[CONTRIBUTION FROM THE DANIEL SIEFF RESEARCH INSTITUTE]

The Photochemical Deamination of Amino Acids in Water Solution.
Part I

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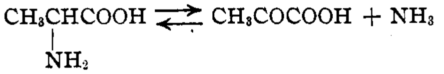
The only method of de-amination of amino acids under physiological conditions is the oxidative de-amination, *e. g.*



$$CH_3\underset{NH_2}{\underset{|}{C}}HCOOH \rightleftarrows CH_3COCOOH + NH_3$$

which has been thoroughly investigated by many authors. The ordinary hydrolysis of amino acids

$$CH_3\underset{NH_2}{\underset{|}{C}}HCOOH + H_2O \qquad CH_3\underset{OH}{\underset{|}{C}}HCOOH + NH_3$$

has never been observed in biological systems and even against treatment with acid or alkali, the amino acids exhibit such a marked resistance that occasionally the view has been expressed that the occurrence of ammonia in the hydrolysis of proteins is not due to hydrolysis of amino, but rather of amide groups. (That this view is not correct has been demonstrated by Melville[1] in the case of glutamine containing peptides.)

Therefore, an observation made by Henri, Weizmann and Hirshberg[2] seemed to be worthy of further study. These authors reported that in aqueous solution glycine is hydrolyzed under the influence of ultraviolet rays according to the above scheme. The experiments have been extended to include alanine, betaine and aspartic acid. In all these cases the photolysis occurred markedly. The kinetics of the reactions could be studied easily, using the conductivity change or the *p*H change accompanying the photolysis, or, in a more accurate way, determining the amount of ammonia formed during the reaction. As a qualitative test showing whether the desired reaction has occurred or not, the color reaction with 2,7-dioxynaphthalene proved useful; it may be mentioned that the reaction is by no means specific for glycolic acid, as was assumed by Eegriwe,[3] but is a group test for α-hydroxy acids.

The attempt to become familiar with the details of the photolytic reaction meets with one main difficulty: one does not know, *a priori*, which is the reactive form of the amino acid molecules. In this connection, the observation of Henri, Weizmann and Hirshberg[4] is noteworthy that the effective radiation in the case of glycine has a wave length of $\lambda < 2265$ Å. Since, according to a recent paper of Henri and Lasareff,[5] methylamine shows an ultraviolet absorption between 2529 and 1986 Å., the most intense bands lying between 2320 and 2100 Å. the conclusion seems justified that the radiation effective in our photolysis is that absorbed by the C–N linkage, in other words, the photochemical primary process involves that linkage. Two arguments appear to show that the photolysis does not occur or, at least, does not occur markedly on the "Zwitter-ions." (1) The speed of the photolysis depends on the *p*H of the medium; at neutral reaction, the reaction velocity has a minimum; it increases both to the acid and to the alkaline side, giving a graph (Fig. 1) very similar to those obtained for the hydrolysis of certain esters[6] or the bromination of certain aliphatic ketones.[7] Since one knows[8] that at the neutral point the relative amount of

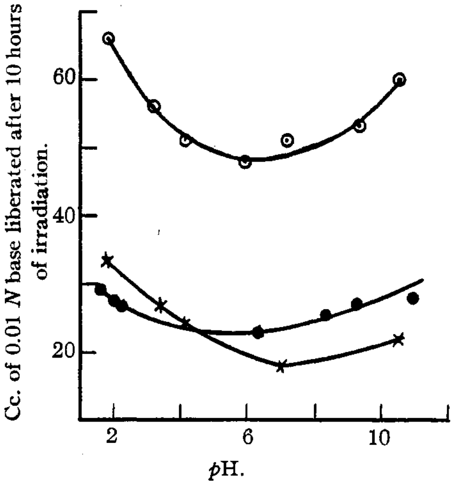


Fig. 1.—Photolysis of alanine 0.05 *N*, ⊙; betaine, 0.05 *N*, ●; aspartic acid, 0.01 *N*, ×.

