TABLE III

INDIUM TRIALKYL SULFONIUM HALIDES

Formula	Indium, % Calcd.	Indium, % Found	Chlorine or iodine, % Calcd.	Chlorine or iodine, % Found	Water Calcd.	Water Found
$4(CH_3)_3SCl \cdot 2InCl_3 \cdot 3H_2O$	24.25	24.20 24.27	37.45	37.63 37.19	5.71	5.88
$(CH_3)_3SI \cdot InI_3$	16.40	16.64 16.68	72.57	72.31 72.08		
$(C_2H_5)_3SI \cdot InI_3$	15.47	15.75 15.63	68.46	68.37 68.63		

150° and 145–147°, respectively. In ether these iodides decompose slowly with the liberation of iodine.

Optical Properties.—Indium trimethylsulfonium chloride crystallized in such irregularly twinned monoclinic prisms that the refractive indices could not be determined. Indium trimethyl sulfonium iodide yielded anisotropic crystals whose indices of refraction were greater than that of methylene iodide. Indium triethylsulfonium iodide gave such extremely minute crystals that the optical properties could not be determined.

Summary

Various double chlorides, bromides, iodides and sulfates of indium with the corresponding substituted ammonium and sulfonium salts have been prepared, their formulas determined, and when possible their optical properties.

BOULDER, COLORADO RECEIVED JANUARY 27, 1936

[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY, UNIVERSITY OF NEBRASKA COLLEGE OF MEDICINE]

The Oxidation of Amino Acids with Sodium Hypobromite

BY ALVIN H. FRIEDMAN AND SERGIUS MORGULIS

Introduction

The problem of the oxidation of the amino acids in the body is one still awaiting a satisfactory solution. The mechanism of the oxidation is not accessible to direct experimentation. The close relationship among the amino acids, ketonic acids and hydroxy acids furnishes a basis for the interpretation of the mode of ultilization of amino acids by the body. Experiments on perfusing the liver of various animals with different amino acids have demonstrated the presence of ketonic acids in the perfusate. The deamination of the amino acids in the liver is also well established. Amino acids may also be decarboxylated in the organism, as is shown by the presence in the body fluids of histamine and tyramine.

The oxidation of the amino acids *in vitro* has been the subject of many investigations, both from a purely chemical standpoint and from the point of view of its bearing on the mechanism of the amino acid metabolism in the organism. Amino acids are resistant to hydrolyzing agents, and they do not react readily with reducing agents, but are very reactive with oxidizing agents in general.[1,2,3,4]

The production of aldehydes from the amino acids has been demonstrated by many authors with a diversity of oxidizing agents.[5,6,7] Langheld[8] was the first to study the effect of sodium hypochlorite on the amino acids and found that carbon dioxide, ammonia, and "C-armere Aldehyde" are produced in the reaction. Dakin's[9] work on the reaction of amino acids with sodium hypochlorite followed Langheld's. Dakin used sodium-*p*-toluene sulfonchloramide (chloramine-T) and found that although this reagent contained no free sodium hypochlorite, it acted in the same manner as would a solution of sodium hypochlorite, chlorination of the amino group to give an N-chloro amino acid being the first product which decomposes yielding ammonia, carbon dioxide and the corresponding aldehyde.

The reaction of the amino acids with sodium hypobromite has not been very extensively studied. Jolles[10] long ago noticed that when glycine is acted upon by sodium hypobromite a compound is formed which is precipitable with phosphotungstic acid. This compound, as can

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(1) W. Heintz, *Ann. Chem. Pharm.*, **145**, 214 (1868).

(2) R. M. Herbst and H. T. Clarke, *J. Biol. Chem.*, **104**, 769 (1934).

(3) K. Kraut and F. Hartmann, *Ann. Chem. Pharm.*, **133**, 101 (1865).

(4) A. Strecker, *ibid.*, **75**, 37 (1850).

(5) Sh. Akrabi, *Proc. Imp. Acad. Japan*, **3**, 672 (1927).

(6) E. S. Hill, *J. Biol. Chem.*, **95**, 197 (1932).

