

TABLE IV

GAS IMPERFECTION CORRECTIONS TO THE VAPOR HEAT CAPACITIES OF *n*-OCTANE AND 2,3,4-TRIMETHYLPENTANE

	C_p , cal./deg. mole		$C_p^1 - C_p^0$, cal./deg. mole	Berthelot
	760 mm.	361 mm.		
130° <i>n</i> -Octane	58.53	58.06	0.89	0.57
2,3,4-Trimethylpentane	60.19	59.58	1.16	.51
		Av.	1.0	
190° <i>n</i> -Octane	65.15	64.86	0.55	.38
2,3,4-Trimethylpentane	66.52	66.20	0.61	.34
		Av.	0.6	
250° <i>n</i> -Octane	70.55	70.57	-0.04	.26
2,3,4-Trimethylpentane	72.50	72.41	0.17	.23
		Av.	0.1	

From the average gas imperfection corrections, the heat capacities in the ideal gas state are calculated from the experimental values and are given together with the experimental values in Table II.

The heat capacities of all the branched chain compounds studied here are higher than those of the normal compound. The differences between the heat capacity of the branched isomers and that of the normal compound are seen to be less than 3%.

TABLE V

COMPARISON OF THE CALCULATED AND OBSERVED IDEAL GAS STATE HEAT CAPACITIES IN CAL./DEG. MOLE OF *n*-HEXANE, *n*-HEPTANE AND *n*-OCTANE

	Calcd.		Differ- ence	Increment	
	Calcd.	Obsd.		Calcd.	Obsd.
403.2°K. <i>n</i> -Hexane	44.30	43.67	0.63	7.20	6.99
<i>n</i> -Heptane	51.50	50.66	.84	7.20	7.4
<i>n</i> -Octane	58.7	58.1	.6		
473.2°K. <i>n</i> -Hexane	50.25	49.84	.40	8.13	7.81
<i>n</i> -Heptane	58.38	57.65	.73	8.13	8.3
<i>n</i> -Octane	66.51	66.0	.5		

Comparison with Other Data.—The vapor heat capacities of *n*-hexane⁵ and *n*-heptane⁶ have recently been accurately measured and a comparison of these values with the values for *n*-octane obtained here is of interest. As a basis for the comparison, the heat capacities calculated by Pitzer⁷ and published by the A. P. I. Research Project 44⁸ are also included in Table V.

The calculation of the thermodynamic functions requires, essentially, the assumption that the functions for a given *n*-paraffin can be obtained from those of the *n*-paraffin of one less carbon atom by the addition of an appropriate, and calculable, increment. Furthermore, it is required that this increment be independent of chain length for *n*-paraffins of more than four carbon atoms.

Although the observed heat capacities of Table V are not known to sufficient accuracy to test the constancy of the CH₂ heat capacity increment, they do confirm the general correctness of the calculated value of this quantity. The calculated heat capacities, however, are all of the order of 1% higher than the experimental values.

Acknowledgments.—The author wishes to express his appreciation to Dr. Wm. Weltner for his work on the construction of the gas calorimeter and to Professors K. S. Pitzer and Wm. D. Gwinn for their help and advice with the experimental work and preparation of the manuscript.

(5) G. Waddington and D. R. Douslin, *THIS JOURNAL*, **69**, 2275 (1947).

(6) G. Waddington, S. S. Todd and H. M. Huffman, *ibid.*, **69**, 22 (1947).

(7) K. S. Pitzer, *J. Chem. Phys.*, **8**, 711 (1940); *Ind. Eng. Chem.*, **36**, 829 (1944).

(8) Selected Values of Properties of Hydrocarbons, Circular of the National Bureau of Standards C 461, U. S. Government Printing Office, Washington, D. C., 1947.

