

[CONTRIBUTION FROM THE PHYSICO-CHEMICAL LABORATORY OF THE NEW YORK STATE EXPERIMENT STATION]

Splitting the CONH Linkage by Means of Ultraviolet Light¹

BY D. C. CARPENTER

Any method which would enable us to break selectively the peptide (CONH) linkage between certain of the various amino acids of a protein, should bring forth a certain amount of information on the arrangement of the individual amino acids within the molecule. Inasmuch as the specific properties of proteins such as insulin probably depend on the presence of a certain sequence of grouping of amino acids in the molecule, a method bearing on the finding of these groupings is of considerable interest and importance. From recent results it appears that ultraviolet light may be a useful tool for opening up the protein molecule in such work.

Rideal and Mitchell² showed that stearic anilide $C_{17}H_{35}CONHC_6H_5$ when irradiated as a monolayer with light of wave length 2350–2400 and 2483 Å. was decomposed into stearic acid and aniline. In this experiment the benzene ring functioned as the chromophore group. Inasmuch as the bond energy of the C–N linkage (48.6 kcal./mole)³ is the weakest linkage in the compound, it is not surprising that breakage occurs at this point. It might be concluded that Rideal and Mitchell's experiment applied directly to the breakage of peptides in a similar manner. However, in the peptides and proteins the benzene ring or other light absorbing group is contained in a side chain and situated several atoms away from the point of splitting. To be of any use in splitting peptides or the main peptide chain in proteins, the matter arises as to whether light energy absorbed by the chromophore group can pass through several CH_2 groups and still cause splitting of the CONH linkage. Norrish and Appleyard⁴ irradiated methyl butyl ketone, $CH_3CH_2CH_2CH_2COCH_3$, at 2200–3000 Å., where the CO group is the chromophore, and found that $CH_3CH=CH_2$ and CH_3COCH_3 were produced. This showed that energy is able to travel along a chain and cause breakage of a molecule at a distant point. How far energy may travel in this way has not been established.

(1) Approved by the Director of the New York State Experiment Station for publication as Journal Paper No. 348.

(2) Rideal and Mitchell, *Proc. Roy. Soc. (London)*, **159A**, 206 (1937).

(3) Pauling and Niemann, *This Journal*, **61**, 1862 (1939).

(4) Norrish and Appleyard, *J. Chem. Soc.*, 874 (1934).

In the present paper⁵ we have confirmed the observations of Rideal and Mitchell with respect to the photolysis of stearic anilide. In addition to this we have prepared benzylstearylamine ($C_{17}H_{35}CONHCH_2C_6H_5$) and β -phenylethylstearylamine ($C_{17}H_{35}CONH(CH_2)_2C_6H_5$) and examined the photolysis of these compounds containing one and two CH_2 groups, respectively, interposed between the chromophore and the keto-imino linkage. We have found that both of these latter compounds undergo photolysis at 2537 Å. and therefore it may be expected that peptides and proteins having side chains bearing chromophore groups will behave similarly, and the CONH groups adjacent to the side chain undergo photolysis and the light absorbing amino acid is liberated from the peptide or protein molecule. This behavior would conform to observations that the free amino groups in proteins are increased by irradiation.⁶

Experimental

Preparation of Materials

Stearic Anilide.—This compound was prepared by converting pure stearic acid m. p. 69.3° (cor.) into the acid chloride by adding small portions dissolved in anhydrous chloroform to thionyl chloride and refluxing over a steam-bath for one-half hour. The thionyl chloride was used in 35% excess of the calculated amount. The excess thionyl chloride and chloroform were removed by distillation *in vacuo* over a water-bath.

Stearyl chloride (8.2 g.) was treated with 2.5 g. of aniline dissolved in 30 cc. of dry benzene and warmed on the steam-bath for two to three minutes. The benzene solution was decanted into a separatory funnel and washed successively with water, 5% hydrochloric acid solution, 5% sodium hydroxide solution and finally with water. The benzene was evaporated and the stearic anilide recrystallized several times from petroleum ether, alcohol and finally twice from acetone; 9.0 g. of stearic anilide in white needles m. p. 94° (cor.) was obtained.

Benzylstearylamine.—This compound was prepared in analogous fashion from benzylamine b. p. 184° (cor.) and stearyl chloride, and after recrystallizing as above was obtained as white flakes, m. p. 98.6° (cor.).