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[1] Melville, *Biochem. J.*, **29**, 178 (1935).

[2] Henri, Weizmann and Hirshberg, *Compt. rend.*, **198**, 168 (1934).

[3] Eegriwe, *Z. anal. Chem.*, **89**, 121 (1932).

[4] Henri, Weizmann and Hirshberg, *Compt. rend.*, **199**, 1205 (1934).

[5] Henri and Lasareff, *ibid.*, **200**, 829 (1935). Compare also Herzberg and Koelsch, *Z. Elektrochem.* **39**, 572 (1933); Woo and Liu, *J. Chem. Physics*, **3**, 544 (1935); Emeléus and Jolley, *J. Chem. Soc.*, 1612 (1935).

[6] Ingold, *et al.*, *J. Chem. Soc.*, 1039 (1930); 2035 (1931).

[7] Watson and Yates, *ibid.*, 1207 (1932).

[8] Compare Schlenk and Bergmann, "Textbook," p. 450.

molecules occurring in the "Zwitter-ion" form is highest, that form cannot be the carrier of the photolysis reaction. (2) If one compares the photolysis of betaine with that of the unmethylated amino acids we have investigated, one will find that the photolysis of betaine is markedly slower (see *e. g.*, Fig. 1, where a 0.1 *N* solution of betaine is compared with a 0.01 *N* solution of aspartic acid).

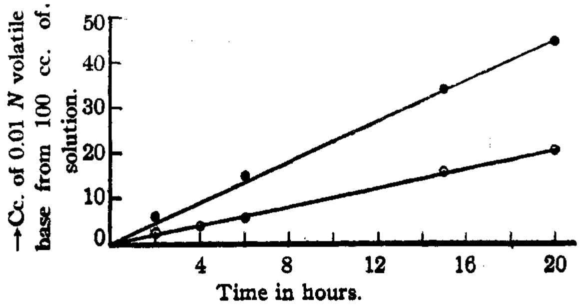

Fig. 2.—Speed of the photolysis of 0.01 *N* betaine (lower curve); 0.01 *N* aspartic acid (upper curve).

We suggest that the normal form of the amino acid molecule is "photo-active"; its C–N bond is split not by water molecules, but by H^+ and OH^-.

The quantum yield of the photolysis has been determined in several experiments by comparison with the photochemical decomposition of monochloroacetic acid. For glycine and alanine the quantum yield has been found to be about equal to unity, which corresponds to the case of, say, monochloroacetic acid.[9] It is surprising that the quantum yield for betaine under the same conditions is only 0.5. It is rather difficult to explain that figure definitely, but it may be due to the fact that in betaine there are two different kinds of C–N bonds absorbing the "active" light, one representing the CH_3N groups, one connecting the nitrogen with the trunk of the acetic acid molecule, and that only the latter one by absorption of light can lead to the desired photolytic process, the other one causing just a dissipation of the absorbed light energy.[9a]

As regards the various amino acids investigated, the details will be given in the experimental part of the paper, but a few remarks may be added here. In the case of alanine, the lactic acid formed has been isolated as zinc salt, in the case of betaine the trimethylamine as the chloroaurate. From aspartic acid, crystalline malic acid has been obtained. A few preliminary experiments were made with *l*-tyrosine, but without success, since the alkaline solution immediately turned yellow, afterward brown; the acid solution behaved similarly after a short period of irradiation. (At the neutral point, tyrosine is known to be insoluble in water.) We thought the color may be due to melanine formation, caused by unavoidable traces of oxygen, but the absorption spectrum of our solution was quite different from the absorption spectrum exhibited by tyrosine after treatment with tyrosinase.[10] Therefore, no conclusion can be drawn regarding the color change of our tyrosine solutions.