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[CONTRIBUTION FROM THE INSTITUTE OF POLYMER RESEARCH, POLYTECHNIC INSTITUTE OF BROOKLYN]

Photochemistry of Proteins. XII. A Contribution to the Photochemistry of Amino Acids¹

BY INES MANDL AND A. D. McLAREN²

In the course of study of the comparative rates of inactivation of enzymes and viruses under oxygen and nitrogen it seemed worthwhile to observe the behavior of related substances under similar conditions. More ammonia is liberated from acidic DL-alanine solutions under oxygen than under nitrogen by radiation from an Hanovia quartz mercury vapor lamp. Under nitrogen, the products ammonia, lactic acid and pyruvic acid were formed but no acetaldehyde was found. The product L-lactic acid was obtained from L-alanine in 0.10 *N* hydrochloric acid. After irradiation of L-aspartic acid, L-malic acid was found. The production of optically active hydroxy acids from optically active amino acids is a new observation and requires a new scheme for the photolysis of amino acids.

Introduction

The photochemistry of amino acids has been recently reviewed.³ As far back as 1908 photolysis to ammonia, carbon dioxide and aldehydes in the presence of air was investigated by Neuberg.⁴

(1) From the Ph.D. Thesis of Ines Mandl, Polytechnic Institute of Brooklyn, April, 1949.

(2) For previous papers see A. D. McLaren and P. Finkelstein, *THIS JOURNAL*, **72**, 5423 (1950), and G. Oster and A. D. McLaren, *J. Gen. Physiol.*, **33**, 215 (1950), Paper XI.

(3) A. D. McLaren, "Advances in Enzymology," **9**, 75 (1949).

(4) C. Neuberg, *Biochem. Z.*, **13**, 305 (1908); **29**, 279 (1910).

Henri, Weizmann and Hirshberg⁵ and Weizmann, Bergmann and Hirshberg^{6,7} have found ammonia, carbon monoxide and hydroxy acids among the photolysis products of alanine and aspartic acid irradiated under nitrogen. Their quantum yield data have been recalculated.⁸ In view of the im-

(5) V. Henri, C. Weizmann and Y. Hirshberg, *Compt. rend.*, **198**, 168 (1934); **199**, 1205 (1935).

(6) C. Weizmann, E. Bergmann and Y. Hirshberg, *THIS JOURNAL*, **58**, 1675 (1936).

(7) C. Weizmann, Y. Hirshberg and E. Bergmann, *ibid.*, **60**, 1799 (1938).

portance of reactions undergone by amino acids to the wider field of photochemistry of proteins and the possibility envisaged of using quantum yields under different conditions as a tool in the determination of protein structure, it seemed worthwhile to compare the relative rates of photolysis of alanine under oxygen and nitrogen and to clear up some of the doubtful points regarding the products and mechanism of deamination in the absence of air.

Experimental

A Comparison of the Rate of Photolysis of DL-Alanine-HCl in the Presence and Absence of Oxygen.—Equipment for irradiation of solutions in quartz vessels while being stirred by a stream of oxygen or nitrogen is described elsewhere.³ The solutions were kept cool during the irradiation and all air was carefully excluded in the nitrogen experiments.³

In all cases 0.01 *N* solutions of DL-alanine in 0.01 *N* hydrochloric acid were irradiated by a Hanovia mercury vapor Utility Lamp, without filter, at a distance of 5 cm. from the face of the quartz irradiation cell. This source has appreciable amounts of radiation at 2260 and 2399 Å., *i.e.*, radiant energy corresponding to the long wave length threshold found for the closely related compound, glycine (2265 Å.).⁵ The gas, oxygen or nitrogen, was bubbled through continuously. At the end of the irradiation period the entire solution was removed from the cell and aliquots of 1 or 2 ml. were withdrawn for analysis. In all experiments the extent of photolysis was measured by first aeration and then nesslerization of the ammonia split off.

Under oxygen, for a total time of irradiation of alanine of 1 hour, between 28 and 30% of the total amino nitrogen was found as ammonia in 6 consecutive experiments. Under nitrogen 16–18% NH₃ was split off in each of 5 experiments. After correcting for a blank of 2% (average of 3 results) the amount of photolysis taking place under oxygen-free conditions is seen to be approximately half that under oxygen or more exactly 27 and 15%, respectively, a ratio of 9:5.

