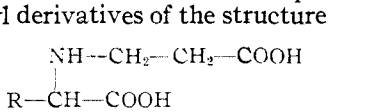
[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY[1]]

Cyanoethylation of α-Amino Acids. III. Hydrolysis of Cyanoethyl Derivatives

BY L. L. McKINNEY, E. A. SETZKORN AND E. H. UHING

N-Cyanoethyl and N-bis-cyanoethyl derivatives of α-amino acids were hydrolyzed with acids and bases to give N-β-carboxyethyl derivatives. Alkali hydrolysis proved to be the most practicable. N-Bis-cyanoethyl derivatives gave monocarboxyethyl derivatives on hydrolysis with one cyanoethyl group being split off. N-β-Carboxyethyl derivatives melt with decomposition and are insoluble in organic solvents. They do not acetylate or benzoylate readily, and attempts to condense them with diamines resulted in brown, low molecular weight resins.

Previous reports of this series have described the preparation of monocyanoethyl and dicyanoethyl derivatives of α-amino acids.[2] This paper describes the hydrolysis of the nitrile group of these derivatives with acids and bases to produce N-β-carboxyethyl derivatives of the structure



With acids, high concentrations and long refluxing time were required for complete hydrolysis and, with the exception of N-(β-carboxyethyl)-tyrosine, the products were tedious to isolate and acid hydrolysis was abandoned for the more practicable alkaline hydrolysis.

Both sodium and barium hydroxides were efficient for hydrolyzing the nitrile groups. Calcium hydroxide did not effect complete hydrolysis even on prolonged boiling. When one equivalent excess of two normal alkali was used, about 6 hours of boiling was required to complete hydrolysis. Fusion of the cyanoethylated amino acids with barium hydroxide octahydrate in accordance with the method of Ford[3] was an efficient method of hydrolysis and the reaction was complete within 30 minutes.

Attempts to obtain N-bis-β-carboxyethyl derivatives by hydrolyzing N-bis-β-cyanoethyl derivatives with barium hydroxide gave over 50% yields of the monocarboxyethyl with none of the bis-carboxyethyl derivative being obtained. Either the second cyanoethyl or the second carboxyethyl group attached to the α-amino group is labile to alkali.

In preparing N-β-carboxyethyl derivatives from amino acids, it is unnecessary to isolate the cyanoethyl derivatives. The over-all yield was markedly improved by hydrolyzing the reaction mixture after treating the amino acid with acrylonitrile. This procedure precluded losses in isolating the cyanoethyl derivatives, and side reactions did not hinder isolation of the carboxyethyl derivatives.

Melting points and solubilities of the N-β-carboxyethyl derivatives of the seven amino acids studied are shown in Table I. They all melt with decomposition and are insoluble in organic solvents.

Several attempts were made to treat the N-β-carboxyethyl derivatives with acetic anhydride, benzoyl chloride and benzene sulfonyl chloride in order to convert the secondary amines into amides.

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) L. L. McKinney, *et al.*, THIS JOURNAL, **72**, 2599 (1950); **73**, 1641 (1951).

(3) J. H. Ford, *et al.*, *ibid.*, **67**, 876 (1945).

TABLE I

PROPERTIES OF N-β-CARBOXYETHYL DERIVATIVES OF α-AMINO ACIDS

N-β-Carboxyethyl derivative of	M.p., °C. (dec.)	Water soly., g./100 ml. Solubility, 7°	Solubility, 75°	Molecular formula	Nitrogen, % Calcd.	Found
Glycine	191–192[a]	2.6	Inf.[b]	$C_5H_9O_4N$	9.52	9.45
DL-Alanine	200–202	10.2	36[c]	$C_6H_{11}O_4N$	8.68	8.60
DL-Valine	233–235	2.4	4.8	$C_8H_{15}O_4N$	7.40	7.38
DL-Leucine	223–225	1.5	2.8	$C_9H_{17}O_4N$	6.89	6.81
DL-Methionine	212–215	1.2	4.0	$C_8H_{15}O_4NS$	6.33	6.36
L-Tyrosine	255–256	0.08	[d]	$C_{12}H_{15}O_5N$	5.53	5.53
DL-Aspartic acid	202–203	1.6	16.5	$C_7H_{11}O_6N$	6.82	6.82

[a] Hydrochloride melted at 120–122°. Dihydrate decomposed at room temp. [b] 1.0% soluble in 50% acetone at 7°. [c] 3.5% soluble in 50% acetone at 25°. [d] Soluble in hot phenol.

