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Photochemistry of Proteins. IX.[1] Photolysis of the Peptide Bond at 2537 Å.[2]

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It is reasonably certain that peptide bonds, aromatic rings and -SS- groups are cleaved and/or oxidized as proteins absorb ultraviolet light. Mitchell[3] suggests that tyrosine absorption in a protein leads to a break of a -CONH- bond adjacent to the chromophore. Although with the model substance stearyl anilide, as a monolayer, no reaction was found to occur at wave lengths at or longer than 2537 Å., egg albumin photohydrolyzed at 2537 Å. as a monolayer.[3b,4] Carpenter[5a] repeated the study of the anilide model, and extended it to benzylstearylamine and β-phenylethylstearylamine in which the benzene ring and the -CONH- linkage are separated by one and two CH_2 groups, respectively. He also reports[5b] cleavage of the peptide bonds in tyrosyl di- and tri-peptides.

The investigation of peptide or hemipeptide model substances in solution under the influence of ultraviolet light appeared worthwhile since such data may eventually lead to an elucidation of two of the problems of protein photolysis: The reason for the small quantum yields observed for the inactivation of enzymes and viruses[6] and the possibility of determining the amino acid sequence of the intact protein by analysis of the fragments resulting from ultraviolet light irradiation.[5b] With these ends in view a study of a series of acylated amino acids $C_6H_5(CH_2)_n$-CONHCH(CH_3)COOH with n equal to 0, 1, 2 and 3 was undertaken. Also a comparison of two isomeric substrates with the chromphore on opposite sides of the -CONH- bond would illustrate the relative efficiency of transfer of energy past 3 C or past 2 C and 1 N atoms. Propionyl-phenylalanine and phenylpropionylalanine were selected. Allen, et al.,[7] had shown that such substrates are cleaved at the -CONH- bond. Also any ammonia found would necessarily be derived through peptide bond splitting.

Experimental

The acylated alanine derivatives were prepared as de-

scribed.[8,9] Propionylphenylalanine was prepared similarly, m. p. 136.5–138°. Anal. Calcd.: C, 65.15; H, 6.78; N, 6.33. Found: C, 65.41; H, 6.87; N, 6.46. Aqueous solutions were usually used, ca. 0.01 M for irradiation (ca. 99% absorption). Twenty-seven times more concentrated solutions were used in the experiments with acetylalanine to absorb 67.3% at 2537 Å. Maxima, minima and molar extinction coefficients at 2537 Å. are collected in Table I together with solubility data at 24.1°.

TABLE I

	Molal solubility	ϵ_{max}	λ	ϵ_{min}	λ	ϵ_{2537} Å.
Acetylalanine						1.28
Benzoylalanine	0.0256	10,850	2290			3000
Benzoyl-β-alanine	.0342	10,150	2280			2280
Phenylacetylalanine	.0400	187	2580	78	2435	142
Phenylpropionyl-alanine	.0138	213	2585	78	2375	177
Phenylbutyrylalanine	.0098	359	2585	170	2340	354
Propionylphenyl-alanine		190	2590	75	2395	145

Irradiation technique, intensity measurements, etc., in the presence of air have been fully described elsewhere.[10] Modifications of these procedures for irradiation under oxygen or nitrogen may be found in the Ph.D. thesis of one of us (I.M.) (see footnote 2).

Analyses of products of photolysis: Ammonia: Aeration of the alkaline solution into 0.05 N sulfuric acid followed by nesslerization, as well as micro diffusion,[11] was used. Amino Acids: For some of the experiments (acetyl-, benzoyl-, phenacetyl-, phenylpropionyl- and phenyl-butyryl-alanine) the ninhydrin color reaction in the modification of Harding and MacLean[12] was adopted for the determination of alanine but since ammonia is known[13] to give the same reaction compound, although the yield is different, curves were constructed to correct for color values[14] due to known concentrations of ammonia (Fig. 1). Since the amount of ammonia present was determined by nesslerization, conversion of the Nessler reading to the ninhydrin value from the plot allowed calculation of the color due to alanine by difference. (For example see Fig. 2.) This procedure is permissible since ammonia and amino acid ninhydrin reaction products were found to have the same absorption spectrum. The color due to alanine was then converted to moles alanine in the usual way.[12] The manometric[15] or titrimetric[16] determinations of the carbon dioxide given off on reaction of amino acids with triketohydrindene hydrate were used in the case of phenyl-propionylalanine and propionylphenylalanine. The results obtained with phenylpropionylalanine agreed by these methods and the colorimetric method agreed. Spectra: Changes in ultraviolet light absorption after irradiation of

