(0.075 mole) of freshly prepared dry yellow mercuric oxide at room temperature. 25.5 g. (0.10 mole) of iodine was added in one portion, and the mixture stirred for 7 hr., then let stand overnight. The insolubles were removed by filtration through Celite,[18] and the filtrate concentrated to dryness *in vacuo*. Recrystallization from 200 ml. of 75% ethanol gave 9.8 g. (34%) of 3,5-dichloro-4-iodoaniline (XXXIV). It was converted directly to the *N*-acetyl derivative (XXXV) by refluxing with excess acetic anhydride in benzene solution. The crude XXXV was purified by recrystallization from ethanol, m.p. 223–224°, yield, 13.6 g. (41% based on starting 3,5-dichloroaniline).

Anal. Calcd. for $C_8H_6Cl_2INO$: C, 29.12, H, 1.83, Cl, 21.49, I, 38.46, N, 4.25. Found: C, 29.03, H, 2.04, Cl, 21.91, 21.67, I, 38.63, N, 4.17.

Preparation of XXXIV *and* XXXV *from 2,6-dichloro-4-nitroaniline.* 1,3-Dichloro-2-iodo-5-nitrobenzene (XXXVI) was obtained in 50% yield from 2,6-dichloro-4-nitroaniline by the method of Körner and Contardi.[22]

The reduction of XXXVI to XXXIV was carried out by the method of West.[23] 15.9 g. (0.05 mole) of 1,3-dichloro-2-iodo-5-nitrobenzene (XXXVI) was dissolved in 250 ml. of methanol containing 2 ml. of concd. hydrochloric acid. The solution was stirred at reflux as 10 g. (0.17 g.-atom) of iron powder was added in small portions. After addition was complete, the mixture was stirred at reflux for another 2 hr. A 1.5-g. sample of solid sodium hydroxide was added to neutralize the acid. The hot suspension was filtered and the residue washed with 100 ml. of hot methanol. The combined filtrate and wash was concentrated *in vacuo* to a dark semi-solid residue. Attempts to isolate pure XXXIV were unsuccessful, so the residue was refluxed with 15 ml. of acetic anhydride in 150 ml. of benzene. The reaction mixture was cooled, the precipitate collected and recrystallized from 50% ethanol to give 3.2 g. (10%) of authentic 3′,5′-dichloro-4′-iodoacetanilide XXXV, m.p. 223–224°. A mixed melting point determination on the 3′-5′-dichloro-4′-iodoacetanilide samples prepared by the above two reaction sequences showed no depression.

Acknowledgment. We wish to thank Dr. H. G. Arlt, Jr., and his staff for the large scale preparation of certain intermediates, Mr. C. Pidacks and Mr. R. D. Mills for the chromatographic studies, Mr. W. Fulmor and Mr. G. Morton for the ultraviolet absorption spectra, Miss A. Craig for the bioassays, and Mr. L. Brancone and his staff for the microanalyses. Dr. A. W. Vogel and his associates determined the antineoplastic activity in mice.

PEARL RIVER, N. Y.

(22) G. Körner and Contardi, *Atti accad. Lincei*, 22I, 823; *Chem. Abstr.*, **8**, 73 (1914).

(23) R. West, *J. Chem. Soc.*, **127**, 494 (1925).

[CONTRIBUTION FROM THE DIVISION OF PROTEIN CHEMISTRY, THE INSTITUTE FOR MUSCLE DISEASE, INC.]