Isolation of the Products of Photolysis in an Oxygen-Free Atmosphere.—In order to isolate and identify products of photolysis, a quartz flask of 350-ml. capacity equipped with an inlet and outlet tube for nitrogen was employed. Solutions of 3 g. of alanine in 333 cc. of 0.10 *N* HCl, *i.e.*, 0.10 *N* solutions were irradiated for 4 hours. An ammonia determination of an aliquot showed decomposition of 20%. The separation procedure adopted⁹ involved direct precipitation of the carbonyl compound hydrazones by adding 1.5 g. of 2,4-dinitrophenylhydrazine in 90 cc. of 2 *N* HCl to the irradiated solution.

Some of the hydrazone was suspended in sodium carbonate solution. It dissolved completely, showing that only pyruvic acid but no acetaldehyde had originally been present. This was also confirmed by a negative sodium nitroprusside-piperidine reaction on an aliquot. The dissolved hydrazone was reprecipitated with acetic acid and the entire amount was then dissolved in sodium carbonate until a dark color was obtained, the solution serving as its own indicator. After filtration, reprecipitation with 50% acetic acid, standing in the ice-box, and filtering, the hydrazone was dried in a desiccator and weighed as pyruvic acid hydrazone. The yield of 0.5 g. (5%) corresponds to one-fourth of the ammonia found; melting point 215–216.5° (reported in the literature⁹ as 216°); found *N*, 20.58; calcd., *N*, 20.7.

The filtrate from the hydrazone precipitation was neutralized with sodium hydroxide and then cooled in the ice-box, filtered, decolorized with charcoal and refiltered. The clear-water liquid obtained was evaporated almost to dryness. The residue was adsorbed on anhydrous sodium sulfate and mixed in a mortar with sirupy phosphoric acid until the fine powder was acid to congo paper.¹⁰ The powder was dried in a desiccator overnight and then extracted in an ordinary Soxhlet apparatus with ether. When extraction was complete the ether was evaporated off; the residue was dissolved in water and zinc oxide was added. After heating

on the water-bath the excess zinc oxide was filtered off; the filtrate was evaporated almost to dryness and filtered. The zinc lactate obtained was washed with methanol, recrystallized from hot water and dried. Drying in the oven at 108° gave the anhydrous salt which was treated with nitric acid and heated to yield zinc oxide. The ratio of ZnO:Zn lactate found (0.071:0.206) agreed very well with theory (81:243), proving that the zinc salt really was the lactate. The absolute amount of lactic acid obtained, however, corresponded to slightly less than half the ammonia.

Stereochemical Investigations.—(1) Investigating the photolysis of L-aspartic acid under nitrogen, Weizmann, *et al.*,⁶ report almost complete racemization to DL-malic acid. Unless the mechanism proceeds via pyruvic acid, which is not believed by the authors to be the case, such racemization points strongly to the presence of ions or free radicals.¹¹ The mechanism proposed by them is based on this reasoning, so that it appeared of considerable interest to test to what extent such racemization is an obligatory step of amino acid photolysis.

The investigation was therefore extended to L(+)-alanine: 3 g. of L-alanine was irradiated for 4 hours and the products were isolated exactly as described for DL-alanine. No significant differences in either products or yield were observed. The zinc lactate obtained (0.4 g. corresponding to 0.25 g. of lactic acid) indicates 8.3% decomposition of alanine. After recrystallization from hot water an aliquot was analyzed in a 2-dm. microtube with a Ventzke saccharimeter; 0.144 g. in 5 ml. water corresponded to 0.25 g. of the water-free salt per 10 ml. An optical rotation of $[\alpha]_D -8.5^\circ$ was found. The theoretical $[\alpha]_D$ for L(-) zinc lactate is -8° to -8.4° .¹² Contrary to the findings of Weizmann, *et al.*,⁶ optimum optical activity has thus been found under these conditions. It is not possible that differences in solubility led to a complete separation of the optically active salt, since the racemate is less soluble and would have crystallized out first.