(7) O. Warburg, *Science*, **61**, 577 (1925).

(8) K. Langheld, *Ber.*, **42**, 392 (1909).

(9) H. D. Dakin, *Biochem. J.*, **10**, 319 (1916); **11**, 79 (1917).

(10) H. Jolles, *Z. physiol. Chem.*, **31**, 391 (1900).

be inferred from the work of Dehn[11] is cyanate. He observed also that the nitrogen of the amino acid was not liberated as free nitrogen. Van Slyke,[12] using hypobromite for the gasometric determination of urea in the Van Slyke and Niell apparatus, mentions that among the sources of nitrogen gas, besides urea and ammonia, may be uric acid and creatinine. According to our results, creatinine would play only a very insignificant part, but creatine liberates a much greater proportion of its nitrogen as a gas. Goldschmidt, Wiberg, Nagel and Martin[13] discuss the degradation of the amino acids, poly-peptides and diketopiperazines with sodium hypobromite and show that this is similar to the reaction with sodium hypochlorite, aldehydes and nitriles being formed. Our experiments have led us to similar conclusions, as will be indicated later by the modified scheme which was developed to elucidate the mechanism of this reaction.

Experimental

We have adopted for this work a comparative method of procedure, each amino acid being studied under the same conditions. This form of procedure compares the amino acids among themselves, though possibly not under conditions comparable with those of other workers. To determine the ratio of moles sodium hypobromite used up per mole amino acid we resorted to the following procedure. The reagents, including the hypobromite, were prepared according to Rappaport[14] or Teorell,[15] as was described in a previous paper.[16] The amino acid solutions were made up from the pure amino acids, the purity of the sample being checked by its nitrogen content. Two solutions containing 0.01 and 0.10 mg. of N per cc. were prepared always fresh and the nitrogen content was determined. For the analysis a desired amount of amino acid solution was pipetted into a 50-cc. Erlenmeyer flask, treated with an excess of sodium hypobromite, and allowed to stand at room temperature for ten minutes from the time of the first addition of the hypobromite reagent. The rest of the procedure is described in our previous paper.[16] For the calculation, the difference between the blank and titration value was multiplied by 0.03502/mg. N. (1.00 cc. 0.005 N $Na_2S_2O_3$ = 0.0000025 mole; 1.00 mg. N = 0.0000714 mole amino acid.) In this way the number of moles of hypobromite per mole amino acid was determined in the case of the mono amino acids and the results are presented in Table I.

TABLE I

AVERAGE MOLES NaOBr PER MOLE SUBSTANCE

Substance	Unbuffered NaOBr	Buffered NaOBr	Buffered NaOBr 60° C.	% N liberated as gas (unbuffered NaOBr)
Ammonium sulfate	3.00	3.00	3.00	100
Urea	2.86	0.48	3.06	93–95
Phenyl urea	3.20	(5.26–5.55)[a]		4.3
Semicarbazide–HCl	2.20	2.20		67–75
Guanidine carbonate	4.00	0.50		97
Glycine	2.97	(1.95–2.40)		1.6–3.0
Alanine	(2.06–2.40)	(1.95–2.40)		22
Amino-*n*-butyric acid	2.04–2.20	2.03		12.5–13.0
Amino-*n*-valeric acid	2.04	2.00		7.3
Valine	2.02	2.00		4.6
Norleucine	2.26	2.26		7.7
Leucine	2.09	2.07	2.07	5.9
Aminocaprylic acid	2.32			60.8 (alk.)[b]
Phenylalanine	(2.25–2.50)	2.07	2.07	9.8
Proline	(1.80–2.70)	1.03		8.1
Aspartic acid	(3.60–4.50)	2.30–3.16	3.74	1.1
Glutamic acid	(2.01–2.25)	2.01	1.995	11.3
Cysteine·HCl	6.78	5.18		0
Arginine	4.80	4.60		49.2
Histidine·2HCl	5.40	6.30		2.5
Sarcosine	1.10	0.03		6.5
Uric acid		2.34	15.4	
Creatine	3.96	3.00	5.1	66
Creatinine	0.54	0.27	1.8	0

[a] Values in parentheses represent limits of variation when these were so wide that averaging of results could not be made. [b] Insoluble in neutral solutions.