Synthesis of β-Cyano-L-alanine and γ-Cyano-α-L-aminobutyric Acid, Dehydration Products of L-Asparagine and L-Glutamine; a New Synthesis of Amino Acid Nitriles

CHARLOTTE RESSLER AND HARRIET RATZKIN

Received January 13, 1961

Syntheses of two amino acids, β-cyano-L-alanine and γ-cyano-α-L-aminobutyric acid, are presented. These compounds correspond to L-asparagine and L-glutamine in which the β- and γ-carboxamides are replaced by nitrile groups. Carbobenzoxy-L-asparagine and carbobenzoxy-L-glutamine which served as starting materials were smoothly dehydrated to carbobenzoxy-β-cyano-L-alanine (II) and carbobenzoxy-γ-cyano-α-L-aminobutyric acid with *N,N′*-dicyclohexylcarbodiimide in a new route which had been suggested by a side reaction previously encountered in the synthesis of asparagine peptides. Selective removal of the carbobenzoxy group without reduction of the nitrile group was effected with sodium in anhydrous ammonia to yield β-cyano-L-alanine and γ-cyano-α-L-aminobutyric acid. In contrast, treatment of II with sodium in ammonia containing methanol yielded the reduction product α,γ-diaminobutyric acid, and with excess sodium in ammonia an unidentified less basic substance was obtained in addition. Characteristic color reactions with ninhydrin are presented for these amino acids along with other data.

During the synthesis of an asparagine-containing cyclic pentapeptide structurally related to the ring moiety of oxytocin an unusual side reaction was observed.[1] On countercurrent distribution of the cyclic pentapeptide product an additional pentapeptide was found. Examination of this showed that in place of the asparagine moiety a residue of α,γ-diaminobutyric acid was present. It appeared that the asparagine moiety had been partially converted by the coupling agent[2] tetraethyl pyrophosphite into a reducible form, so that after a further synthetic step involving sodium in liquid ammonia α,γ-diaminobutyric acid was formed after hydrolysis in place of aspartic acid and ammonia. The reactivities of the altered asparagine moiety, namely, its hydrolysis to aspartic acid and its reducibility, at least in part, to a basic grouping and the dehydrating nature of the coupling reagent that had been employed suggested that the asparagine-β-carboxamide had been dehydrated to a cyano group although other explanations were possible.

In order to gain further knowledge on this interesting side reaction of peptide synthesis it was de-

(1) C. Ressler, *J. Am. Chem. Soc.*, **78**, 5956 (1956).

(2) Anhydro products of unestablished structure were isolated from similar coupling reactions employing tetraethyl pyrophosphite or *N,N*-dicyclohexylcarbodiimide (D. T. Gish, P. G. Katsoyannis, G. P. Hess, and R. J. Stedman, *J. Am. Chem. Soc.*, **78**, 5954 (1956)).

sired to determine, using a carboxamide-containing compound more amenable to analysis than a polypeptide, whether application of a peptide coupling agent can, indeed, convert a carboxamide to a nitrile. It was also desired to synthesize the amino acid nitrile corresponding to asparagine, *viz.*, β-cyano alanine, and test its properties, especially its reducibility with sodium in ammonia to α,γ-diaminobutyric acid.

We have therefore now treated carbobenzoxy-L-asparagine (I) in pyridine solution with the coupling agent *N,N'*-dicyclohexylcarbodiimide.[3] Evidence of reaction was supplied by precipitation of *N,N'*-dicyclohexylurea. The product which was isolated in 78% yield after recrystallization agreed closely in properties with the carbobenzoxy-β-cyano-L-alanine (II)[4a] obtained from I with *p*-toluenesulfonyl chloride as dehydrating agent, indicating that *N,N'*-dicyclohexylcarbodiimide is, indeed, capable of converting carbobenzoxy asparagine to the corresponding nitrile.

Removal of the carbobenzoxy group from II was accomplished by the sodium in liquid ammonia procedure.[5] By observing anhydrous conditions the protecting group was selectively removed without appreciable reduction of the nitrile group, and crystalline β-cyano-L-alanine (III) was obtained in a yield of 50–60 %. On the other hand, the use of sodium in ammonia containing methanol[4b] led, as established by paper electrophoresis, to the reduction product α,γ-diaminobutyric acid. Excess sodium in ammonia yielded α,γ-diaminobutyric acid and in addition a less basic substance which was not identified. These reactions are illustrated in the accompanying diagram.