N-(β-Carboxyethyl)-acetylmethionine was obtained in low yield. In most cases, however, uncrystallizable sirups were obtained.[4]

Condensation of the dicarboxylic acids with diamines resulted in brown, brittle resins. Resins prepared at 230° were soluble in water but as the temperature was raised to increase molecular weight, solubility was gradually lost, probably the result of cross-linking with the second hydrogen of the α-amino group. Decomposition was also observed at the higher temperatures. The instability of the acids, and cross-linking reactions above 230°, precluded formation of linear, high molecular weight condensation polymers.

Experimental

Hydrolysis of Cyanoethylated Amino Acids. Acid Hydrolysis.—N-(2-Cyanoethyl)-L-tyrosine (0.5 mole) was refluxed for 24 hours with 4 moles of 6 *N* hydrochloric acid. The extent of hydrolysis was followed by removing aliquots, adding magnesium oxide and distilling ammonia. N-(β-Carboxyethyl)-tyrosine precipitated upon neutralizing with sodium hydroxide to *p*H 3.5. The product was washed free of chlorides for a yield of 91%.

Alkaline Hydrolysis.—The monocyanoethyl derivatives of glycine, DL-alanine, DL-leucine, DL-methionine and L-tyrosine were boiled for 6 hours with 2.5 equivalents of 2 *N* barium hydroxide. Barium was removed from the glycine, alanine and leucine derivatives with sulfuric acid. On cooling the filtrate, the N-β-carboxyethyl derivatives of glycine and leucine crystallized. Recrystallization was effected from hot water with a yield of 90 and 61%, respectively. N-(β-Carboxyethyl)-glycine crystallized as the dihydrate. The water of hydration was quantitatively lost on standing for 9 days or by drying in vacuum at 100°. N-(β-Carboxyethyl)-alanine crystallized in long needles on the addition of two volumes of acetone for a yield of 77%. With the methionine and tyrosine derivatives, hydrochloric acid was used to neutralize the alkali and the insoluble N-β-carboxyethyl derivatives were washed free of chlorides with cold water. N-(β-Carboxyethyl)-methionine was recrystallized from

(4) The acyl derivatives were readily prepared from esters by a procedure which will be described in a later report.

hot water yielding 86%. The tyrosine derivative was too insoluble and was not recrystallized. This yield was 77%.

N-(β-Carboxyethyl)-valine was prepared by the fusion technique of Ford[3]: A mixture of N-(2-cyanoethyl)-DL-valine (10.64 g.) and 2.4 equivalents of barium hydroxide octahydrate was heated on a steam-bath for 30 minutes during which time the melt solidified. The solid was suspended in water and sulfuric acid added to remove barium. The product crystallized upon reducing the volume, the yield being 73%.

The monocyanoethyl derivatives of tyrosine and aspartic acid were also hydrolyzed by boiling 6 hours with 2 *N* sodium hydroxide. Four moles of sodium hydroxide was used for each mole of cyanoethyltyrosine while three moles of alkali was used with each mole of cyanoethylaspartic acid. Since the N-β-carboxyethyl derivatives were insoluble in cold water, the products were readily obtained by neutralizing the alkali with hydrochloric acid and washing free of chlorides. N-(β-Carboxyethyl)-aspartic acid was recrystallized from hot water for a yield of 60%. N-(β-Carboxyethyl)-tyrosine was quite insoluble (Table I) and was not recrystallized. Analysis indicated a pure product, yield 85%.

Dicyanoethyl derivatives of DL-alanine and DL-methionine were subjected to hydrolysis with barium hydroxide in the same manner as described above for the monocyanoethyl derivatives. The yield of N-(β-carboxyethyl)-alanine was 75% and of N-(β-carboxyethyl)-methionine was 56%. None of the dicarboxyethyl derivatives was found.

Hydrolysis of Reaction Mixtures.—Acrylonitrile reacted with amino acids as previously described.[2] The barium salts of glycine, DL-alanine, DL-valine and DL-aspartic acid were used and the sodium salts of DL-methionine and L-tyrosine were employed to effect the reaction with acrylonitrile. About 500 ml. of water was used with each mole of amino acid. After completion of the cyanoethylation reaction, an additional two equivalents of barium hydroxide was added to neutralize the new carboxyl group formed from the nitrile and to allow an excess of one equivalent. For example, with one mole of glycine, 0.5 mole of barium hydroxide was used for the cyanoethylation and an additional mole was added for the hydrolysis. The mixture was boiled 5 hours and the N-β-carboxyethyl derivatives isolated as described above for the hydrolysis of cyanoethyl derivatives with barium hydroxide. Yields of the N-β-carboxyethyl derivatives, based on the amino acid, were as follows: glycine 70%, alanine 80%, valine 50%, methionine 80%, tyrosine 85% and aspartic acid 50%.