The experiments with aspartic acid were made on an optically active (levorotatory) preparation which gave us the opportunity of dealing with the question of the configurational relationship between the starting material and the end-product of the photolysis. The malic acid obtained was nearly completely racemic and only a negligible levorotation could be observed. According to the view of Polanyi and co-workers,[11] racemization can only occur in substitution reactions of the type suggested above when the trunk of the molecule passes a stage where it exists as a positively charged ion or a free radical. Obviously, we cannot decide definitely between these alternatives, but in view of the above conclusion that in this reaction hydrogen and hydroxyl *ions* take part, the former possibility seems more reasonable. The photochemical stretching of the C–N bond leads to the formation of ions RCH^+COOH and NH_2^-, which stabilize by the reactions

$$RCH^+COOH + OH' \longrightarrow RCH(OH)COOH$$
$$NH_2^- + H^+ \longrightarrow NH_3$$

Experimental Part

The **preparations** used were commercial samples which were purified by the usual methods. Betaine was prepared from its hydrochloride according to Husemann and Marmé.[12] The solutions of the amino acids in doubly distilled water were **irradiated** in two ways: (1) the quartz lamp was surrounded by two jackets, the first containing running water, the second the solution; (2) the solution was filled into a quartz cell which was irradiated by the direct light of a horizontal mercury arc (without any light filters) at a certain distance. After definite times, the am-

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(9) L. Farkas and O. H. Wansbrough-Jones, *Z. physik. Chem.*, **18B**, 124 (1932).

(9a) For theoretical discussion of low quantum efficiency, see Thompson and Linnett, *J. Chem. Soc.*, 1453 (1935).

(10) Florence, Enselme and Pozzi, *Bull. soc. chim. biol.*, **17**, 290 (1935). Compare also Lignad, *Strahlentherapie*, **34**, 605 (1921).

(11) Polanyi and Meer, *Z. physik. Chem.*, **19B**, 164 (1932); Bergmann, Polanyi and Szabo, *ibid.*, **20B**, 161 (1933); *Trans. Faraday Soc.*, **32**, 843 (1936); Bergmann and Polanyi, *Naturwiss.*, **21**, 378 (1933); see also Ingold and Hughes, *J. Chem. Soc.*, 244 (1935); Hughes, *ibid.*, 255 (1935).

(12) Husemann and Marmé, *Ann.*, Suppl., **3**, 246 (1864).

monia formed was determined in the usual way; the conductivity and the *p*H were measured, the latter with a glass electrode.

An absolute determination of the **quantum yield** proved tedious, since the reactions are relatively slow. Therefore, the reaction was compared in a rather crude way with the photo-decomposition of monochloroacetic acid for which the quantum yield is equal to unity. A parallel ultraviolet light beam was obtained from the light of an ordinary mercury lamp (Heraeus lamp) through a quartz lens of f = 10 cm. At a constant distance a quartz cell of 10 cm. length and 46 cc. volume was placed in the path of the light beam. The lamp worked constantly at 220 volts and 4 amperes.

We compared the amount of monochloroacetic acid decomposed in a second cell after passing (a) water, (b) the investigated solution. The difference between the two figures gave the quantity of energy absorbed in the photodecomposition of the solution investigated. The following results were obtained:

Glycine	0.93	0.88	0.86	0.85	0.82
Alanine	1.02	.98	.95	1.00	
Betaine	0.46	.49	.52		

In order to demonstrate our procedure in the kinetic experiments, the data obtained in some experiments may be given here.

TABLE I

BETAINE

100 cc. of a 0.1 *N* solution was irradiated with the horizontal mercury lamp (220 v., 9 amp.). The distance between the lamp and the cell was 13 cm.

Time, hours	Conductivity, measured at 30°	*p*H	0.01 *N* trimethylamine, cc.
0	3.24×10^{-5}	6.2	0
2	7.43×10^{-5}	6.4	2.2
4	9.2×10^{-5}	6.62	4.2
6	11.2×10^{-5}	6.9	6.0
8	14.9×10^{-5}	7.1	8.2
10	18.3×10^{-5}	7.25	10.5
15	30.4×10^{-5}	7.55	16.0
20	40.0×10^{-5}	7.8	21.2

A part of the same solution was not irradiated; during the same period no change in conductivity or *p*H occurred.