In some cases the reaction mixture was warmed to 60° and the results of these determinations were likewise tabulated.

We also determined the volume of nitrogen gas liberated from the amino acids under the influence of sodium hypobromite. This we have done in the Van Slyke and Niell apparatus with the closed manometer. The pressure obtained using ammonium sulfate containing 0.3 mg. N was

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[11] W. N. Dehn, THIS JOURNAL, **31**, 1230 (1909).

[12] D. D. Van Slyke, *J. Biol. Chem.*, **83**, 449 (1929).

[13] S. Goldschmidt, E. Wiberg, F. Nagel and K. Martin, *Ann. Chem. Pharm.*, **456**, 1 (1927).

[14] F. Rappaport, *Mikrochemie*, **8**, 49 (1933).

[15] T. Teorell, *Biochem. Z.*, **248**, 246 (1932).

[16] S. Morgulis and A. H. Friedman, *Bull. soc. chim. biol.*, in press.

designated p_n, that of a water blank p_0, and the pressure developed by the substance under examination, likewise containing 0.3 mg. N, was designated p_x. The per cent. of nitrogen liberated was then calculated according to the formula

$$\text{Percentage N} = \frac{p_0 - p_x}{p_0 - p_n} \times 100$$

The percentage of nitrogen of the various compounds examined liberated as gas is recorded in Table I.

We found that the mixture remaining from the reaction with the hypobromite contained carbon dioxide, also liberated from the amino acid. We determined this carbon dioxide by a macroprocedure. An aqueous solution of 0.20 g. of the amino acid was treated with 2 moles of hypobromite (0.1 *N*). After ten minutes, excess of potassium iodide and sulfuric acid were added, the carbon dioxide was aspirated into barium hydroxide, and the barium carbonate estimated acidimetrically. Appropriate blank determinations gave completely negative values. When checked against pure sodium carbonate, this procedure was found to have an error of less than −3%.

The relative amount of carbon dioxide so determined corresponds to one carbon atom from the amino acid, with the exception of glycine, which yielded two. With less hypobromite than that corresponding to the microanalytical results, less carbon dioxide was obtained, but the use of an excess of the reagent was without effect.

The Action of Sodium Hypobromite on Individual Amino Acids

Glycine.—This amino acid reacts with three moles of hypobromite, very little nitrogen being set free in the reaction. After the reaction has proceeded for a few minutes only traces of ammonia are present, but more is liberated on standing. The nitrogen of the glycine is precipitable by either phosphotungstic or phosphomolybdic acids after reacting with sodium hypobromite, which is not true of the original amino acid. The reagents precipitate cyanates but not cyanides, and cyanate can be isolated from the reaction mixture according to the procedure outlined by Dehn.[11] The whole of the carbon content of the amino acid is liberated as carbon dioxide on standing, which presumably results from hydrolysis of the cyanate formed, since cyanate hydrolyses on standing in aqueous solution. The molecular rearrangement of the cyanate formed may perhaps account for the urea found by some workers (Herbst, Clarke and others) on the oxidation of glycine. All this evidence points to the equation

$$CH_2NH_2COOH + 2NaOBr \longrightarrow HCN + CO_2 + 2H_2O + 2NaBr$$

Dehn has shown that HCN is oxidized by hypobromite to HCNO

$$HCN + NaOBr \longrightarrow HCNO + NaBr$$

and HCNO on standing hydrolyses, setting free ammonia and carbon dioxide.

Summing up these equations, the entire reaction may be shown as follows

$$CH_2NH_2COOH + 3NaOBr \longrightarrow HCNO + CO_2 + 2H_2O + 3NaBr \longrightarrow NH_3 + 2CO_2 + 3NaBr + H_2O$$

Only slight traces of formaldehyde or formic acid were found in the reaction mixture.

