THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 236, No. 3, March 1961
Printed in U.S.A.

The Bromodecarboxylation of Amino Acids: Formation of Nitriles*†‡

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(Received for publication, July 19, 1960)

In previous papers from this laboratory (1, 2) it has been reported that α-amino acids undergo a quantitative decarboxylation in aqueous solution when treated with *N*-bromosuccinimide. Under the conditions used (a temperature of 30° and a pH of 3 to 5), the reaction was complete in 30 minutes. Anomalies in behavior were observed with glycine and β-alanine. The consumption of *N*-bromosuccinimide was typically somewhat more than 2 moles per mole of amino acid and was as high as 8 moles per mole in the case of histidine, tyrosine, and tryptophan. The reagent was consumed with great rapidity—in less than 5 minutes at room temperature. It was also shown that certain of the amino acids evolved nitrogen in amounts that corresponded to from 20% to 100% of the α-amino nitrogen present in the parent compound.

We have now completed a study of the nongaseous reaction products which are herein identified as nitriles and aldehydes. It should be mentioned that Schönberg *et al.* (3) reported that *N*-bromosuccinimide, when used in the bromodecarboxylation of alanine, leucine, and phenylglycine, yields aldehydes of one less carbon atom than the parent compounds, but these authors did not discuss the fate of the amino nitrogen.

EXPERIMENTAL PROCEDURE

Apparatus and Reagents

Mass spectrometric analyses were performed with a Consolidated Engineering Corporation mass spectrometer, model 21-401, and later with a model 21-103C.

Gas chromatography studies were carried out with apparatus built by the Stanford Research Institute. The volatile fractions in the exhaust gas stream were collected in U-tubes immersed in liquid nitrogen.

Under the conditions used, the fractionating properties of the tetrahydroxyethyl ethylenediamine columns used for aqueous solutions allowed the nitriles to precede the water vapor fraction by several minutes. The concentration of each volatile component was recorded as a peak on a strip-chart recorder. The area under each peak was determined with a planimeter (Keuffel and Esser, No. 4236), and this area was empirically related to the concentration of the particular component. The peak areas produced by solutions of authentic compounds which were chromatographed under the same conditions were used as reference standards. The standard solutions were prepared in concentrations to produce peak areas approximately the same as those produced by the samples. For the analysis of nonaqueous solutions we used columns of fire brick coated with Dow-Corning silicone oil No. 710.

Amino acid and ammonia determinations were performed with a Beckman-Spinco amino acid analyzer model 120 (4, 5).

N-Bromosuccinimide was purchased from Arapahoe Chemicals, Inc., and was used without further purification.

The amino acids were obtained from Mann Research Laboratories, Inc.

EXPERIMENTS AND RESULTS

Nitriles—The technique of gas-liquid chromatography made possible the isolation and quantitative determination of the nitriles formed in the reaction between *N*-bromosuccinimide and amino acids. A typical experiment follows.

DL-Norvaline (117.2 mg, 1 mmole) and powdered *N*-bromosuccinimide (356 mg, 2 mmoles) were weighed in a test tube calibrated at 1 ml. The reagents were well mixed before the reaction was initiated by the addition of water (about 0.5 ml). At the end of 1 hour, crystals of sodium thiosulfate were added to decolorize the solution which was then made up to 1 ml with water. The solution was shaken with *o*-xylene (2 ml), and a sample of the extract was injected into the gas chromatography column. The principal volatile component was collected and analyzed in the mass spectrometer which revealed that this fraction consisted of *n*-butyronitrile, the fragmentation pattern being identical with that recorded in "the Index" (6) as American Petroleum Institute (A.P.I.) spectrum 236.

A similarly conducted experiment yielded benzyl cyanide (α-tolunitrile) from phenylalanine. The nitrile was identified by mass spectrometric analysis (fragmentation pattern identical with A.P.I. spectrum 510) as well as by comparison with an authentic sample of the nitrile chromatographed under the same conditions.

Six amino acids were quantitatively analyzed for the volatile products that resulted from the reaction with *N*-bromosuccinimide. In each case, samples of 0.02 ml of the reaction solution were injected into the column. The concentration of each nitrile fraction was recorded and, at the same time, a sample of the fraction was collected for mass spectrometric analysis and the fragmentation pattern was compared with the spectra recorded

* From the thesis submitted by George William Stevenson in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

† The investigation was supported by a research grant (C-2341) from the National Institutes of Health, United States Public Health Service.

‡ The authors are indebted to Dr. Lloyd Moss of the Stanford Research Institute for his generous assistance in connection with some of the analyses.

TABLE I

Determination of aldehydes and nitriles by gas chromatography

All water solutions were fractionated in a column (180 × 0.5 cm) of tetrahydroxyethyl ethylenediamine. All other solutions were fractionated in a column (180 × 0.5 cm) packed with fire brick particles coated with Dow-Corning silicone oil No. 710. The carrier gas was helium which was adjusted to a flow rate of 30 ml per minute. Sample volume was 0.02 ml. Yields (Table II) were obtained by comparing the peak areas of the fractions from 1 mole of amino acid with the peak area from 1 mole of authentic compound (considered as 100%).