β -Phenylethylstearylamine.— β -Phenylethylamine of b. p. 194.5–196° (cor.) prepared by the method of Ladenburg⁷ was condensed with stearyl chloride as above and

(5) Carpenter, *Science*, **89**, 251 (1939).

(6) Fort and Lloyd, *J. Soc. Dyers and Colourists*, **30**, 73 (1914); Fort, *ibid.*, **32**, 184 (1917).

(7) Ladenburg, *Ber.*, **19**, 782 (1886).

after several recrystallizations was obtained as white needles, m. p. 91.8° (cor.).

Absorption Spectrum.—The absorption spectrum of each of the compounds was determined in anhydrous ethyl alcohol as solvent by means of a Bausch and Lomb ultraviolet sectorphotometer, suitable dilutions being employed to cover the absorption range. The absorption spectra are shown in Fig. 1. Comparison of our data for stearic anilide with the results of Rideal and Mitchell shows good general agreement for the maximal absorption region, but the latter's results show somewhat greater absorption throughout the entire range. The band is fairly flat between 2360 and 2500 Å. with a maximum close to 2420 Å.

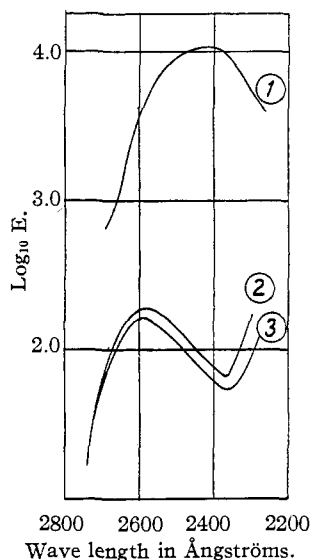


Fig. 1.—Absorption spectra; 1, stearic anilide; 2, benzylstearylamine; 3, β -phenylethylstearylamine.

The absorption spectra for benzylstearylamine and β -phenylethylstearylamine are close together and considerably less than for stearic anilide, the introduction of the first CH_2 group between the ring and the fatty acid residue accounting for most of the decreased absorption and the shift of the maximum toward the visible range. Both of these compounds have an absorption maximum close to 2580 Å.

Irradiation Experiments.—Rideal and Mitchell employed a large quartz monochromator for obtaining monochromatic light. A monochromator of sufficient size to be useful was not available to us, consequently as a source of light in our experiments we have used a quartz-mercury lamp with a combination of light filtering solutions⁸ in quartz containers. Rideal and Mitchell record that in the photolysis of stearic anilide no reaction can be detected at 2537 Å. while reactions occur at 2483 Å. They put the threshold for the photochemical reaction near 2500 Å. With the system of light filters we have used, both the 2483 and 2537 Å. lines are transmitted, so we are unable to say definitely whether the photochemical effects we have observed are due to the 2483 or the much more intense 2537 Å. line. We have not observed any ef-

fects when using the 2650 and 2700 Å. lines, which are both transmitted when employing a different combination of filter solutions.

In our experiments, mono-layers of the substances have been formed by spreading dilute benzene solutions onto normal or tenth normal hydrochloric acid solutions contained in a Pyrex glass trough. The collapse areas of the films have been measured and the theoretical molecular areas calculated. These results are contained in Table I and show that the respective molecules

TABLE I
MOLECULAR AREAS OF SUBSTANCES

	Molecular area, sq. Å.	
	Found	Calcd.
Stearic anilide	23.9	23.7
Benzylstearylamine	30.7	30.4
β -Phenylethylstearylamine	28.6	28.1

are oriented as shown in Fig. 2. In the latter figure the benzene ring is shown with dotted lines but the plane of the ring actually lies at right angles to the plane of the paper as is shown by the solid line. Calculation of the molecular area of

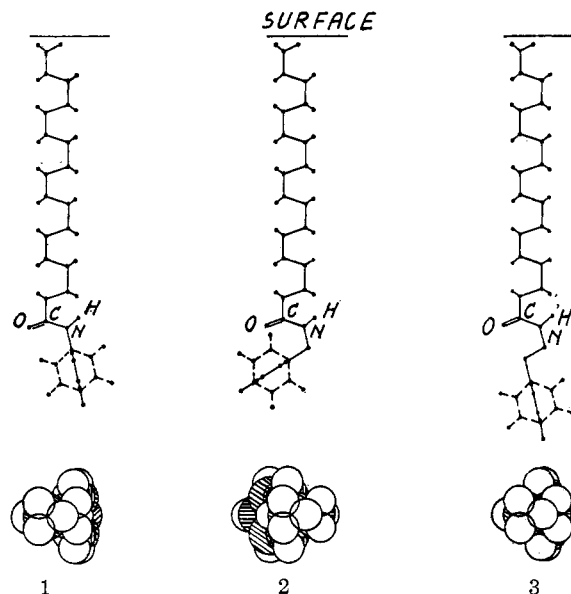


Fig. 2.—Monolayers on 0.1 *N* hydrochloric acid; 1, stearic anilide; 2, benzylstearylamine; 3, β -phenylethylstearylamine.