The ammonia formed in the second part of the reaction is not determined by our usual procedure (results reported in Table I) because within the ten minutes the hydrolysis does not proceed to any extent, but under the conditions of the carbon dioxide determination, as shown in Table II, the hydrolysis is essentially completed.

TABLE II

CARBON LIBERATED FROM THE AMINO ACIDS BY HYPOBROMITE

Substance	Percentage of C set free Found	Calcd.	Theoretical value corresponds to C atoms
Sodium carbonate	97.2	100.0	1
Sodium carbonate	98.3	100.0	1
Glycine	97.0	100.0	2
Alanine	30.9	33.3	1
Amino-*n*-valeric acid	20.3	20.0	1
Amino-*n*-caproic acid	15.8	16.7	1
Aminocaprylic acid	12.3	12.5	1
Phenylacetic acid	8.8	11.1	1
Proline	15.4	20.0	1
Glutamic acid	17.3	20.0	1
Sarcosine	30.0	50.0	1
Creatine	14.6	25.0	1
Creatinine	0	25.0	1
Aminocaprylic acid +			
1 mole OBr^-	5.83	12.5	1
+ 3 moles OBr^-	12.3	12.5	1

Alanine.—The amino acid liberates 22% of its nitrogen as gas and this reaction requires at least 2.11 moles of hypobromite. This is due to the fact that each mole of ammonia set free requires 1.5 moles of hypobromite to oxidize it to free nitrogen. Were all the alanine converted to acetaldehyde and ammonia, this should require 2.5 moles of hypobromite. This is the reason why amino acids generally require more than two moles of hypobromite, the amount in excess depending on the amount converted to aldehydes and hence liberating ammonia. When the reaction mixture resulting from the action of hypobromite on alanine is distilled, drops of acetonitrile can be observed in the first portion of the distillate, which dissolves in the water distilled over. Acetic acid has been determined by its odor from the hydrolysis of the acetonitrile with sulfuric acid. We have isolated the acetaldehyde produced from alanine by distilling it from the neutral solution after removing the bromine, and determined the melting points of its 2,4-dinitrophenylhydrazone and its dimethyldihydroresorcinol derivative.

The isolation of the nitriles was accomplished by extracting the alkaline reaction mixture three times with ether.

distilling off the ether, shaking with sodium bisulfite and separating, washing and drying. The nitriles were determined by hydrolysis in alcoholic solution with sulfuric acid. The products formed were the ethyl ester of the corresponding acid and a precipitate of ammonium sulfate. The esters were separated by the addition of water and the boiling point of the distillate was determined. The following amino acids were tested in this manner: amino-*n*-butyric acid, amino-*n*-valeric acid, valine, norleucine, leucine, amino-*n*-caprylic acid and phenylalanine. It was observed that as the carbon chain of the amino acids became longer, the tendency to form aldehydes became less marked, while the tendency to form nitriles became greater. This is also manifested by the fact that the ratio of moles of hypobromite per mole of amino acid approaches 2.00 more nearly as the carbon chain increases and less of the nitrogen is liberated as gas.

Sarcosine.—This substance reacts with the hypobromite reagent, its nitrogen being at least partially split off in the form of methylamine. The methylamine was isolated and identified by its reaction with 2,4-dinitrochlorobenzene, and also by the preparation of its hydrochloride compound. Some free nitrogen is also produced, which probably comes from the methylamine, and carbon dioxide is liberated in the reaction with hypobromite.

Creatine.—Creatinine is practically unaffected by the reagent. Creatine, however, is acted upon by hypobromite splitting off two-thirds of the nitrogen. This is probably not a simple reaction, and, from the available data, it seems probable that the primary reaction is the liberation of the two free nitrogen atoms of the guanidine group. The extent of the decarboxylation of the remaining free carboxyl group and the resultant oxidation of the resulting sarcosine depends upon the reaction, being favored by increased alkalinity. From the resulting mixture in buffered solution it should be possible to isolate either the resulting N-carboxy-N-methylglycine, or sarcosine.

