XXIII. AMINO-ACIDS, ACYL-AMINO-ACIDS, DIPEPTIDES, ACYL-DIPEPTIDES, AND DERIVATIVES OF THESE COMPOUNDS

II. EFFECTS OF IRRADIATION WITH CATHODE RAYS AND ULTRAVIOLET LIGHT¹

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(Received 17 October 1936)

THE present study was undertaken to gain some information as to the nature of the effect of radiant energy on the matter of living cells. Solutions of several amino-acids and of a large number of derivatives of amino-acids and dipeptides, the absorption curves of which are given in the foregoing paper, were submitted to the action of cathode rays and of ultraviolet light. Absorption measurements were made on the solutions in order to see whether the compounds had undergone any change under the influence of the radiations used. That such was invariably the case can be seen from the figures presented here. The absorption curve of any irradiated solution, when compared with that of the original solution, was usually found to lie on the side of the greater wave-lengths. This was indicative of the occurrence of some deep-seated reactions.

It may be recalled that α -amino-acids are decomposed under the influence of sunlight in the presence of oxygen. The photochemical reaction has been summarily represented by the following equation:

This photo-oxidation takes place in aqueous solution in the presence of metal salts [Neuberg, 1908; 1910] or metal oxides [Gopala & Dhar, 1934], and also in the absence of any such catalysts [Ganassini, 1912; 1913]. Peptones and proteins [Neuberg, 1908; 1910] also yield aldehydes, ammonia and carbon dioxide, a fact which can be explained only by assuming that at some stage of the process hydrolysis of the peptide linkages takes place.

Having exposed various amino-acids to the light of a mercury vapour quartz lamp, Lieben & Urban [1931] reported that the rate of deamination varies, as

¹ Presented before the Third Summer Conference on Spectroscopy and its Applications at the George Eastman Research Laboratories of Physics, Massachusetts Institute of Technology, 16 July 1935.

was expected, from compound to compound, and also that the ammonia cleavage proceeds more rapidly in an acid than in an alkaline medium.

In a series of articles, Becker [1933; 1934] and Becker & Freytag [1932] have shown that X-rays produce photochemical changes of a nature similar to those occurring with ultraviolet light. Becker has found several amino-acids and egg albumin to be unstable under the conditions he used. Henri *et al.* [1934] have studied the photochemical decomposition of glycine. They measured the rate of formation of the ammonia and were able to detect the presence of glycollic acid in an aqueous solution of glycine which had been exposed to ultraviolet light. This observation is of particular interest since it shows that amino-acids may undergo a direct hydrolytic deamination. It has been the theory of Wieland & Bergel [1924] that under the conditions in which an amino-acid yields ammonia, carbon dioxide and the corresponding aldehyde, in an equimolecular ratio, no primary intermediate other than an imino-acid is formed. The latter, according to Wieland & Bergel, then undergoes a decarboxylation (at least when its solution is in contact with active charcoal).

A picture of the various reactions that may take place when aqueous solutions of glycine ($R=H$) and the secondary amino-acids are exposed to light in the presence of oxygen is given below:

We have submitted to the action of cathode rays only such amino-acids as entered into the structure of the other compounds available for study (dipeptides, acetyl-dipeptides and derivatives thereof).

Since, on photo-oxidation, aldehydes are formed from secondary amino-acids, ketones should be obtained from tertiary amino-acids. This can be expressed by the following equation:

Hitherto no tertiary amino-acid had been submitted to the action of radiations of any kind. We have found that solutions of α -aminoisobutyric acid are unstable (Fig. 2, *C*, *D* and *E*) towards cathode rays and ultraviolet light; ammonia is liberated and what appears to be acetone is formed (sodium nitroprusside test). Since a tertiary amino-acid does not contain an α -hydrogen atom, it cannot yield an imino-acid, nor can the tertiary hydroxy-acid that must be formed on deamination lead to an α -keto-acid. Apparently, there remains no other possibility than the one that the hydroxy-acid is decomposed into carbon

dioxide and secondary alcohol, the latter giving on oxidation the corresponding ketone.

We did not attempt to keep the aqueous solutions of α -aminoisobutyric acid (or those of its derivatives) free of oxygen in order to be able to detect the formation of isopropyl alcohol. It is well known [Tian, 1911; Duane & Scheuer, 1913] that water is decomposed by ultraviolet light and other radiations into hydrogen and oxygen, the latter combining with water to give hydrogen peroxide.