(2) In view of the unexpectedness of our finding, Weizmann's work with L-aspartic acid was repeated to decide whether the reaction was a general one. It was thought conceivable that the stereochemical behavior of aspartic acid might be different from that of other amino acids.¹³ Solutions of L-aspartic acid, 0.10 *N* in 0.10 *N* HCl were irradiated in an atmosphere of nitrogen. After 8 hours, samples were withdrawn and the optical activity was determined by an adaptation of a method developed by Walden.¹⁴ The small rotation of active zinc malate may have led Weizmann to underestimate the amount of activity. Walden's method has been designed to avoid such an error. It is based on the formation of uranyl complexes which increase the rotation several hundred fold and thus greatly facilitates analyses. Roberts¹⁵ in a recent study has shown that under otherwise identical conditions the rotation of aspartic acid in the presence of uranyl salts is not only much smaller but also in the opposite direction, so that isolation of malic acid is not necessary. In a typical experiment 1.5 ml. of uranyl nitrate solution (10 g./10 ml.) was added to 15 ml. of irradiated solution, corresponding to 0.2 g. of aspartic acid, and the volume was made up to 25 ml. Pure L-malic acid treated in this way has been reported by Walden¹⁴ to give a maximum rotation of $[\alpha]_D -405^\circ$ and by Holmberg¹⁶ to give a maximum rotation of $[\alpha]_D -436^\circ$. 0.10 *N* L-aspartic acid (non-irradiated) in 0.10 *N* HCl in the presence of uranyl nitrate showed a value of $+0.4^\circ$ in good accord with the data of Roberts.¹⁵

The irradiated solution showed $\alpha = -0.9^\circ$ or $[\alpha]_D (-1.3 \times 100)/(0.8 \times 2) = -81^\circ$. Taking into account that approximately 20% of the original aspartic acid has been decomposed ($100 - (100 \times 81)/436$) the blank should be somewhat less positive and the corrected value for $\alpha = -1.2^\circ$

(11) M. Polanyi and N. Meer, *Z. physik. Chem.*, **19B**, 164 (1932); E. Bergmann, M. Polanyi and A. Szabo, *ibid.*, **20B**, 161 (1933); *Trans. Faraday Soc.*, **32**, 843 (1936).

(12) C. Neuberg and M. Kobel, *Ann. Acad. Scient. Fenni*, **A29**, No. 8 (1927).

(13) Pringsheim (*Z. physiol. Chem.*, **65**, 89 (1910)) reports racemization of the related asparagine by simple boiling and yeast (F. Ehrlich, *Biochem. Z.*, **63**, 390 (1914)) symmetrically attacks aspartic acid unlike most other amino acids.

(14) P. Walden, *Ber.*, **30**, 2889 (1897).

(15) I. S. Roberts, *Exp. Med. and Surg.*, **5**, 251 (1947).

(16) B. Holmberg, *J. prakt. Chem.*, [2] **88**, 564 (1913).

(8) E. Simon and C. Neuberg, *Biochem. Z.*, **232**, 479 (1931).

(9) C. Neuberg and M. Kobel, *ibid.*, **210**, 494 (1929).

(10) C. Neuberg and L. Landstein, *Ber. Berl. physiol. Ges.*, **114** (1922).

or $[\alpha]_D -75^\circ$. To compute the percentage L-malic acid the maximum value of Holmberg¹⁵ is considered to correspond to 100% L-malic acid. By simple proportionality ($75/436 = x/100 = 17$) it is found that 17% of the original L-aspartic acid is decomposed to L-malic acid. The more sensitive methods of polarization thus established a substantial retention of optical activity also in the case of L-aspartic acid.

Results and Discussion

Under nitrogen pyruvic and lactic acids were obtained from alanine, but no acetaldehyde. The occurrence of side reactions which may include the dismutation assumed by Wiegand^{17a} and condensations between pyruvic acid and ammonia or ammonium pyruvate to acetylaminopropionic acid discovered by De Jong^{17b} might account for the lack of correspondence between the amount of ammonia split off and the sum of the other products isolated. Photolysis of the keto acid could further diminish the yield.

