[CONTRIBUTION FROM THE CHEMICAL LABORATORY, FACULTY OF SCIENCE, TOKYO UNIVERSITY]

Near Infrared Spectra of Compounds with Two Peptide Bonds and the Configuration of a Polypeptide Chain. II

BY SAN-ICHIRO MIZUSHIMA, TAKEHIKO SHIMANOUCHI, MASAMICHI TSUBOI, TADAO SUGITA, KAZUO KUROSAKI, NOBORU MATAGA AND REISUKE SOUDA

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Near infrared spectra of acetyl-DL-alanine N-methylamide and acetyl-DL-proline N-methylamide have been measured in carbon tetrachloride solutions. The spectra of acetylglycine N-methylamide and acetyl-DL-leucine N-methylamide have been remeasured. From the experimental results it has been concluded that acetyl-DL-proline N-methylamide exists in one molecular configuration (B form), while each of the other three substances exists in two molecular configurations (E and B forms). The configurations of glycine, alanine, leucine and proline residues in a polypeptide chain have been discussed.

Introduction

As reported in our previous papers[1,2] acetylglycine N-methylamide and acetylleucine N-methylamide exist in two molecular forms E and B in carbon tetrachloride solutions (see Fig. 1). The population ratio of these two forms was found to change strikingly with temperature. This was explained as due on one hand to the energy difference between these two molecular species and on the other hand to the entropy difference between them.

We have also prepared acetylalanine N-methylamide and acetylproline N-methylamide and have made near infrared measurement of these substances. At the same time the spectra of acetylglycine N-methylamide and acetylleucine N-methylamide have been remeasured in order to obtain more detailed information about these substances. These experimental results will be discussed in the present paper in relation to the configuration of a polypeptide chain.

Preparation of Materials.—The four acetyl-DL-amino-acid N-methylamides measured in this experiment have been prepared by dissolving anhydrous methylamine into the cooled methanol solution of the corresponding ethyl esters of acetyl-DL-aminoacids and setting the solution aside for two weeks at room temperature. Acetylglycine N-methylamide and acetyl-DL-alanine N-methylamide were recrystallized from the mixture of ethanol and ethyl acetate to constant m.p. of 158 and 162°, respectively, acetylleucine N-methylamide from ethyl acetate to that of 160° and acetyl-DL-proline N-methylamide from carbon tetrachloride to that of 93°.

Experimental Results.—Figures 2 and 3 show the absorption curves in the 3 μ region obtained for carbon tetrachloride solutions of acetylglycine N-methylamide (AGNA), acetyl-DL-alanine N-methylamide (AANA), acetyl-DL-leucine N-methylamide (ALNA) and acetyl-DL-proline N-methylamide (APNA) which will be abbreviated hereafter as shown in the parentheses. Curve A of Fig. 2 refers to a concentration of 0.0002 mole/l. and a path length of 10 cm. and curve B to a concentration of 0.0001 mole/l. and a path length of 20 cm. The temperature of the cell was kept at 30°. As ALNA and APNA are more soluble in carbon tetrachloride than the other two substances, we could measure the spectra for ALNA and APNA in the wider range of concentration as shown in Fig. 3 in which curve A refers to a concentration of 0.05 mole/l., curve B to that of 0.0025 mole/l., curve C to that 0.001 mole/l. and curve D to that of 0.0005 mole/l. The temperatures at which these measurements were made were 30 and 60° and the absorption path length was chosen as 0.1 cm. for A, 2 cm. for B, 5 cm. for C and 10 cm. for D so that the product of concentration and path length was kept constant as in the case of Fig. 2.

(1) S. Mizushima, T. Shimanouchi, M. Tsuboi, T. Sugita, E. Kato and E. Kondo, THIS JOURNAL, **73**, 1330 (1951).

(2) S. Mizushima, T. Shimanouchi, M. Tsuboi and R. Souda, *ibid.*, **74**, 270 (1952).