TABLE II

(2) ALANINE

100 cc. of a 0.1 *N* solution was irradiated as above

Time, hours	Conductivity, measured at 30°	*p*H	NH_3, mg.	NH_3, %
0	3.38×10^{-5}	6.4	0	0
1	7.17×10^{-5}	6.85	0.017	0.20
2	9.14×10^{-5}	7.10	.034	.40
4	19.7×10^{-5}	7.50	.072	.75
6	23.8×10^{-5}	7.75	.10	1.2
15	44.6×10^{-5}	7.95	.27	3.2
100 cc. of a 0.05 *N* solution as above, but with a stronger lamp.				
0	7.33×10^{-6}	5.65	0	0
1	28.6×10^{-6}	6.0	...	...
4	71.1×10^{-6}	6.65	0.61	4.5
6	103.0×10^{-6}	7.25	1.1	8.0
8	200.0	7.65	1.5	11.0
100 cc. of a 0.02 solution as before				
0	7.37×10^{-6}	5.5	0	0
2	24.3×10^{-6}	6.7	0.2	1.49
3	33.2×10^{-6}	6.9	.29	2.12
6	73.6	7.2	.61	4.5
10	148.0×10^{-6}	7.5	1.3	10.0

TABLE III

(3) ASPARTIC ACID

100 cc. of a 0.01 *N* solution was irradiated as above; distance 10 cm.

Time, hours	*p*H	0.01 *N* NH_3, cc.
0	3.2	0
2	3.5	5.5
6	3.9	15.2
9	4.1	19.5
15	4.5	33.5
20	5.1	45.0

TABLE IV

Change of absorption during irradiation of alanine

(a) 0.02 *N* SOLUTION

λ, Å.	ε before irradiation	ε after 10 hours of irradiation
2150	17.5	17.5
2225	8.74	13.5
2240	6.72	8.74
2264	4.40	5.85
2280	3.36	4.40
(b) 0.05 *N* SOLUTION		ε after 8 hours of irradiation
2144	28.08	28.08
2195	10.80	21.6
2240	6.92	10.8
2260	5.40	6.92
2328	1.58	2.81
2350	...	2.16
2360	...	2.05
2370	...	1.58

Isolation of the Irradiation Products.—The solution obtained by photolysis of *alanine* was concentrated, acidified with sulfuric acid, then extracted several times with ether. The ether was distilled off and the residue taken up with water and heated with zinc carbonate on the water-bath. The excess of the carbonate was filtered off and the solution evaporated in a desiccator. The residue was identified by micro-analysis. Calcd. for $C_6H_{10}O_6Zn{\cdot}3H_2O$: C, 24.2; H, 5.4; Zn, 22.0. Found: C, 24.7; H, 5.4; Zn, 22.9.

The trimethylamine formed by irradiation of a *betaine* solution was distilled off—after addition of a few drops of alkali—into dilute hydrochloric acid. The acid solution was concentrated on the water-bath and a solution of gold chloride added. The precipitate was filtered off, washed with distilled water, dried to constant weight and analyzed. Calcd. for $C_3H_9NCl_4Au$: C, 9.0; H, 2.3; N, 3.5; Au, 49.4. Found: C, 9.5; H, 2.6; N, 3.7; Au, 49.2. The malic acid obtained from *aspartic acid* by irradiation was isolated in the same way as lactic acid. The crystalline product was recrystallized from a mixture of acetone and carbon tetrachloride; m. p. 121°. The analysis did not agree very well with theory (hygroscopicity?): Calcd. for $C_4H_6O_5$; C, 35.8; H, 4.5. Found: C, 36.2; H, 5.5.

Summary

1. In water solution glycine, alanine, betaine and aspartic acid are hydrolyzed under the influence of ultraviolet light, giving the corresponding hydroxy acids.