The formation of lactic acid on irradiation under nitrogen agrees with the findings of Weizmann, Bergmann and Hirshberg.⁶ Checking was nonetheless desirable because it was felt that the above authors placed too much reliance on a color test which is not sufficiently specific. The Eegriwe color reaction¹⁸ for glycolic acid, which Weizmann, *et al.*, found to be applicable to lactic acid as well, exists in two modifications, both described by Eegriwe himself. Weizmann makes use of the violet-red shade observable on heating the hydroxy acid with sulfuric acid and 2,7-dihydroxynaphthalene, while a fluorescence appears when the reagent is allowed to stand in sulfuric acid for some hours or heated on the water-bath. In the latter case 2,7-dihydroxynaphthalene-3,6-disulfonic acid forms¹⁹ and is the actual reagent. As in all similar color reactions the mechanism involves oxidative degradation of the hydroxyacid to the lower aldehyde and aldehydes must of necessity react in the same way. No distinction is thus possible by Eegriwe's reaction between acetaldehyde, pyruvic acid and lactic acid or other related compounds that undergo the same reaction.

Weizmann, *et al.*,⁶ also isolated zinc lactate, but unfortunately the presence of pyruvic acid was not excluded. Both zinc pyruvate and zinc lactate crystallize with three molecules of water, so that the salts differ by only 2 hydrogen atoms, which would hardly show up in the analyses.

The formation of pyruvic acid in the oxygen-free nitrogen atmosphere cannot be unequivocally accounted for. Conceivably lactic acid might be converted to pyruvic acid by nascent oxygen. References to the formation and decomposition of H_2O_2 under ultraviolet irradiation abound in the older literature.^{20,21} Piffault²² points out that at

(17) (a) C. Wiegand, *Pflüger's Arch. ges. Physiol.*, **223**, 378 (1929); (b) A. W. K. De Jong, *Rec. trav. chim.*, **19**, 259 (1900); **23**, 131, 147 (1904); see also Erlenmeyer and J. Kunlin, *Ann.*, **307**, 146 (1899).

(18) E. Eegriwe, *Z. anal. Chem.*, **89**, 123 (1932).

(19) Beilstein's "Handbuch d. org. Chem.," IV. Aufl., XI, Julius Springer, Berlin, 1918, p. 309.

(20) H. Thele, *Ber.*, **40**, 4914 (1907); M. Kernbaum, *Compt. rend.*, **149**, 273 (1909); A. Tian, *ibid.*, **152**, 1012 (1911). See also H. Aughlon, *ibid.*, **152**, 398 (1911); J. P. Becker and P. Szendró, *Pflüger's Arch.*, **228**, 755 (1931).

(21) More recently, J. H. Clark (in B. M. Duggar, "Biological Effects of Radiation," McGraw-Hill Book Co., Inc., New York, N. Y., 1936, p. 303) maintains that such a mechanism can be effective at very

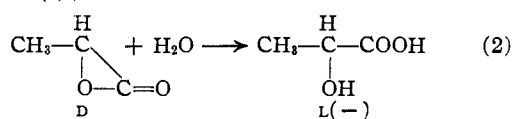
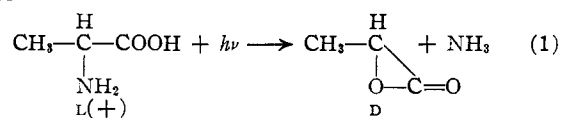
short wave lengths ($\sim 2000-1800 \text{ \AA}$.) irradiation of aqueous solutions under nitrogen leads to the formation of oxygen according to equation 1



He suggests that some of the alanine reacts with nascent oxygen to become pyruvic acid, part of which decomposes to CO_2 and aldehyde.

Decomposition of the aldehyde to methane and CO as well as combination of the CO_2 with H_2 would account for the gases rich in CO_2 found by Henri, Weizmann and Hirshberg.⁵ Hydrogenation of the pyruvic acid to the corresponding hydroxy acid could not, however, give optically active lactic acid. The ionic mechanism postulated by Weizmann, *et al.*,⁶ equally prohibits the formation of the optically active products found by us and indeed racemization was the basic assumption for their scheme. Our stereochemical results prove that the optical configuration of both L-alanine and L-aspartic acid was retained on photo-deamination under the conditions applied.