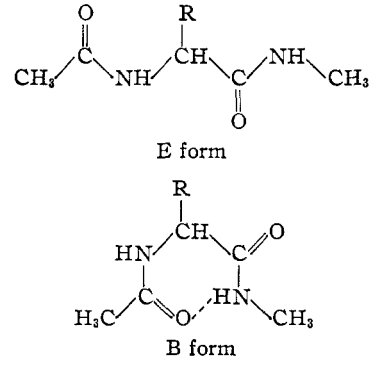

Fig. 1.—Two molecular configurations of $CH_3CONHCHR-CONHCH_3$.

Discussion

Figure 1 shows the two absorption peaks in the 3 μ region observed for each of the three substances in carbon tetrachloride solutions. The relative height of the 3.0 μ peak with respect to the 2.9 μ peak dropped, when the concentration was lowered from 0.0002 mole/l. (curve A of Fig. 2) to 0.0001 mole/l. (curve B). This is due to the fact that the 3.0 μ band arises partly from the N–H group involved in intermolecular hydrogen bond. However, as stated in a previous paper,[2] this band must be due in the main to the presence of B form with *intramolecular* hydrogen bond, since there should be much more conspicuous intensity change of the 3.0 μ band on dilution, if this band would entirely be due to the N–H group involved in *intermolecular* hydrogen bond.

It is worthy of note that the relative intensity of the 3.0 μ band decreases in the order of AGNA, AANA and ALNA; in other words the intensity of this band decreases as the size of the side chain becomes larger. We can conclude from this fact that the hydrogen-bonded NH group becomes less abundant in the order stated above, although we cannot distinguish at the present stage between the *intramolecular* and *intermolecular* hydrogen bond.

As the concentration of the solution is raised the intensity of the 2.9 μ band becomes weaker until this band becomes hardly detectable, while the intensity of the 3.0 μ band becomes stronger (see Fig. 3) and the wave length of the peak of this band is shifted gradually from 2.98 to 3.03 μ. At the same time the 3.22 μ band began to appear. All these phenomena are characteristic to the molecule with peptide bond in which N—H and C=O bonds

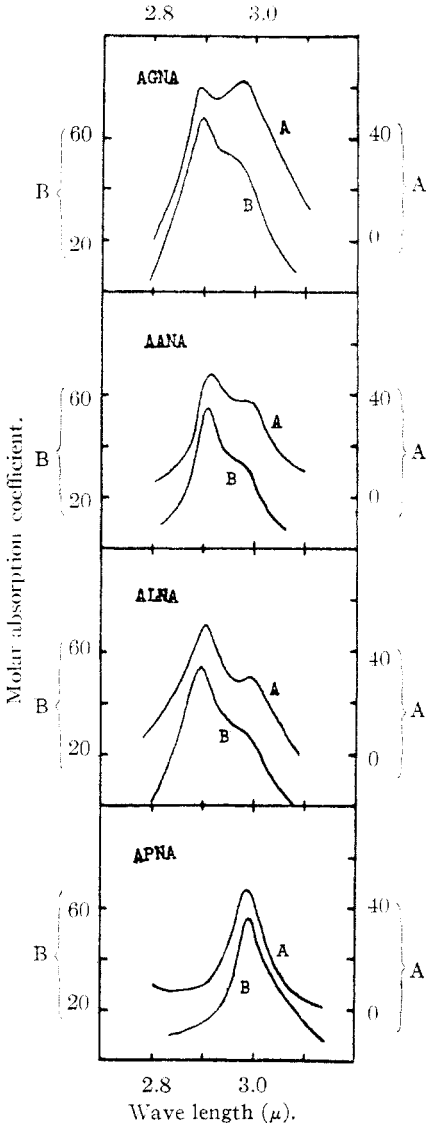

Fig. 2.—Absorption curves of carbon tetrachloride solutions in the 3μ region at 30°: A, 0.0002 mole/l. × 10 cm.; B, 0.0001 mole/l. × 20 cm.

are in the *trans* position with respect to each other[3] and this is in conformity with the molecular configurations of ALNA presented by us.