(1) For the previous paper in this series see E. H. Kaplan, E. D. Campbell and A. D. McLaren, Biochim. et Biophys. Acta, in press (1950).

(2) From the M.S. Thesis (1948) of Beatrice Levy and Part II of the Ph.D. Thesis (1949) of Ines Mandl at the Polytechnic Institute of Brooklyn. Presented in part at the New York meeting of the A. C. S., September, 1947.

(3) (a) J. S. Mitchell, Nature, 137, 509, 1936; (b) Proc. Roy. Soc. (London), A155, 696, (1936).

(4) J. S. Mitchell and E. K. Rideal, ibid., A167, 342 (1938).

(5) (a) D. C. Carpenter, THIS JOURNAL, 62, 289 (1940); (b) J. Franklin Inst., 232, 76 (1941).

(6) I. Mandl and A. D. McLaren, Arch. of Biochem., 21, 408 (1949).

(7) A. J. Allen, R. E. Steiger, M. A. Magill and R. G. Franklin, Biochem. J., 31, 195 (1937).

(8) B. Levy and A. D. McLaren, THIS JOURNAL, 71, 1512 (1949).

(9) R. L. M. Synge, Biochem. J., 33, 1913 (1939).

(10) A. D. McLaren and S. Pearson, J. Polymer Sci., 4, 45 (1949).

(11) E. J. Conway, "Microdiffusion Analysis and Volumetric Error," Crosby Lockwood & Son, Ltd., London, 1947; E. J. Conway and A. Byrne, Biochem. J., 27, 420 (1933).

(12) V. J. Harding and R. M. MacLean, J. Biol. Chem., 25, 337 and 319 (1916).

(13) C. Neuberg, Biochem. Z., 56, 500 (1913).

(14) A Klett–Summerson photoelectric colorimeter was employed.

(15) D. D. Van Slyke, R. T. Dillon, D. A. MacFayden and P. Hamilton, J. Biol. Chem., 141, 627 (1941).

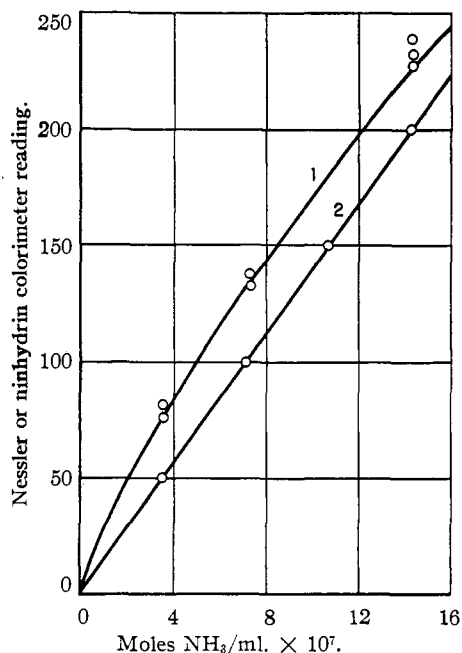(16) D. D. Van Slyke, D. A. McFayden and P. Hamilton, ibid., 141, 671 (1941).

Fig. 1.—Ammonia color value conversion graph: 1, ninhydrin reaction; 2, Nessler reaction.