Compound	Solvent	Effluent time	Column temperature
		min	°C
Fraction I from alanine	Decalin	0.8	110
Acetaldehyde	Decalin	0.8	110
Fraction II from alanine	Water	3.3	110
Acetonitrile	Water	3.3	110
Fraction I from α-aminobutyric acid	Decalin	2.1	110
Propionaldehyde	Decalin	2.1	110
Fraction II from α-aminobutyric acid	Water	2.8	119
Propionitrile	Water	2.8	119
Fraction I from norvaline	Xylene	2.2	114
Butyraldehyde	Xylene	2.1	114
Fraction II from norvaline	Water	6.2	110
Butyronitrile	Water	6.2	110
Fraction I from valine	Xylene	1.7	120
Isobutyraldehyde	Xylene	1.6	120
Fraction II from valine	Water	2.7	129
Isobutyronitrile	Water	2.7	129
Fraction I from leucine	Xylene	2.3	164
Isovaleraldehyde	Xylene	2.3	164
Fraction II from leucine	Water	6.2	164
Isovaleronitrile	Water	6.2	164
Fraction I from phenylalanine	Benzene	3.8	220
Phenylacetaldehyde	Benzene	3.9	220
Fraction II from phenylalanine	Water	5.6	230
α-Tolunitrile	Water	5.6	230

TABLE II

*Yields of nitriles and aldehydes produced in reaction of N-bromosuccinimide with amino acids**

Compound	Vitrile†	Aldehyde†
DL-Alanine	Acetonitrile (38%)	Acetaldehyde (30%)
DL-α-Amino-n-butyric acid	Propionitrile (40%)	Propionaldehyde (10%)
DL-Norvaline	n-Butyronitrile (42%)	n-Butyraldehyde (5%)
DL-Valine	Isobutyronitrile (46%)	Isobutyraldehyde (3%)
DL-Leucine	Isovaleronitrile (44%)	Isovaleraldehyde (4%)
DL-Phenylalanine	Benzyl cyanide (51%)	Phenylacetaldehyde (6%)

* Mole ratio of *N*-bromosuccinimide to amino acid = 2:1.

† The figures in parenthesis indicate the yields of the compounds based on 1 mole of the parent amino acid. The identity of the nitriles and aldehydes was confirmed by mass spectrometric analyses performed by the Stanford Research Institute.

in the A.P.I. Index. The results of the gas chromatography studies are presented in Tables I and II.

Aldehydes—Aldehydes were quantitatively estimated by extraction of each decolorized reaction solution with *o*-xylene or nonane and injection of a sample of the extract into a column containing fire brick coated with the silicone oil previously mentioned. The peak area was compared with the area produced by a similarly treated solution of the authentic aldehyde.[1] The results of these experiments are included in Tables I and II.

Ammonia—Ascending paper chromatography was employed in an effort to detect the presence of ammonia in the reaction medium. Whatman No. 1 filter paper was used in conjunction with a solvent system of *n*-butanol, acetic acid, and water in volume ratios of 75:10:15. The ninhydrin solution was prepared according to Moore and Stein (7). Aqueous solutions of amino acids (0.02 M) were treated with *N*-bromosuccinimide in a mole ratio of 10:1, and the reaction was allowed to proceed for 30 minutes.[2] To chromatography paper 20 μl of each of these solutions were applied together with standard samples. The chromatograms were run for 24 hours and then developed with ninhydrin. A colored spot corresponding to ammonia was not found in any of the experimental runs.

A further attempt to detect ammonia in the reaction medium of amino acids treated with *N*-bromosuccinimide was made by ion exchange chromatography. The 15-cm × 0.9-cm column was filled with a specially processed sulfonated styrene-8% divinylbenzene copolymer resin. The temperature was maintained at 50°. The eluting solvent was 0.2 M citrate buffer at pH 5.28.

DL-Leucine (13.12 mg, 100 μmoles) and *N*-bromosuccinimide (178 mg, 1000 μmoles) were added to water (5 ml) and allowed to react for 24 hours at room temperature. A 1-ml sample was mixed with 0.2 M citrate buffer (3 ml) at pH 2.2, and 2 ml of this buffered solution were applied to the ion exchange column.

Almost all of the naturally occurring α-amino acids were treated in a similar manner. Ammonia was not detected in the reaction medium in any case, even though the α-amino nitrogen initially present corresponded to a potential 10 μmoles of ammonia.

Although ammonia has been reported by others to be among the reaction products of the bromodecarboxylation of amino acids, in our experience it may be found only if insufficient amounts of *N*-bromosuccinimide are used or if the reaction products in solution are exposed to conditions (*e.g.* long standing or heating) that cause hydrolysis of the nitrile.