To account for all the facts observed in this study it is believed that a common intermediate which may give rise to all the products formed and allows retention of optical configuration must be postulated. In analogy to the first order reaction of α -bromopropionic acid to give lactic acid of the same configuration²⁴ we propose an α -lactone as the most likely intermediate. The steps would be



A simple nucleophilic replacement would yield inverted D-lactic acid^{24,25} but a 2-step mechanism can account for apparent retention of configuration. Both steps cause inversion. First an intermediary α -lactone of the opposite configuration is formed. Then hydrolysis of this lactone re-establishes the original configuration. Other reactions of the α -lactone could give rise to carbon monoxide, methane, pyruvic acid²⁶ and acetaldehyde (in the presence of air), four of the known products of the photochemical reaction. Under nitrogen step (3a) is probably favored over (3b)

short wave lengths and long irradiation times only. Although like A. S. Allen, R. E. Steiger, M. A. Magill and R. G. Franklin (*Biochem. J.*, **31**, 195 (1937)) we could not confirm the presence of H_2O_2 in irradiated amino acid solutions, this negative result might be due to the extremely rapid rate of photodecomposition or reaction with other products.

(22) C. Piffault, *Compt. rend. soc. biol.*, **136**, 713 (1944).

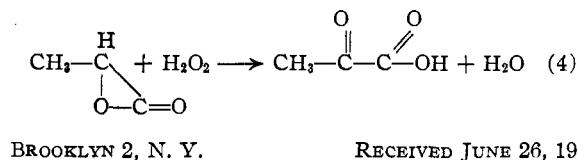
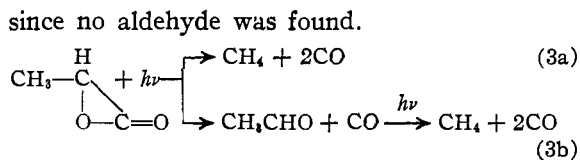
(23) D. Berthelot and H. Gaudechon (*Compt. rend.*, **152**, 262 (1911)) found CO , CO_2 , CH_4 and H_2 among the photolysis products of lactic acid and G. R. Burns (*THIS JOURNAL*, **51**, 3165 (1929)) also reports CO , CO_2 and saturated and unsaturated hydrocarbons.

(24) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, pp. 176-178.

(25) J. Kenyon and H. Phillips, *Trans. Faraday Soc.*, **26**, 451 (1930).

(26) A. Benrath (*Ann.*, **382**, 222 (1911)) and also D. Ganassini (*Giorn. Farm. Chim.*, **61**, 439 (1913)) under different conditions suggested the formation of pyruvic acid from lactic acid irradiated in the presence of oxygen, but conclusive proof has never been advanced.

since no aldehyde was found.



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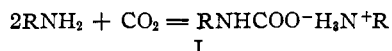
[CONTRIBUTION FROM THE WEIZMANN INSTITUTE OF SCIENCE]

The Chemical Structure of Some Diamine Carbamates¹

BY EPHRAIM KATCHALSKI, CHAJA BERLINER-KLIBANSKI AND ARIE BERGER

One mole of carbon dioxide reacts with one mole of ethylenediamine or hexamethylenediamine to yield the corresponding carbamate. These diamine carbamates give with diazomethane the *N,N'*-dicarbomethoxydiamines (V), the *N*-monocarbomethoxydiamines (IV) and the free diamines. This result is interpreted by the assumption that the carbamate of an aliphatic diamine consists of a mixture of an intramolecular salt of the *N*-monocarboxydiamine (II) and of an "intermolecular" salt of the diamine-*N,N'*-dicarboxylic acid (III) with the free diamine.

Whilst the formula I of the products resulting from the action of carbon dioxide on ammonia and primary aliphatic monoamines has been well established^{1a,3}



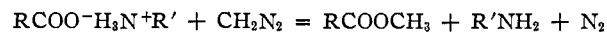
little seems to be known on the structure of compounds derived from carbon dioxide and aliphatic diamines. The present investigation deals with the constitution of diamine carbamates, ethylenediamine and hexamethylenediamine carbamates serving as model compounds.

Crystalline products formed readily when dry carbon dioxide was passed through a cold alcoholic solution of the diamines. The products are very soluble in water, giving strongly alkaline solutions. By measuring the amount of combined carbon dioxide (Van Slyke²) liberated upon addition of mineral acids,³ and by determination of the amino groups regenerated (Linderstrøm-Lang⁴), it could be shown that under the experimental conditions used, one mole of carbon dioxide reacted with one mole of the diamine.