Glutamic Acid.—This is acted upon by hypobromite with the formation of aldehydopropionic acid and cyanopropionic acid. The acid is extracted from the bromine-free reaction mixture with ether. Upon evaporation the acid crystallizes and is purified by washing with chloroform. The melting point is somewhat low, 47–48° (uncorr.). It can be hydrolyzed very easily to succinic acid, and the succinic acid extracted in fairly pure state from the evaporated acid reaction mixture with ethyl acetate. Upon recrystallization, the yield of pure succinic acid, m. p. 185°, has been found to be nearly quantitative.

Aspartic Acid.—This substance apparently does not react in a similar manner, as may be seen from Table I. This reaction, which we have not thoroughly investigated, is probably much more complex.

Miscellaneous Substances.—Dakin has shown that histidine probably yields cyanomethylglyoxaline, but his findings have not been confirmed. We have not had an opportunity of testing this hypothesis in our studies of histidine and arginine.

The amines, ethylamine and propylamine, are attacked very slowly with liberation of small quantities of nitrogen, as was shown by Dehn and others.

Glycolic and lactic acids are acted upon very slightly by sodium hypobromite, thus ruling out the possibility that they are intermediates in the formation of aldehydes. This is also true of the oxidation of these substances by silver oxide, as was shown by Herbst and Clarke.

Semicarbazide is acted upon incompletely, the reaction being similar to that upon urea. Varying quantities of nitrogen are liberated by the hypobromite, depending upon the reaction time, more being liberated upon standing. In twenty minutes up to 74% of the nitrogen may be liberated.

Guanidine carbonate is completely broken down whereby 97% of its nitrogen is liberated as gas.

Phenyl urea is attacked by hypobromite, but only a small quantity of the nitrogen is liberated as gas. It is mostly converted to aniline, as is also true for N-phenylglycine.

The alkalinity has a great influence on this reaction. Wright[17] recognizes that the reaction of the medium has an effect on the chlorination of substances by hypochlorite, stating that the acid reaction favors chlorination, while the alkaline reaction promotes oxidation of substances by hypochlorite. We observed that the greater the alkalinity, the greater the tendency toward the production of aldehydes at the expense of the nitriles. We studied this especially with the amino acids alanine, amino-*n*-caproic acid, and amino-*n*-caprylic acid. Increasing the alkalinity in each case resulted in more nitrogen being given off as gas. We also measured the nitrile which we were able to isolate. As can be seen from the data in Table III the nearer the reaction of the solution is to neutrality the greater the yield of nitrile. In order to keep the solution neutral it was necessary to add base from time to time, since the carbon dioxide set free during the reaction tends to liberate bromine as it turns the solution acid.

TABLE III

Amino acid	Alkalinity	% Aldehyde	% Nitrile
Alanine	0.5 *N*	44	
	0.1 *N*	34	
	Neutral ⟶ slight alk.	20	
Amino-*n*-caproic acid	2.0 *N*		20
	0.1 *N*		40
	Neutral ⟶ slight alk.		80
Amino-*n*-caprylic acid	2.0 *N*		20
	0.5 *N*		44
	Neutral ⟶ slight alk.		84

The temperature at which the reaction takes place has an influence on this reaction. Although this phase has not been thoroughly investigated, the reaction at higher temperature is apparently in the same direction as under the influence of greater alkalinity. That is, the number of moles of hypobromite per mole of amino acid is increased, with the liberation of more nitrogen, more of the amino acid being converted to aldehyde. The tendency to react with more hypobromite at higher temperatures is especially obvious with substances which do not simply form nitrile or