From the comparison of the results of the present experiment with that of the previous one[3] it will be seen that the molecular association is much stronger in ALNA than in N-alkylacetamides. For example, Fig. 3 shows that in the solution of ALNA at 30° there is a considerable number of associated molecules at the concentration of 0.001 mole/l. at which nearly all the molecules of N-alkylacetamides were found to exist in single state. This is explained as due to the fact that E form of ALNA can make double hydrogen bonds and, therefore, this substance shows stronger association as compared with N-alkylacetamides whose molecules associate through single hydrogen bond.[3] It will easily be seen that AGNA and AANA behave

(3) S. Mizushima, T. Shimanouchi, S. Nagakura, K. Kuratani, M. Tsuboi, H. Baba and O. Fujioka, THIS JOURNAL, **72**, 3490 (1950); M. Tsuboi, *Bull. Chem. Soc. Japan*, **22**, 215, 255 (1949).

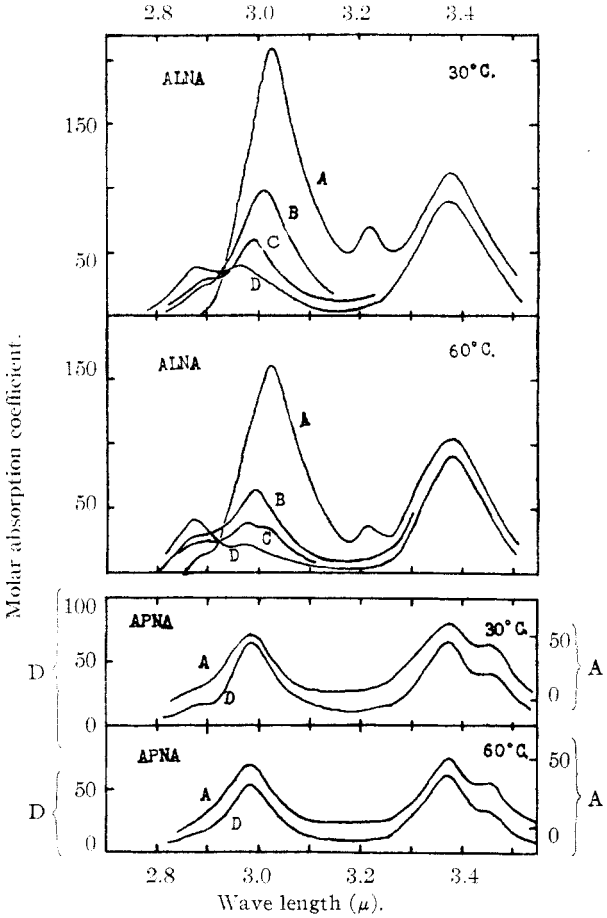

Fig. 3.—Absorption curves of carbon tetrachloride solutions in the 3μ region; A, 0.05 mole/l. × 0.1 cm.; B, 0.0025 mole/l. × 2 cm.; C, 0.001 mole/l. × 5 cm.; D, 0.0005 mole/l. × 10 cm.

similarly as ALNA in their molecular association.

As will be seen from Figs. 2 and 3, APNA behaves quite differently from the other three substances, AGNA, AANA and ALNA—in the concentration range from 0.0001 mole/l. to 0.05 mole/l. and at 30° APNA showed only one absorption peak at 2.98 μ and the molar absorption coefficient of this band was found almost independent of concentration. This band remained unchanged when the temperature was raised as high as 60°. Therefore, we can conclude that the molecule of APNA exists only in B form or the folded form shown in Fig. 4 and the absorption band stated above arises from the N–H group involved in *intramolecular* hydrogen bond. Any other molecular configurations without this bond seems much more unstable as compared with B form.

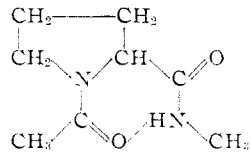

Fig. 4.—Molecular configuration of acetylproline N-methylamide.

As stated in our previous papers[1,2] the object of this series of researches is to determine the structure

of such kinds of simple molecules as would permit the reliable prediction of reasonable configuration of the polypeptide chain. In this connection the result of the present experiment would be used to derive a conclusion that glycine, alanine and leucine residues take both E and B forms in a polypeptide chain, while proline residue takes only B-form.