Derivatives of glycine and of secondary and tertiary amino-acids, whether or not their molecules carried a free COOH or a free NH_2 group, were found to

Table I

Name of compound	Formula of compound
1. Glycine	$\text{NH}_2\text{CH}_2\text{COOH}$
2. <i>dl</i> -Alanine	$\text{NH}_2\text{CH}(\text{CH}_3)\text{COOH}$
3. <i>dl</i> -Valine	$\text{NH}_2\text{CH}(\text{CH}(\text{CH}_3)_2)\text{COOH}$
4. <i>dl</i> -Leucine	$\text{NH}_2\text{CH}(\text{CH}_2\text{CH}(\text{CH}_3)_2)\text{COOH}$
5. <i>dl</i> - β -Phenylalanine	$\text{NH}_2\text{CH}(\text{CH}_2\text{C}_6\text{H}_5)\text{COOH}$
6. α -Aminoisobutyric acid	$\text{NH}_2\text{CCH}_3(\text{CH}_3)\text{COOH}$
7. Acetyl- α -aminoisobutyric acid	$\text{CH}_3\text{CONHCCH}_3(\text{CH}_3)\text{COOH}$
8. Benzoyl- α -aminoisobutyric acid	$\text{C}_6\text{H}_5\text{CONHCCH}_3(\text{CH}_3)\text{COOH}$
9. Benzenesulphonyl- α -aminoisobutyric acid	$\text{C}_6\text{H}_5\text{SO}_2\text{NHCCH}_3(\text{CH}_3)\text{COOH}$
10. Acetyl- <i>N</i> -methyl- α -aminoisobutyric acid	$\text{CH}_3\text{CON}(\text{CH}_3)\text{CCH}_3(\text{CH}_3)\text{COOH}$
11. Benzoyl- α -aminoisobutyrylmethyl-amide	$\text{C}_6\text{H}_5\text{CONHCCH}_3(\text{CH}_3)\text{CONHCH}_3$
12. Benzenesulphonyl- α -aminoisobutyryl-amide	$\text{C}_6\text{H}_5\text{SO}_2\text{NHCCH}_3(\text{CH}_3)\text{CONH}_2$
13. Glycyl- <i>dl</i> -leucine	$\text{NH}_2\text{CH}_2\text{CONHCH}(\text{CH}_2\text{CH}(\text{CH}_3)_2)\text{COOH}$
14. Acetyl- <i>dl</i> -alanylglycine	$\text{CH}_3\text{CONHCH}(\text{CH}_3)\text{CONHCH}_2\text{COOH}$
15. Acetylglycyl- <i>dl</i> -alanine	$\text{CH}_3\text{CONHCH}_2\text{CONHCH}(\text{CH}_3)\text{COOH}$
16. Acetyl- <i>dl</i> -valylglycine	$\text{CH}_3\text{CONHCH}(\text{CH}(\text{CH}_3)_2)\text{CONHCH}_2\text{COOH}$
17. Acetylglycyl- <i>dl</i> -valine	$\text{CH}_3\text{CONHCH}_2\text{CONHCH}(\text{CH}(\text{CH}_3)_2)\text{COOH}$
18. Acetyl- <i>dl</i> -leucylglycine	$\text{CH}_3\text{CONHCH}(\text{CH}_2\text{CH}(\text{CH}_3)_2)\text{CONHCH}_2\text{COOH}$
19. Acetylglycyl- <i>dl</i> -leucine	$\text{CH}_3\text{CONHCH}_2\text{CONHCH}(\text{CH}_2\text{CH}(\text{CH}_3)_2)\text{COOH}$
20. Acetylglycyl- α -aminoisobutyric acid	$\text{CH}_3\text{CONHCH}_2\text{CONHCCH}_3(\text{CH}_3)\text{COOH}$
21. Acetyl- <i>dl</i> -alanyl- α -aminoisobutyric acid	$\text{CH}_3\text{CONHCH}(\text{CH}_3)\text{CONHCCH}_3(\text{CH}_3)\text{COOH}$
22. Acetyl- α -aminoisobutyryl- α -aminoisobutyric acid	$\text{CH}_3\text{CONHCCH}_3(\text{CH}_3)\text{CONHCCH}_3(\text{CH}_3)\text{COOH}$
23. Acetyl- <i>dl</i> - α -phenylalanylglycine	$\text{CH}_3\text{CONHCCH}_2\text{C}_6\text{H}_5(\text{CH}_3)\text{CONHCH}_2\text{COOH}$
24. Acetyl- <i>dl</i> - α -phenylalanyl- <i>dl</i> -alanine	$\text{CH}_3\text{CONHCCH}_2\text{C}_6\text{H}_5(\text{CH}_3)\text{CONHCH}(\text{CH}_3)\text{COOH}$
25. Acetyl- <i>dl</i> - α -phenylalanyl- α -aminoisobutyric acid	$\text{CH}_3\text{CONHCCH}_2\text{C}_6\text{H}_5(\text{CH}_3)\text{CONHCCH}_3(\text{CH}_3)\text{COOH}$
26. Acetyl- <i>dl</i> - β -phenylalanyl- α -aminoisobutyric acid	$\text{CH}_3\text{CONHCH}(\text{CH}_2\text{C}_6\text{H}_5)\text{CONHCCH}_3(\text{CH}_3)\text{COOH}$
27. Acetylglycyl- <i>dl</i> -leucylanilide	$\text{CH}_3\text{CONHCH}_2\text{CONHCH}(\text{CH}_2\text{CH}(\text{CH}_3)_2)\text{CONHC}_6\text{H}_5$
28. Acetyl- <i>dl</i> - α -phenylalanylglycyl-anilide	$\text{CH}_3\text{CONHCCH}_2\text{C}_6\text{H}_5(\text{CH}_3)\text{CONHCH}_2\text{CONHC}_6\text{H}_5$
29. Acetyl- <i>dl</i> - α -phenylalanyl- <i>dl</i> -alanyl-anilide	$\text{CH}_3\text{CONHCCH}_2\text{C}_6\text{H}_5(\text{CH}_3)\text{CONHCH}(\text{CH}_3)\text{CONHC}_6\text{H}_5$
30. Acetyl- <i>dl</i> - α -phenylalanyl- α -aminoisobutyrylamide	$\text{CH}_3\text{CONHCCH}_2\text{C}_6\text{H}_5(\text{CH}_3)\text{CONHCCH}_3(\text{CH}_3)\text{CONH}_2$
31. Acetyl- <i>dl</i> - β -phenylalanyl- α -aminoisobutyrylanilide	$\text{CH}_3\text{CONHCH}(\text{CH}_2\text{C}_6\text{H}_5)\text{CONHCCH}_3(\text{CH}_3)\text{CONHC}_6\text{H}_5$