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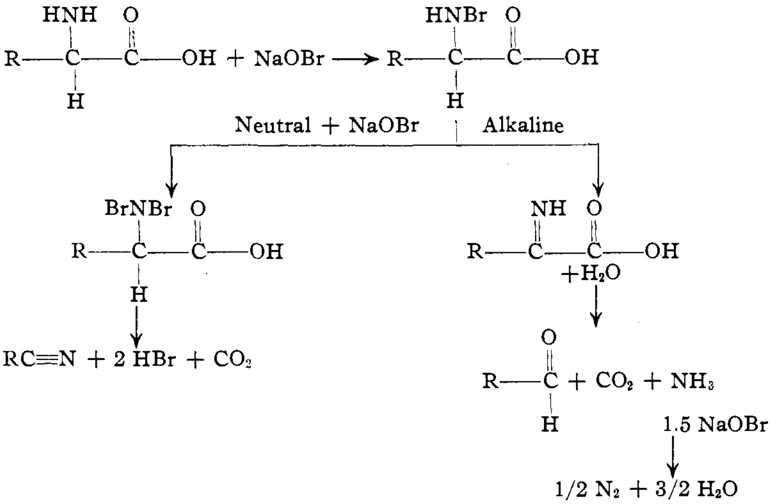
[17] N. Wright, *Biochem. J.*, **20**, 524 (1926).

aldehyde, but with substances, such as uric acid, in which the oxidation products are of a more complex nature.

From the data here presented and the work of previous workers we suggest the following scheme for the oxidation of amino acids by sodium hypobromite. This embodies Langheld's, Dakin's and Goldschmidt's results, with certain modifications based on our own experiments.



The first step of the reaction is bromination of the amino group. In alkaline solution there is a greater tendency for this group to split off hydrogen bromide with the probable formation of an imine, which hydrolyzes to the aldehyde, ammonia and carbon dioxide. The formed ammonia reacts further with 1.5 moles of hypobromite reagent. In less alkaline solution there is a greater tendency for the formation of the dibromo substitution product and subsequent nitrile formation.

Summary

The amino acids react with sodium hypobromite to form aldehydes or nitriles with one carbon atom less than the original amino acid, ammonia and carbon dioxide. The extent of the aldehyde or nitrile production depends upon the alkalinity, and the greater the alkalinity the greater the tendency for the production of aldehyde at the expense of nitrile. Furthermore the longer the carbon chain of the amino acid the greater is the amount of nitrile formed. A quantitative study of the amounts of nitrogen and of carbon dioxide liberated as well as of the amounts of sodium hypobromite reacting with the various amino acids and some derivatives has been made under varying experimental conditions.

OMAHA, NEBRASKA RECEIVED MARCH 16, 1936

[CONTRIBUTION FROM UNIVERSAL OIL PRODUCTS COMPANY RESEARCH AND DEVELOPMENT LABORATORIES]

Alkylation of Paraffins with Olefins in the Presence of Aluminum Chloride

BY V. N. IPATIEFF, ARISTID V. GROSSE, HERMAN PINES AND V. I. KOMAREWSKY

As previously stated the catalytic alkylation of paraffins[1] consists in the direct addition of an olefin to a paraffin, for instance

$$\underset{\text{Hexane}}{C_6H_{14}} + \underset{\text{Ethylene}}{C_2H_4} \xrightarrow{\text{Catalyst}} \underset{\text{Octanes}}{C_8H_{18}} \xrightarrow{\text{Catalyst}} \underset{\text{Decanes}}{C_{10}H_{22}}, \text{ etc.}$$

Both normal and *i*-paraffins can be alkylated in the presence of aluminum chloride, in contrast to boron fluoride.[1] Paraffins from butane to dodecane have been used in our experiments and the reaction is general for paraffins with the possible exception of methane and ethane, which, up to the present, have not been alkylated. Olefins that can alkylate in the presence of aluminum chloride are ethylene, propylene and butylenes; higher molecular weight olefins have not been used yet.

In the presence of aluminum chloride, the main reaction of alkylation is complicated by side reactions. The elucidation of these side reactions was important for the understanding of general hydrocarbon reactions involving aluminum chloride (especially cracking) and was accomplished step by step, investigating the action of aluminum chloride on individual paraffins and olefins, as described in separate articles.[1,2,3]

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(1) V. N. Ipatieff and A. V. Grosse, THIS JOURNAL, **57**, 1616 (1935).

(2) V. N. Ipatieff and A. V. Grosse, *Ind. Eng. Chem.*, **28**, 461 (1936).

(3) V. N. Ipatieff and A. V. Grosse, THIS JOURNAL, **58**, 915 (1936).