DISCUSSION

The nitriles and aldehydes resulting from the reaction of *N*-bromosuccinimide with amino acids corresponded to the decarboxylated parent amino acids; *i.e.* the nitriles and aldehydes contained one less carbon atom than the original amino acid from which each was derived. Higher yields of the nitrile were

[1] It should be mentioned that aldehydes were so fully retained on columns of tetrahydroxyethyl ethylenediamine that quantitative estimation with such columns was not possible. We also tried reduction of the aldehydes to the corresponding alcohols with potassium borohydride. These could readily be fractionated on such columns, but the yields of both alcohols and nitriles were so diminished as to necessitate abandonment of this procedure.

[2] Buffered solutions were not used. The initial pH in all cases approximated pH 4 to 4.5. In the course of the reaction, the pH fell to a final value of pH 2 to 2.3.

observed with an increase in length of the carbon chain of the amino acid—a relationship which is inverse to that observed in connection with aldehyde yields. In all of the cases studied, the yields of the nitriles were greater than those of the corresponding aldehydes.

The absence of ammonia from the reaction medium is in agreement with a previous finding (2) that ammonium ions are rapidly and quantitatively oxidized to nitrogen by *N*-bromosuccinimide at 30°. According to titration studies, one mole of ammonia requires 1.5 moles of *N*-bromosuccinimide for this reaction, a value which agrees with theoretical calculations.

The reaction of ammonium salts with *N*-bromosuccinimide explains the relationship between the formation of aldehydes and the liberation of gaseous nitrogen. The conversion of an α-amino acid to an aldehyde with one less carbon must involve the cleavage of the carbon-nitrogen bond, thus releasing ammonia. The ammonia is subsequently oxidized to nitrogen in yields corresponding to the amount of aldehyde formed. A strict stoichiometric relationship between yields of aldehyde and nitrogen does not occur because of the partial spontaneous hydrolysis of some of the nitriles—a reaction which yields additional ammonia and hence nitrogen.

The reaction pathway suggested by Schönberg, Moubasher, and Barakat (3) accounts for the formation of aldehyde only. From the mole ratio of *N*-bromosuccinimide to amino acid used, it was indicated that about 1 mole or less of *N*-bromosuccinimide was sufficient to decarboxylate each mole of amino acid. However, titration studies on the consumption of the reagent (2) show that at least 2 moles of *N*-bromosuccinimide are required for complete reaction with amino acids such as alanine, leucine, and phenylalanine. In addition, the formation of ammonium ions from the reaction of free amino acids would require a further consumption of *N*-bromosuccinimide. Since it has been reported that ammonium ions are rapidly oxidized to nitrogen by *N*-bromosuccinimide (2), ammonium ions would be present in appreciable amounts only when insufficient *N*-bromosuccinimide was used for the decarboxylation of an amino acid. Ammonium ions or ammonia would also arise from any further treatment of the reaction medium that would cause hydrolysis of the nitriles.

$$RCH(NH_2)COOH \xrightarrow{NBS} \underset{I}{[RCH(NH_2)Br]} + CO_2$$

$$[RCH(NH_2)Br] \xrightarrow[-2HBr]{NBS} RCN$$

$$\downarrow H_3O^+$$

$$RCHO + NH_4Br \xrightarrow{1.5\ NBS} \tfrac{1}{2}N_2 + \tfrac{5}{2}HBr$$

All the evidence from this present study and from the work of previous investigators suggests the following scheme for the oxidation of amino acids by *N*-bromosuccinimide.

The fact that *N*-bromosuccinimide is stable in water for over 24 hours (2) indicates that hypobromous acid which would oxidize amino acids is not first formed. In either case, however, the bromine would enter the reaction as positively charged bromine. The initial point of attack is assumed to be the α carbon rather than the amino group because the reaction still occurs when both hydrogen atoms of the amino group are substituted with alkyl groups (8).

Hydrolysis of the intermediate I would lead to the formation of an aldehyde and ammonia. The ammonia would then consume 1.5 moles of *N*-bromosuccinimide and evolve 0.5 mole of nitrogen. This reaction pathway is most characteristic of the lower amino acids (short side chains). Further bromination of I, instead of hydrolysis, may result in the formation of a dibromo intermediate which then loses 2 moles of hydrobromic acid and forms a nitrile. The formation of nitriles is greatest from the longer chain amino acids.

SUMMARY

N-Bromosuccinimide reacts with aqueous solutions of some α-amino acids at room temperature to form the nitrile corresponding to the decarboxylated parent compound. In addition, smaller amounts of aldehyde with one carbon less than the original amino acid may be formed. The formation of the aldehyde is accompanied by the liberation of ammonia which is rapidly oxidized to nitrogen by excess *N*-bromosuccinimide.

This study provides a new method for the conversion of α-amino acids to nitriles with one less carbon than the amino acid. The reaction proceeds rapidly and in good yield in aqueous media at room temperature.

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J. Biol. Chem. 1961, 236:715-717.