undergo a degradation under the influence of cathode rays or of ultraviolet light. Since ammonia was liberated from all compounds (the tests for ammonia were usually made on the solutions submitted to the rapid, drastic action of the cathode rays), hydrolysis to simple molecules liable to give off ammonia undoubtedly took place in every instance. In the case of the derivatives which contained only —CO—NH—CR'(R)— groupings (see Table I, compounds number 7, 8, 11, 22, 25, 30) there must have been first hydrolysis to give a free amino-acid:

The other compounds examined contained at least one —CO—NH—CH(R)— or one $\text{—CO—NH—CH}_2\text{—}$ grouping and perhaps were dehydrogenated before any amino-acid was set free by hydrolysis. If this were the case, the breakdown of the large molecules probably took place along the following lines:

The list of compounds examined is given in Table I.

EXPERIMENTAL METHODS

In preparing the solutions, the dry substances were weighed in 10 ml. volumetric flasks, dissolved in the appropriate solvent (water or 95% alcohol), and the flasks were filled to the mark at room temperature.

For the experiments with ultraviolet light, the solutions were irradiated in small quartz test-tubes of about 0.7 ml. capacity (4 mm. in internal diameter) placed at a distance of 10 cm. from a mercury arc (Hanovia Alpine Sun Lamp). The solutions, cooled by a fan, were irradiated for 3 hours.

For the irradiation with cathode rays, 2 ml. of solution were placed in a small glass dish of diameter 22 mm. This was placed directly below the aluminium window of the cathode ray tube (the surface of the liquid was 1.0 cm. below the window), and the solution, cooled by immersion in ice water, was irradiated for 5 min. The cathode ray tube used was of the Coolidge type, operating on 120 kv. and provided with an aluminium window 0.001 inch thick supported by a grating.

The absorption spectra were taken with a Bausch and Lomb quartz spectrograph; Hilger echelon cells were used (see Part I).

The ammonia tests were made as follows. The solutions to be tested were made acid with hydrochloric acid and any alcohol, aldehyde or ketone present was removed by distillation. The residues were cooled, made alkaline with magnesia, and the ammonia (or methylamine from those compounds containing an NHCH_3 radical) was distilled into cold water. Nessler's reagent was then added to the distillates.