In accordance with formula I, it can be assumed that in these *diamine carbamates*, the carbon dioxide is linked by covalent bonds to half of the original amino groups in the form of an *N*-carboxylic acid, which is neutralized by the other half of the amino-groups as ammonium ions. Indeed, formula (II, $n = 2$), which satisfied the analytical data, has been ascribed recently⁵ to the compound between ethylenediamine and carbon dioxide described in 1900.⁶ Obviously, however, (II, $n = 2$)

is not the only structure in accord with the analytical findings. Also in the "bimolecular" diammonium salt of an *N,N'*-dicarboxydiamine (III, $n = 2$), half of the original amino groups appear in the form of *N*-carboxylic acids, while the other half neutralizes these groups; on treatment with mineral acids also (III) would give equimolar amounts of diamine and carbon dioxide. Obviously, the analysis would fit also any mixture of (II) and (III).⁷ If one can assume that both amino-groups of a symmetric diamine molecule have an equal chance of being converted into NHCOO^- or NH_3^+ , the carbamate would contain equal weights of (II) and (III); if it be assumed, however, that the chance of the amino groups to form NHCOO^- and NH_3^+ is different from the above but in accordance with the analytical data, other weight ratios of (II) to (III) can be calculated.

In order to investigate the presence and relative ratio of (II) and (III) in the carbamates of ethylenediamine and hexamethylenediamine, use was made of the reaction between diazomethane and ammonium salts of carboxylic acids⁸



which would convert (II) into the monocarbomethoxy derivative (IV), and (III) into an equimolar mixture of the dicarbomethoxy derivative (V) and the original free diamine.

In fact, both for ($n = 2$) and ($n = 6$), the compounds (IV), (V) and the free diamine were formed in the reaction between an ethereal diazomethane solution and the carbamates. For comparison, (V, $n = 2$) was prepared from ethylenediamine and methyl chloroformate, (IV, $n = 2$) by amination of *N*-carbomethoxybromoethylamine according to Délépine,⁹ and (V, $n = 6$) from hexamethylenediamine and methyl chloroformate.

Further evidence was obtained for the presence of (III) in the diamine carbamates by the isolation of the calcium salts of *N,N'*-dicarboxyethylene-

(1) This paper is part of the dissertation presented by Chaja Berliner-Klibanski to the Hebrew University, Jerusalem, in partial fulfillment of the degree of Ph.D.

(1a) Cf. V. Meyer and P. Jacobson, "Lehrbuch der organischen Chemie," Vol. I, Part 2, Leipzig, 1913, p. 1370; F. Fichter and B. Becker, *Ber.*, **44**, 3481 (1911); C. Faurholt, *J. chim. phys.*, **22**, 1 (1925).

(2) D. D. Van Slyke and J. M. Neill, *J. Biol. Chem.*, **61**, 523 (1924); J. P. Peters and D. D. Van Slyke, "Quantitative Clinical Chemistry," Vol. II, 1932, p. 283.

(3) M. Frankel and E. Katchalski, *THIS JOURNAL*, **65**, 1670 (1943).

(4) K. Linderstrøm-Lang, *Z. physiol. Chem.*, **173**, 32 (1928).

(5) J. F. Mulvaney and R. L. Evans, *Ind. Eng. Chem.*, **40**, 393 (1948).

(6) E. Schering, German Patent 123,138 (1900); *Chem. Zentr.*, **72**, II, 519 (1901).

(7) W. Siefken, *Ann.*, **562**, 6 (1948), suggested the presence of structures (II) and (III) in order to explain the reaction between the carbamates of aliphatic diamines and phosgene.

(8) M. Frankel and E. Katchalski, *THIS JOURNAL*, **66**, 763 (1944).

(9) M. Délépine, *Compt. rend.*, **120**, 501 (1895); **124**, 292 (1897); *Bull. soc. chim.*, [3] **17**, 290 (1897); [4] **31**, 108 (1922); cf. A. Galat and G. Elion, *THIS JOURNAL*, **61**, 3585 (1939).