The reaction sequence was also carried out starting with carbobenzoxy-L-glutamine. Since carbobenzoxy-γ-cyano-α-L-aminobutyric acid separated as an oil, it was extracted into ethyl acetate and was dried and treated directly with sodium in ammonia. The yield of γ-cyano-α-L-aminobutyric acid[6] was somewhat lower than III, and this may have been due to difficulty in obtaining anhydrous conditions. γ-Cyano-α-L-aminobutyric acid, like β-cyano-L-alanine, was rather soluble in water, and the water-ethanol mixtures usually employed for amino acid isolations were found not very satisfactory. Both compounds were isolated instead from aqueous dioxane from which they crystallized well with sharp decomposition points. Potentiometric titration of β-cyano-L-alanine showed an unusually low apparent dissociation constant for both the carboxylic acid group and the ammonium group, the cyano substituent having effected an increase in the the acidity of the $-COOH$ group and a decrease in the basicity of the $-NH_2$ group, as was seen by comparing the *p*Ka values with those of asparagine. The infrared spectra (potassium bromide disk) of both amino acids showed characteristic nitrile absorption in the 4.5 μ region.

Reaction of the amino acid nitriles with ninhydrin was of some interest. With ninhydrin in acetone on paper or in *n*-butyl alcohol solution at 100° for fifteen minutes, β-cyano-L-alanine, like β-aminopropionitrile,[7] produced a green color,[8] while γ-cyano-α-L-aminobutyric acid gave a blue-purple color. Further heating in solution changed the green color obtained with β-cyano-L-alanine to a gray-lavender. The color obtained with β-aminopropionitrile remained qualitatively unchanged. Under the more hydrolytic and reducing conditions of the ninhydrin reaction as used in the automatic analysis of amino acids[9] only the more usual purple color was obtained with all three nitriles. The absorption spectra of the ninhydrin reaction products of γ-cyano-α-L-aminobutyric acid, β-cyano-L-alanine, β-aminopropionitrile, and leucine under the latter conditions are shown in Fig. 1 and suggest that the strong maximum at 320 mμ might be of aid in characterizing the configuration in which an amino group is in position beta to a cyano group. The green ninhydrin color with absorption maximum at 655 mμ under the butyl alcohol conditions described earlier may be of similar use, although additional examples under both conditions are desirable. A report on the biological properties of β-cyano-L-alanine and γ-cyano-α-L-aminobutyric acid will be presented separately.

The mildness and ease of the dehydration reaction and the reasonable yield and purity of II from I would suggest that the carbodiimide procedure might have general useful application for the synthesis of nitriles. In particular, the low degree of reactivity with the aliphatic hydroxyl group usually encountered with the use of *N,N'*-dicyclohexylcarbodiimide would appear to offer advantages over existing methods for converting carboxamides to nitriles which employ acyl or arylsulfonyl chlorides[10,4a] or phosphorus oxychloride.[11] How-

(3) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

(4) (a) M. Zaoral and J. Rudinger, *Collection Czechoslov. Chem. Communs.*, **24**, 1993 (1959); (b) as in a synthesis of ornithine from toluenesulfonyl-γ-cyano-α-aminobutyric acid, *loc. cit.*

(5) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

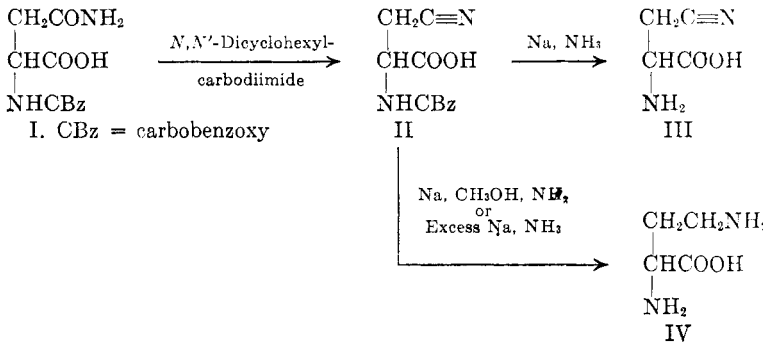
(6) Preliminary experiments were carried out by Mr. Paul A. Redstone.