N-(β-Carboxyethyl)-acetylmethionine.—Five grams (0.0226 mole) of N-(β-carboxyethyl)-methionine was dissolved in 100 ml. of hot water and 50 ml. (0.5 mole) of acetic anhydride added in two equal portions while stirring. After 30 minutes, the solution was evaporated to dryness under reduced pressure and 50 ml. of dioxane was added to the residue. Evaporation to dryness was repeated to remove residual acetic acid. The residue was taken up in 20 ml. of water and a precipitate of 0.7 g. of unacetylated material was discarded. Acetone (10 ml.) was added to the clear filtrate and the solution set at 4° for 3 hours. The crystals weighed 3.5 g. and contained 5.16% nitrogen (calcd. 5.29). Upon recrystallization from hot water, 3 g. of the crude crystals gave 1.15 g. of the product which melted at 146–148°.

Anal. Calcd. for $C_{10}H_{17}O_5NS$: C, 45.5; H, 6.87; N, 5.29; neut. equiv., 264.3. Found: C, 45.5; H, 6.39; N, 5.28; neut. equiv., 264.6.

Unsuccessful Attempts at Acylation.—Treatment of aqueous solutions of other N-β-carboxyethyl derivatives with acetic anhydride yielded the starting materials. Glacial acetic acid–acetic anhydride suspensions yielded a sirup on heating. Benzoylation by the Schotten–Baumann technique and benzenesulfonylation by the Hinsberg method also gave sirups from which we were unable to isolate the desired acylated derivative.

Condensation with Diamines.—Equimolar quantities of the N-β-carboxyethyl derivatives of glycine, alanine and leucine were heated under nitrogen with both ethylenediamines. The temperature was raised slowly over a period of 6 hours to 230° and then high vacuum applied for 2 hours while the temperature was raised to 250°. In each case browning occurred at 200–225°. As the temperature was increased above 230°, solubility in water was lost and the resin became infusible. The resins were dark brown, brittle solids.

PEORIA 5, ILLINOIS RECEIVED NOVEMBER 5, 1951

[CONTRIBUTION FROM THE CHEMISTRY LABORATORY, UNIVERSITY OF NOTRE DAME]

The Influence of Sulfide, Sulfoxide and Sulfone Groups on the Saponification of Ethyl Benzoate[1]

BY CHARLES C. PRICE AND JOSEPH J. HYDOCK[2]

The ethyl esters of *m*- and *p*-methylmercapto-, -methylsulfinyl- and -methylsulfonylbenzoic acids have been prepared and characterized. Their rates of saponification in 56% acetone and 95% *n*-butyl cellosolve and their ultraviolet absorption spectra have been measured and interpreted.

In order to obtain information concerning the influence of sulfide, sulfoxide and sulfone groups on aromatic systems, ethyl benzoate derivatives with these substituents in the *m*- and *p*-positions have been prepared and their rates of saponification measured. The influence of these groups on the ultraviolet spectra is also reported.

Experimental[3]

Phenyl methyl sulfide was prepared by the method of Bourgeois and Abraham.[4] The yield of purified product was 23 g.; b.p. 193.5–195.0° (737 mm.); $n^{25}D$ 1.5835 (lit. $n^{25}D$ 1.5832).

***m*- and *p*-Methylmercaptobenzoic Acids.**—Both of these acids were prepared by following, in part, the procedure of Allen and MacKay.[5] The diazotized aminobenzoic acids were treated with sodium disulfide to give the bis-carboxyphenyl disulfides.

The disulfide was reduced to the mercaptobenzoic acid by sodium sulfide and subsequently methylated with methyl sulfate by the method of Brand, Gabel and Rosenkranz.[6] The *m*- and *p*-methylmercaptobenzoic acids can be recrystallized from 50% methanol. Both are white crystalline solids. Based on the reactant aminobenzoic acid the yields averaged 45–55%.

Compound, acid	M.p., °C. Obsd.	Lit.	Ref.
m-Methylmercaptobenzoic	126.0–127.0	126	6
p-Methylmercaptobenzoic	191.5–192.0	190	7

Sulfones.—Phenyl methyl sulfone and *m*- and *p*-methylsulfonylbenzoic acids were prepared from the corresponding sulfides by the general method recommended by Gilman and

(1) Presented at the International Congress of Pure and Applied Chemistry, New York, September, 1951.

(2) The Coca-Cola Company Fellow, 1947–1949.

(3) Unless otherwise stated, all melting points are corrected. Microanalyses by Micro-Tech Laboratories, Skokie, Ill.

(4) E. Bourgeois and A. Abraham, *Rec. trav. chim.*, **30**, 413 (1911).

(5) C. F. H. Allen and D. D. MacKay, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 580.

(6) K. Brand, W. Gabel and E. Rosenkranz, *Ber.*, **70**, 305 (1937).

(7) S. Smiles and D. C. Harrison, *J. Chem. Soc.*, **121**, 2025 (1922).