2. The kinetics and quantum yields of these reactions have been measured.

3. The following mechanism has been suggested.

(a) $\underset{NH_2}{RCHCOOH} \longrightarrow RCH^+COOH + NH_2^-$

(b) $RCH^+COOH + OH^- \longrightarrow \underset{OH}{RCHCOOH}$

(c) $NH_2^- + H^+ \longrightarrow NH_3$

4. The hydrolysis of optically active aspartic acid is accompanied by racemization, which is in accordance with the above mechanism.

5. The behavior of tyrosine has been studied qualitatively.

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[CONTRIBUTION FROM THE DANIEL SIEFF RESEARCH INSTITUTE]

Cyclopentenotriphenylene

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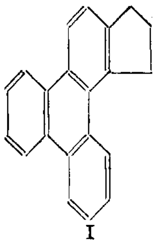
Jacobs and Elderfield[1] obtained by dehydrogenation of strophanthidin, *inter alia*, a hydrocarbon, the analysis of which gave figures corresponding to the formula $C_{21}H_{16}$. On the basis of a strophantidine formula which has since been abandoned, the authors suggested the structure of cyclopentenotriphenylene (I). In connection with other experiments we thought it interesting to synthesize the above hydrocarbon; its properties were quite different from those of the hydrocarbon obtained by the American authors. Our synthesis parallels the preparation of cyclopentenophenanthrene by Cook and co-workers.[2] Their work gave us some valuable information about the formation and structure of various by-products.



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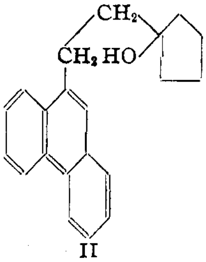

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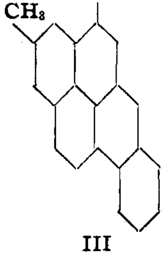
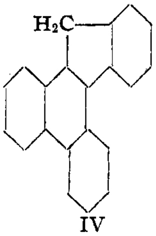


III



IV

β-(9-Phenanthryl)-ethanol was easily prepared from 9-phenanthrylmagnesium bromide and ethylene oxide or ethylene chlorohydrin and converted into the corresponding chloride. Its magnesium derivative reacted with cyclopentanone to give (9-phenanthrylethyl)-cyclopentanol (II). In accordance with the observations of Cook, we tried to convert (II) directly into (I) with glacial acetic acid and concentrated sulfuric acid, but without success, and also none of the two crystalline hydrocarbons resulting from the crude reaction product by subsequent dehydrogenation was the desired product (I). The first had the formula $C_{21}H_{14}$ and was characterized by a vermillion picrate. It is most probable that it corresponds to 2-methylpyrene[2a] obtained by Cook in the corresponding experiment in the naphthalene series, and therefore is 7-methyl-1,2-benzpyrene (III). We hope to confirm this suggestion, by spectroscopic comparison of our product with benzpyrenes of known structure, as 1,2- and 4,5-benzpyrene recently described by Cook and Hewett[3] and Fieser and Fieser,[4] respectively. The second isolated hydrocarbon, which occurred only in minor quantities, turned yellow on keeping. It resembles in this respect the complex fluorene derivatives prepared by Cook and Hewett;[2b] we therefore assume that it has the structure of 1,2,3,4-dibenzofluorene (IV) and is analogous to chrysofluorene isolated by Cook in his experiments in the naphthalene series. The corresponding fluorenone has recently

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[1] Jacobs and Elderfield, *J. Biol. Chem.*, **107**, 143 (1934).

[2] Cook, *et al.*, *J. Chem. Soc.*, (a) 1098 (1933); (b) 365 (1934).

[2a] Barry, Cook, *et al.*, *Proc. Roy. Soc.* (London), **B117**, 321 (1935).

[3] Cook and Hewett, *J. Chem. Soc.*, 398 (1933).

[4] Fieser and Fieser, THIS JOURNAL, **57**, 782 (1935).