(7) J. T. Garbutt and F. M. Strong, *Abstracts 128th Meeting Am. Chem. Soc.*, Minn., Sept. 1955, p. 26 A.

(8) A green ninhydrin color on paper previously obtained with asparagine in another laboratory (C. Ressler, *J. Am. Chem. Soc.*, **82**, 1641 (1960)) has not been obtained here; it may have been due to the presence of trace impurities but remains unexplained.

(9) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

(10) J. Mitchell, Jr., and C. E. Ashby, *J. Am. Chem. Soc.*, **67**, 161 (1945); Q. E. Thompson, *J. Am. Chem. Soc.*, **73**, 5841 (1951); F. A. Hochstein *et al.*, *J. Am. Chem. Soc.*, **75**, 5455 (1953); C. R. Stephens, E. J. Bianco, and F. J. Pilgrim, *J. Am. Chem. Soc.*, **77**, 1701 (1955).



ever, the usefulness of the reaction for the preparation of nitriles other than amino acid nitriles remains to be determined.

With regard to the usefulness of *N,N'*-dicyclohexylcarbodiimide as a coupling reagent in the synthesis of asparagine and glutamine peptides in view of the facile dehydration to nitriles demonstrated with this reagent, it may be pointed out that the conditions described below were chosen to favor the formation of nitrile. With a competing reactive amino group available for coupling to form a peptide, with a limited amount of reagent and base and with other solvents, the formation of asparagine and glutamine containing peptides might well be favored with this reagent, and has, indeed, been accomplished; production of nitriles as coproducts may however be anticipated. In selecting a suitable coupling procedure for preparing asparagine and glutamine peptides it may be useful to assess the extent of the side reaction by reducing the crude coupling product with Na-CH_3OH-NH_3 on a micro scale and chromatographically determining the amount of formed α,γ-diaminobutyric acid or ornithine.

The situation which originally led to the identification of α,γ-diaminobutyric acid after peptide synthesis and hydrolysis[1] has not been investigated directly. However, the reactions described here with carbobenzoxy asparagine, namely, dehydration by a coupling agent to form a nitrile and partial reduction of the latter with excess sodium in ammonia to α,γ-diaminobutyric acid are consistent with and could provide an explanation for the previous observations.

EXPERIMENTAL[12]

Carbobenzoxy-L-asparagine (I) *and carbobenzoxy-L-glutamine.* These compounds were prepared using 2*N* sodium hydroxide instead of the more commonly used magnesium oxide or sodium bicarbonate. Approximately 2 moles of the base and 1.2 moles of carbobenzoxy chloride were added in portions within 90 min. with vigorous stirring to a 16% aqueous solution of the amino acid amide. The *p*H was maintained throughout at approximately 8, and the temperature was kept below 25°. When base was no longer readily consumed, its addition was discontinued, and stirring was continued for another hour. The reaction mixtures were worked up in the usual way by extraction with ether followed by acidification to *p*H 1 and filtration. L-Asparagine hydrate, 30 g., yielded 51 g. of crude product, m.p. 162–163°. After one recrystallization from methanol, 43 g. (81%) of carbobenzoxy-L-asparagine was obtained, m.p. 164–165°, $(\alpha)_D^{22}$ −6.5° (*c* 1, 1*N* sodium bicarbonate); reported m.p. 165°.[13a] L-Glutamine, 20 g., yielded 25.8 g. of crude product, m.p. 135–137°. After one recrystallization from water, 23.3 g. (63%) of carbobenzoxy-L-glutamine was obtained, m.p. 137–138°; $(\alpha)_D^{23}$ −8.9° (*c* 1, 1*N* sodium bicarbonate), $(\alpha)_D^{24}$ −6.8° (*c* 2, absolute ethanol); reported m.p. 137°,[13a] 135° [13b]; reported rotation $(\alpha)_D^{20}$ +5.8° (*c* 2, ethanol).[13b] Both compounds were dried and were used without further purification for the following reactions.