Hydrogen peroxide was detected by means of the titanium sulphate reagent. This was prepared by heating titanium oxide (TiO_2) with twice its volume of concentrated sulphuric acid, cooling and diluting with ice water. A yellow colour (due to TiO_3) is formed when this reagent is added to a solution containing a peroxide.

In order to detect acetone in the irradiated solutions, the sodium nitroprusside test was used. To 2 ml. of the solution to be tested were added 2 drops of a freshly prepared 1% solution of sodium nitroprusside and then 2 drops of a 10% sodium hydroxide solution. When acetone is present, the solution becomes orange, changing to a clear yellow in 20 min.

EXPERIMENTAL RESULTS

The results have been graphically represented. In Fig. 1 is presented a summary of the effects of cathode irradiation upon the various compounds studied. The compound numbers correspond to those in Table I. The increase in absorption of ultraviolet light at an extinction coefficient of 2.0 produced by irradiation of the solutions with cathode rays for 5 min. is indicated. The products produced by irradiation of compounds containing benzene nuclei absorb light of wave-length longer than 2600 Å. (at the extinction coefficient and concentrations indicated), while the products from compounds containing only aliphatic radicals absorb light of wave-length shorter than 2600 Å. Similar compounds give similar shifts in absorption, e.g. the anilides behave alike.

In order to simplify the presentation of the data, curves showing typical changes in absorption spectra produced by cathode and ultraviolet irradiation have been selected.

In Fig. 2 are shown the effects of cathode rays upon a secondary amino-acid, valine, and of cathode and ultraviolet rays upon a tertiary amino-acid, α -aminoisobutyric acid. Primary, secondary and tertiary amino-acids are unstable to radiations.

Several derivatives of α -aminoisobutyric acid have been irradiated (Figs. 3, 4 and 5) in aqueous and alcoholic solutions. The effect of ultraviolet light is similar to that of cathode rays, but the action is less marked.

The effect of cathode rays upon a dipeptide is shown in Fig. 6, *A* and *B*.

Fig. 1.

Fig. 2.

Fig. 1. Increase in the absorption of light at an extinction coefficient of 2.0 produced by irradiation of solutions of the various substances with cathode rays for 5 min. Compounds numbers 1, 2, 3, 4, 5, 6, 13, 14, 18 and 19 were irradiated as 0.05 *M*. The others were irradiated as 0.01 *M*.

Fig. 2. *dl*-Valine. 0.05 *M* in water. *A*, non-irradiated. *B*, irradiated with cathode rays for 5 min. α -Aminoisobutyric acid. 0.05 *M* in water. *C*, non-irradiated. *D*, irradiated with cathode rays for 5 min. *E*, irradiated with ultraviolet light for 3 hours.

Fig. 3.

Fig. 4.

Fig. 3. Acetyl-*N*-methyl- α -aminoisobutyric acid. 0.01 *M* in water. *A*, non-irradiated. *B*, irradiated with cathode rays for 5 min. *C*, irradiated with ultraviolet light for 3 hours.

Fig. 4. Benzoyl- α -aminoisobutyric acid. 0.01 *M* in water. *A*, non-irradiated. *B*, irradiated with cathode rays for 5 min. *C*, irradiated with ultraviolet light for 3 hours.

Fig. 5.

Fig. 6.

Fig. 5. Benzenesulphonyl- α -aminoisobutyric acid. 0.01 M in EtOH. A , non-irradiated. B , irradiated with cathode rays for 5 min. C , irradiated with ultraviolet light for 3 hours.

Fig. 6. Glycyl- DL -leucine. 0.05 M in water. A , non-irradiated. B , irradiated with cathode rays for 5 min. Acetyl- DL -alanyl- α -aminoisobutyric acid. 0.01 M in water. C , non-irradiated. D , irradiated with cathode rays for 5 min.

Fig. 7.

Fig. 8.

Fig. 7. Acetyl- DL - α -phenylalanylglycine. 0.01 M in EtOH. A , non-irradiated. B , irradiated with cathode rays for 5 min. C , irradiated with ultraviolet light for 3 hours.

Fig. 8. Acetyl- DL - α -phenylalanyl- DL -alanylanilide. 0.01 M in EtOH. A , non-irradiated. B , irradiated with cathode rays for 5 min. C , irradiated with ultraviolet light for 3 hours.

Fig. 9.

Fig. 10.

Fig. 9. Acetyl-*DL*- α -phenylalanyl- α -aminoisobutyrylamide. 0.01 *M* in EtOH. *A*, non-irradiated. *B*, irradiated with cathode rays for 5 min. *C*, irradiated with ultraviolet light for 3 hours.