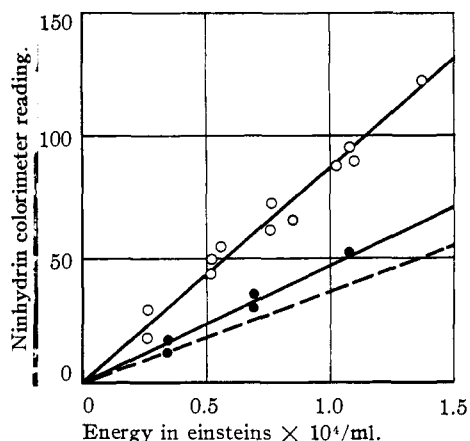Fig. 2.—Photolysis of benzoyl-DL-alanine in air: O, alanine + NH₃; ●, NH₃; ----, alanine (calculated).

phenylpropionylalanine and its isomer under oxygen or nitrogen point to a substantial decomposition of the aromatic ring (Figs. 3 and 4). *Other tests:* All solutions, including those of propionylphenylalanine, remained perfectly colorless, even after prolonged periods of irradiation, whereas phenylalanine solutions become rapidly yellow when air is present. Millon reactions for possible oxidation to hydroxyphenyl derivatives were consistently negative. Ammoniacal silver nitrate was reduced in all cases. Eegriwe reactions with 2,7-dihydroxynaphthalene[17] were positive but since this test is based on oxidation of hydroxy acids to aldehydes this may mean the presence of acetaldehyde or pyruvic acid as well as lactic acid, resp., the corresponding phenyl compounds.

Results and Discussion

Quantum yields were determined in all cases by use of the formula Φ = (moles liberated/cc./
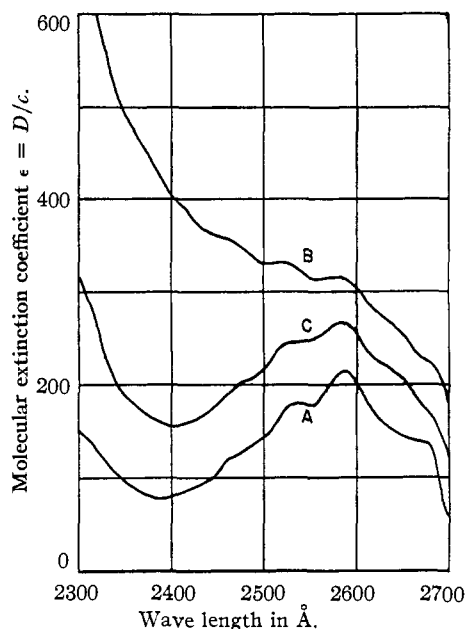
(17) E. Eegriwe, *Z. anal. Chem.*, **89**, 123 (1932).

Fig. 3.—Ultraviolet absorption spectrum of phenylpropionylalanine: A, original; B, irradiated in O₂ 2 hr., 10% decomposed; C, irradiated in N₂ 2 hr., 8% decomposed.
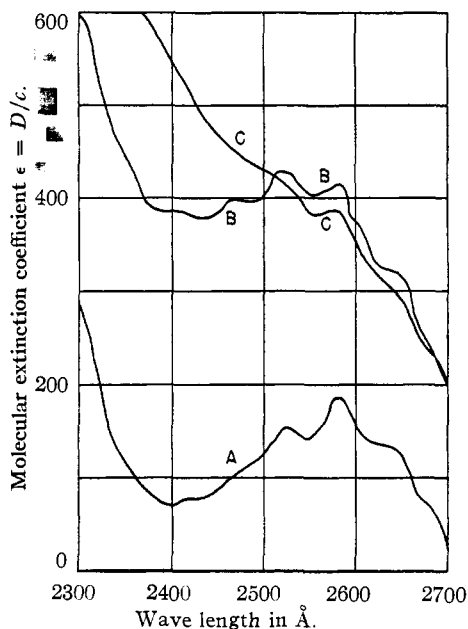

Fig. 4.—Ultraviolet absorption spectrum of propionylphenylalanine: A, original; B, irradiated in air 2.5 hr., 4.5% decomposed; C, irradiated in N₂ 4 hr., 7.2% decomposed.