Carbobenzoxy-β-cyano-L-alanine (II). A solution of 15 g. of carbobenzoxy-L-asparagine in 75 cc. of redistilled pyridine was maintained at 16–20° and to this a solution of 12.2 g. of *N,N'*-dicyclohexylcarbodiimide in 38 cc. of pyridine was added in portions with magnetic stirring over 30 min. After not longer than 3 hr. at this temperature the precipitated dicyclohexylurea was removed by filtration and the filtrate was concentrated to a small volume *in vacuo* at a pressure of 1 mm. The mixture was again filtered, and the filtrate was concentrated to a thick sirup. Dilution with water caused the separation of some solid which was filtered off after 1 hr. in the cold. The filtrate was then acidified with 6*N* hydrochloric acid with the separation of white crystals, wt. 12 g. (86%); m.p. 130–131°. The product was recrystallized from dry ethylene dichloride, wt. 10.9 g. (78%), m.p. 131–132°; $[\alpha]_D^{22}$ −45.2° (*c* 0.96, dimethylformamide). For comparison the compound was also prepared as described by dehydration with *p*-toluenesulfonyl chloride.[4a] This product melted a little higher at 133–134°; $[\alpha]_D^{25}$ −44.2° (*c* 0.93, dimethylformamide); reported m.p. 133–134°.[4a]

Anal. Calcd. for $C_{12}H_{12}N_2O_4$: C, 58.1; H, 4.87; N, 11.3. Found: C, 57.8; H, 5.22; N, 11.1.

β-Cyano-L-alanine (III). Sodium in small pieces was added to 700 cc. of liquid ammonia in a 1-l. round bottom flask fitted for magnetic stirring and protected with a drying tube of Ascarite. When a blue color appeared throughout the liquid addition of II was started. II (7.5 g.) and sodium (1.9 g.) were introduced alternately in portions, sodium being added in amount each time until a blue color was attained. When addition was complete, 37 g. of Dowex 50 (4%) in the form of its dried ammonium salt was carefully introduced. The ammonia was then allowed to evaporate with stirring. The solids were freed of residual ammonia on the water pump. The resin was then stirred well with water and filtered, and the procedure was repeated until the filtrate no longer gave a green color reaction on paper with 0.15% ninhydrin

(11) R. Delaby, G. Tsatsas, and X. Lusinchi, *C. r.*, **242**, 2644 (1956).

(12) Melting points were determined in capillaries and are corrected. They depended on the rate of heating. Samples were heated rapidly to 10° below the melting point and then at a rate of 2° per minute.

(13) (a) M. Bergmann and L. Zervas, *Chem. Ber.*, **65**, 1192 (1932); (b) R. A. Boissonnas, St. Guttmann, P.-A. Jaquenoud, and J.-P. Waller, *Helv. Chim. Acta*, **38**, 1491 (1955).