Fig. 10. Acetyl-*DL*- α -phenylalanyl-*DL*-alanylanilide. *A*, non-irradiated. 0.01 *M* in EtOH. *B*, irradiated with ultraviolet light for 3 hours as 0.001 *M* solution. *C*, irradiated with ultraviolet light for 3 hours as 0.00025 *M* solution. All curves plotted as 0.01 *M*.

Fig. 11.

Fig. 12.

Fig. 11. Acetyl-glycyl-*DL*-leucyl-anilide. 0.005 *M* in EtOH. Irradiated with ultraviolet light.

Fig. 12. Acetyl-*DL*- α -phenylalanyl-*DL*-alanine. 0.01 *M* in EtOH. Irradiated with ultraviolet light.

The absorption curves of aqueous solutions of acetyl-di-peptides are shifted towards the longer wave-lengths by irradiation with cathode rays (Fig. 6, *C* and *D*). Ultraviolet light produces a similar shift in all cases where it is applied. The same type of shift of the absorption spectra is observed with alcoholic solutions of acetyl-di-peptides (Fig. 7).

Fig. 13.

Fig. 14.

Fig. 13. Acetyl-glycyl-*dl*-leucylanilide. Saturated solution in water. Irradiated with cathode rays.

Fig. 14. Acetyl-*dl*- α -phenylalanyl-*dl*-alanine. 0.01 *M* in EtOH. Irradiated with cathode rays.

The effect of cathode rays upon acetyl-di-peptides in which the hydroxyl of the carboxyl group is replaced by an NHC_6H_5 residue is generally less than that upon the corresponding compounds in which the carboxyl is free (Fig. 8). The effect of cathode or ultraviolet rays upon an acetyl-di-peptide in which the hydroxyl of the carboxyl group is replaced by an NH_2 residue is only slightly different from the effect on the parent substance (Fig. 9, compare Fig. 7).

The absorption spectrum of an irradiated solution of acetyl- α -aminoisobutyryl- α -aminoisobutyric acid is nearly the same as that of a mixture in the equimolecular amounts of acetyl- α -aminoisobutyric acid and α -aminoisobutyric acid that has been irradiated for the same length of time. The absorption of light by an aqueous solution containing a mixture of acetic acid, acetone and ammonium bicarbonate in equimolecular amounts is increased by the action of cathode rays, the spectrum of the irradiated solution somewhat resembling that of the irradiated solution of acetyl- α -aminoisobutyric acid.

In Fig. 10 is given the action of ultraviolet light upon solutions of acetyl-*dl*- α -phenylalanyl-*dl*-alanylalilide; the decomposition is greater in the more dilute solution. The decomposition produced by cathode rays was found to be greater for a 0.01 *M* as compared to 0.05 *M* solution of acetyl-*dl*-alanyl- α -aminoisobutyric acid.

Figs. 11 and 12 show the effect of increasing amounts of ultraviolet radiation upon acetylglycyl-*dl*-leucylanilide and acetyl-*dl*- α -phenylalanyl-*dl*-alanine respectively. The effect of increasing amounts of cathode rays on solutions of acetylglycyl-*dl*-leucylanilide and acetyl-*dl*- α -phenylalanyl-*dl*-alanine is shown in Figs. 13 and 14. It is to be noted in Fig. 13 that, at some time between 8 and 16 min. of irradiation, the solution of acetylglycyl-*dl*-leucylanilide has reached a maximum absorption and that subsequently products with less absorption are formed. The same "receding" effect was noted between 20 min. and 1 hour of irradiation of acetyl-*dl*-leucylglycine with cathode rays.

Irradiation with cathode rays for 5 min. and with ultraviolet light for 3 hours produced no noticeable changes in the absorption spectra of water and of 95 % ethyl alcohol (the solvents used in this investigation). The irradiated solvents were tested for hydrogen peroxide with the titanium sulphate reagent. Positive tests for peroxide were obtained with water and alcohol which had been irradiated with cathode rays, the irradiated alcohol giving a stronger test than the irradiated water; no peroxide was detected in these solvents after they had been treated with ultraviolet light.

SUMMARY

Solutions of several amino-acids and of a large number of derivatives of amino-acids and dipeptides were submitted to the action of cathode rays and ultraviolet light. By spectroscopic measurements, it was found that in all cases the compounds undergo a change under the influence of these radiations, regardless of whether the constituent amino-acids are primary, secondary or tertiary. Ammonia was liberated from all compounds upon irradiation. Possible reaction mechanisms are given.

We are grateful to Dr Ellice McDonald for his encouragement and interest in this work.

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