyzed, and purified by the above procedure. The α-*n*-propylserine was recrystallized from water-ethanol (platelets) and weighed 0.9 g. (62%), m.p. 290° dec.

Anal. Calcd. for $C_6H_{13}NO_3$: C, 48.96; H, 8.90; N, 9.52. Found: C, 49.20; H, 8.91; N, 9.64.

α-Benzylserine. Ethyl 2-acetamido-2-cyano-3-phenyl-propionate (5.2 g.) was reduced, hydrolyzed, and purified by the above procedure. The α-benzylserine was recrystallized from water-ethanol (needles) and weighed 2.5 g. (67%), m.p. 263° dec.

Anal. Calcd. for $C_{10}H_{13}NO_3$: C, 61.52; H, 6.71; N, 7.18 Found: C, 61.29; H, 6.68; N, 7.30.

Paper chromatography of amino acids. The α-substituted serines obtained from the alkyl acetamidocyanoacetates were further characterized by paper chromatography in two systems: (A) *n*-propyl alcohol–water (2:1 by volume) and (B) *sec*-butyl alcohol–88% formic acid–water (100:20:13.3 by volume).

α-Substituent on Serine	R_f of Amino Acids	
	System A	System B
None	0.34	0.24
Methyl	0.42	0.38
n-Propyl	0.70	0.60
Benzyl	0.68	0.64

Reaction with periodate. The cleavage of α-*n*-propylserine by sodium periodate was examined by means of the published procedure[12] for periodate oxidations. Under these conditions, α-*n*-propylserine consistently consumed 1.80 moles of periodate per mole of amino acid. The reaction was complete within 10 min. at 25° and *p*H 8. Prolongation of the reaction for 1 hr. caused no further consumption of periodate. Under the same conditions, threonine consumed 1.96 moles of periodate per mole.

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(8) All evaporations were done under reduced pressure. Melting points were determined in capillaries and are uncorrected. In determining the melting point of amino acids, the bath was preheated to within 30° of the expected melting point.

(9) Brit. Patent 621,477; *Chem. Abstr.*, **43**, 6053 (1949).

(10) Metal Hydrides, Inc., Beverly, Mass.

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