benzylstearylamine assuming the ring swung out to the right (free rotation about the N-C bond) and as distant as possible from the oxygen atom of the CO group, leads to an area of 36.8 sq. Å., which is altogether too high to agree with the experimental value and we may safely conclude that in a compressed film the ring lies on the same side

(8) Bowen, *J. Chem. Soc.*, 76 (1935).

as the oxygen atom. The calculated molecular areas agree within 1-2% with the experimental values and this is about as good agreement as can be expected.

The film potential was measured⁹ at intervals as the irradiation proceeded and the maximum ΔV values eventually came to around 550 mv. Rideal and Mitchell record a ΔV value for stearic acid of 545 mv. This indicates that the decomposition product remaining in the mono-layer is stearic acid in all cases.

The amines resulting from the photolysis remain in the hydrochloric acid solution substrate, as may be expected. Aniline was identified colorimetrically in the hydrochloric acid solution after irradiation for as short a period as ten minutes by the red color produced by diazotizing and coupling with β -naphthol. Benzylamine and β -phenylethylamine were identified as picrates, m. p. 195° (cor.)¹⁰ and 168° (cor.),¹¹ respectively, after prolonged irradiation and frequent renewal of the mono-layer. Mixed melting points of each of these were done with the respective pure picrates prepared from the pure amines and confirmed the identity of the photolysis products. Between 4 and 5 mg. of the respective picrates was actually crystallized after about twenty-four hours of ir-

(9) Schulman and Rideal, *Proc. Roy. Soc. (London)*, **130A**, 259 (1931).

(10) Moureu and Lazennec, *Bull. soc. chim.*, [3] **35**, 1183 (1906); Jerusalem, *J. Chem. Soc.*, 1283 (1909).

(11) Michaelis and Linow, *Ber.*, **26**, 2167 (1893); Weinlagen, *Biochem. J.*, **11**, 275 (1917).

radiation. That the reaction with stearic anilide is a photolysis and not hydrolysis in the presence of hydrochloric acid was definitely proved by inserting a glass filter in the optical system which excluded wave lengths below 3000 Å. Under these conditions no aniline was detected after thirty minutes exposure.

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Summary

The absorption spectra of stearic anilide, benzylstearylamine and β -phenylethylstearylamine have been determined and the molecular areas when spread on hydrochloric acid solution have been measured. These substances as mono-layers have been irradiated at 2483 and 2537 Å. and found to undergo photolysis at the CONH linkage. The products of the reaction have been identified by film potential measurements (stearic acid) and colorimetric tests (aniline) and as crystalline picrates (benzylamine and β -phenylethylamine).

It seems reasonable to expect that peptides and proteins containing amino acids with light-absorbing side chains may undergo breakage of the adjacent CONH linkages in an analogous manner,

GENEVA, N. Y.

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[COMMUNICATION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF MANITOBA]

The Heats of Solution, Heats of Formation, Specific Heats and Equilibrium Diagrams of Certain Molecular Compounds

BY A. N. CAMPBELL AND A. J. R. CAMPBELL

A very great number of studies of the freezing point (or melting point) diagrams of binary organic systems has been made.¹ As a result of these studies it appears that a molecular compound frequently is formed. In this connection the question of the relative stability of these numerous compounds has arisen. This question can only be answered with complete precision when the free energy of formation is known and this cannot be obtained readily for the systems

(1) Cf., for instance, Kremann, *Monatsh.*, **25**, 1215 (1904), and following volumes to 1930.

under consideration, since the calculation requires a knowledge of the behavior of the specific heats from absolute zero upward. It is known, however, that in condensed systems the heat of formation very often approximates to the free energy of formation. It was thought that determinations of the heats of formation via the heats of solution of some of these organic molecular compounds would give at least the order of magnitude of the free energy change involved. Furthermore, if the specific heats, at room temperature, of reactants and products are known, an approximate calcula-