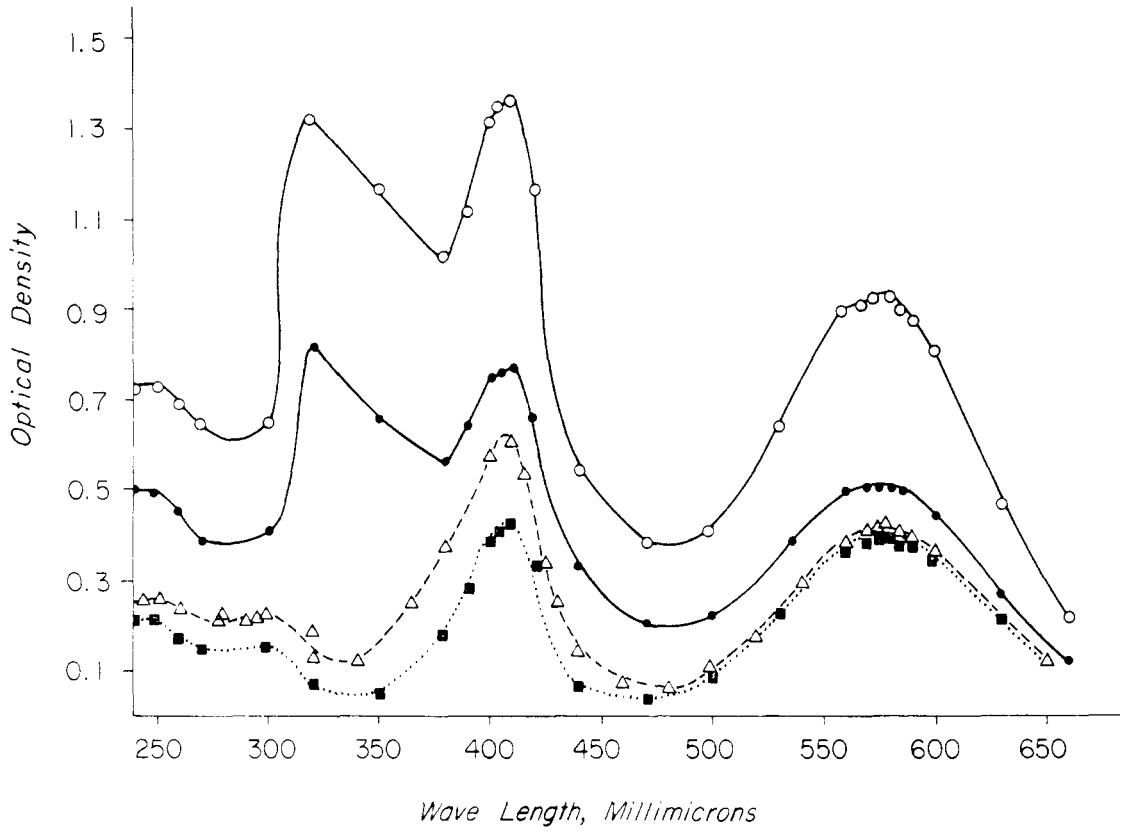


Fig. 1. Absorption spectra of reaction product with ninhydrin (reference 9)

●—● β-cyano-L-alanine, 0.071 μm/cc. × 2; ○—○ β-aminopropionitrile, 0.25 μm/cc.; △——△ γ-cyano-α-L-aminobutyric acid, 0.052 μm/cc.;■···■ leucine, 0.033 μm/cc.

in acetone. The combined extracts were evaporated at room temperature under reduced pressure to approximately 10 cc. The solution was adjusted to *p*H 5 with 6*N* hydrochloric acid. Crystallization of the product as needles usually started at this point and was facilitated by saturating the mixture with purified dioxane and maintaining the *p*H. It was advantageous to separate the solid by the use of centrifugal filtration with a sintered glass funnel which cleanly separated the mother liquor which usually had considerable color from the rather soluble colorless product; wt. 1.77 g. (51%), m.p. 208.5–209° dec. From the mother liquor an additional crop of 241 mg. was obtained, m.p. 206° (total 58%).

For analysis the material was recrystallized several times from water-dioxane at 40°, m.p. 218–218.5° dec., $[\alpha]_D^{26}$ −2.9° (*c* 1.4, 1*N* acetic acid).

Anal. Calcd. for $C_4H_6N_2O_2$: C, 42.1; H, 5.30; N, 24.6. Found: C, 42.4; H, 5.57; N, 24.8.

Paper electrophoresis of both the analytical and the unrecrystallized material in barbital buffer, *p*H 8.5, μ 0.05, using 9 volts/cm. for 3 hr. showed after developing the strip with ninhydrin in acetone a single green spot which was located 5.5 cm. from the origin toward the anode.