hr.)/(einsteins absorbed/cc./hr.). The separate values obtained for ammonia and amino acids, respectively, were added to calculate the total quantum yield for the splitting of the peptide bond. Results, reproducible to within ±5%, are collected in Table II. The quantum yield for

the photolysis of acetylalanine (0.072) is higher than that of any of the other compounds and only ammonia was obtained. The fraction of absorbed energy which disrupts phenyl groups (Figs. 3 and 4) is not known. Possibly the phenyl group in the molecule acts as a stabilizer: the quantum yield for benzoylalanine was lowest (0.0033), that for phenylacetyl- and phenylpropionylalanine about the same and of the same order of magnitude as that for benzoylalanine. Phenylbutyrylalanine gave a ten times higher value (0.044). An alternate explanation lies in the fact that the quantum yield rises with the length of the $(CH_2)_n$ chain. This points to the likelihood of internal photosensitization[18] rather than the traveling of energy along the chain as suggested by Carpenter.[5a] As the chain length increases, folding over of the chromophore to the peptide link is possible for greater fractions of the time. An analogous effect in the case of ketones has been indicated by Davis and Noyes.[19] The well known insulating properties of -CH_2- in absorption spectroscopy[20] suggests that the quantum yield would decline with increase in chain length whereas the reverse effect is observed. The correct interpretation awaits further investigation.

TABLE II

	$\Phi \times 10^3$ amino acid	$\Phi \times 10^3$ ammonia	Total $\Phi \times 10^3$
Acetylalanine in air	None	71.5	71.5
Benzoylalanine in air	1.25	2.09	3.34
Phenylacetylalanine in air	4.77	2.27	7.04
Phenyl- in air	0.89	5.91	6.80
propionyl- in oxygen	0.65	6.3	6.95
alanine in nitrogen	0.12	5.0	5.12
Propionyl- in oxygen	None	5.8	5.8
phenylalanine in nitrogen	None	4.5	4.5
Phenylbutyrylalanine in air	26.8	16.9	43.7

Comparison of the low magnitude of quantum yields for inactivation of enzymes[21] with those for photolysis of the peptide bond supports the hypothesis that absorption of light by any chromophore can lead to inactivation. Thus the low

(18) W. West and W. E. Miller, *J. Chem. Phys.*, **8**, 849 (1940).

(19) W. Davis and W. A. Noyes, THIS JOURNAL, **69**, 2153 (1947).

(20) W. R. Brode, "Chemical Spectroscopy," John Wiley and Sons, Inc., New York, N. Y., 1945.

(21) A. D. McLaren in "Advances in Enzymology," Vol. IX, Interscience Publishers, New York, N. Y., 1949, p. 75.

quantum yields for proteins are not unexpected in view of low yields for splitting of the peptide bond.[10]

Considerable importance may be attached to the finding that although the quantum yields obtained for phenylpropionylalanine were consistently higher than those for propionylphenylalanine, the difference is small enough to warrant the conclusion that about equal numbers of peptide bonds would be cleaved on either side of a chromophore in proteins. This agrees with the view of Mitchell[3b] that the aromatic amino acid residues are split out of the protein chain. Also Carpenter[5b] reports splitting of alanyltyrosylglycine at both peptide linkages. The amounts of ammonia split off under nitrogen fall below those for the same substance under oxygen, indicating a certain amount of photo-oxidation. The ratio of the quantum yields of the two hemipeptides under nitrogen is about the same as that found under oxygen. The complete absence of even traces of phenylalanine is not altogether surprising if the more rapid rate of decomposition of phenylalanine compared to alanine (according to Weizmann[22] 2.7:1) is taken into account.

Summary

Comparative studies were made of the quantum yields for photolysis of acyl derivatives of amino acids. In the series $C_6H_5(CH_2)_nCONHCH(CH_3)COOH$ the quantum yield was found to rise with an increase of n from 0–3. The aliphatic analog acetylalanine gave the highest quantum yield.

Only a small difference exists in the rate of photolysis of the isomeric substances phenylpropionylalanine and propionylphenylalanine, which were investigated under oxygen, under nitrogen and in the presence of air. This may mean that in proteins the energy absorbed by a chromophore is transported with approximately equal efficiency to the peptide bonds at either side past 3 C or 2 C and 1 N atom.

(22) C. Weizmann, Y. Hirshberg and E. Bergmann, THIS JOURNAL, **60**, 1799 (1938).