Further reactions of II *with sodium in ammonia.* A solution of 5 mg. of II in 1 cc. of liquid ammonia containing 100 μl. of methanol was treated with sodium. A portion of the residue was subjected to electrophoresis on paper in barbital buffer for 2.5 hr. Ninhydrin positive material was located only at 5.5 cm. toward the cathode, which corresponded to the position of authentic α,γ-diaminobutyric acid. Another sample of II was dissolved in 1 cc. of liquid ammonia and treated with sodium. A blue color was reached and maintained for 5 min. Analysis of the product using electrophoresis indicated a small amount of β-cyanoalanine, some α,γ-diaminobutyric acid, and additional ninhydrin positive material which was located 1.5 cm. toward the cathode.

γ-Cyano-α-L-aminobutyric acid. A solution of 10 g. of carbobenzoxy-L-glutamine in 45 cc. of pyridine was treated with a solution of 7.65 g. of dicyclohexylcarbodiimide in 22.5 cc. of pyridine, and the reaction was carried out and treated as described for the preparation of II. On acidification a thick oil was obtained which was extracted into ethyl acetate. The extract was dried well over magnesium sulfate and then was filtered, divided in half, and concentrated under reduced pressure. The residues were further dried by azeotroping with anhydrous benzene. To each residue was added approximately 500 cc. of liquid ammonia. Sodium (1.4 g.) was added in portions until a blue color throughout the solution was reached, followed by 35 g. of the dried ammonium salt of Dowex-50. The ammonia was evaporated and the residues were combined and were treated as described for the isolation of β-cyano-L-alanine. γ-Cyano-α-L-aminobutyric acid crystallized as needles from an aqueous solution at *p*H 5 after the cautious addition of dioxane; wt. 1.2 g., (over-all 26%); m.p. 215–216° dec. Several recrystallizations from aqueous dioxane at 80° raised the m.p. to 227.5–229° dec., $[\alpha]_D^{23}$ +4.5° (*c* 1.3, water).

Anal. Calcd. for $C_5H_8N_2O_2$: C, 46.9; H, 6.29; N, 21.9. Found: C, 46.9; H, 6.39; N, 21.7.

Electrophoretic examination as described for β-cyano-L-alanine showed after 3 hr. a single purple spot with ninhydrin which was located 2 cm. from the origin toward the anode. The crude material showed in addition a smaller amount of unidentified material located 1.5 cm. toward the cathode.

Determination of pK. β-Cyano-L-alanine in 0.05*M* solution was titrated against 0.64*N* hydrochloric acid and 0.68*N*

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.

Acknowledgment. Elementary analyses were performed by the Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. The capable assistance of Mr. Gilbert N. Schnirman is acknowledged. This work was supported by a grant from the Muscular Dystrophy Associations of America, Inc.

NEW YORK, N. Y.

(14) A. C. Chibnall and R. K. Cannon, *Biochem. J.*, **24**, 945 (1930).

(15) Obtained from the California Foundation for Biochemical Research.

[CONTRIBUTION FROM THE ROCKEFELLER INSTITUTE]

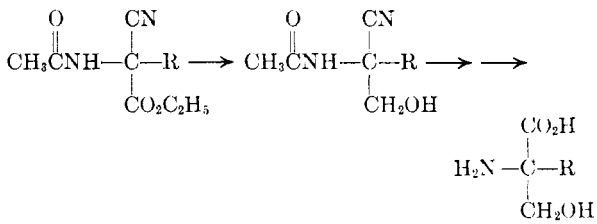
Synthesis of α-Hydroxymethylamino Acids by Means of a Selective Reduction with Lithium Borohydride

JOHN MORROW STEWART

Received January 20, 1961

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-

(1) R. F. Nystrom, S. W. Chaikin, and H. C. Brown *J. Am. Chem. Soc.*, **71**, 3245 (1949).

(2) M. Davis, *J. Chem. Soc.*, 3981 (1956).

(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).