HONGO, TOKYO, JAPAN

[CONTRIBUTION FROM THE NATIONAL BUREAU OF STANDARDS]

The Relation between the Absorption Spectra and the Chemical Constitution of Dyes. XXV. Phototropism and *cis-trans* Isomerism in Aromatic Azo Compounds[1]

BY WALLACE R. BRODE, JOHN H. GOULD AND GEORGE M. WYMAN

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The photochemical *cis–trans* isomerization of a number of amino and hydroxy azo dyes in benzene solution was studied spectrophotometrically. The isomerization reaction was found to take place at such a rapid rate with most of these dyes that, in order to obtain reproducible spectral absorption curves, it was necessary to measure them with the aid of a rotating shutter which permitted the essentially simultaneous irradiation of the solutions during their measurement. The most probable spectral absorption curves of the *cis* forms of these dyes were calculated. The absorption curves of the *cis* forms of two typical dyes are shown and the spectral absorption data for the remaining dyes are tabulated. The spectra of *o*-hydroxyazobenzene and its derivatives were only slightly, or not at all, affected by irradiation, probably because of hydrogen bonding. Correlations between the absorption spectra, chemical structure, and spatial configuration of these dyes are discussed.

Introduction

The photochemical *cis–trans* isomerization of thioindigo dyes dissolved in organic solvents has recently been reported from this Laboratory,[2,3] and the results of that investigation indicated the desirability of re-examining the absorption spectra of organic compounds containing one or more double bonds for evidence of such *cis–trans* isomerizations. Azo dyes were chosen for this investigation because of their wide use in industry and because the phototropic behavior of some azo dyes had been observed previously.[4]

The unstable *cis* isomer of azobenzene was first isolated by Hartley by repeated fractional crystallizations of irradiated solutions of azobenzene.[5,6] The isolation of the *cis* isomers of azobenzene and of a number of its alkyl, nitro and halogen derivatives by the chromatographic separation of irradiated solutions of the appropriate azo compounds was carried out by Cook shortly afterwards.[7,8] Both investigators reported that solutions of 4-amino-, 4-acetylamino- and 4-hydroxyazobenzene (derivatives of which constitute the majority of azo dyes) exhibited considerable darkening in color upon irradiation, thus indicating *trans* to *cis* isomerization. These *cis* isomers appeared to be so short-lived, however, that they could not be estimated photometrically,[6] and, during the attempted chromatographic analyses, only an indefinite "tailing" and the appearance of subsidiary zones indicated their presence, but none could actually be isolated.[7,8]

Preliminary observations in this Laboratory indicated that irradiation of benzene solutions of 4-aminoazobenzene and of 4-dimethylaminoazobenzene by the same technique as had been used for thioindigo dyes[2] results in a change in their absorption spectra, but that there was a rapid reversal of the spectrum in the darkness[9] of the cell compartment during the measurement. In order to overcome this rapid reversal and thus to enable one to obtain reproducible results, a rotating shutter was constructed. The function of this shutter is to permit the irradiation of the solution for a fraction of a second (at the same moment when the measuring beam is cut off by the light chopper of the Cary Spectrophotometer) and, on the other hand, to shut off the irradiating beam when the measuring beam impinges upon the sample photocell.[10]

Experimental

(a) **Purification of Dyes.**—The dyes used for this investigation, which had been prepared by coupling diazotized aromatic amines with the appropriate amino or hydroxy compounds, were purified by repeated recrystallizations from aqueous alcohol. The dyes and their melting points are listed in Table I.

(b) **Preparation of Solutions.**—Approximately 0.010 g. of each dye was weighed and dissolved in 300–400 ml. of freshly distilled benzene and diluted to 500 ml. in a volumetric flask. Appropriate dilutions of these stock solutions were used whenever necessary.

(c) **Measurement of Absorption Spectra.**—The absorption spectra were determined by means of a Cary Recording Quartz Spectrophotometer (Model 12) with matched fused quartz absorption cells and the solvent as the reference standard.

(d) **Irradiation of Solutions.**—The dye solution contained in the absorption cell placed in the sample compartment was first exposed for about 10 minutes to uninterrupted filtered radiation from a 100-watt incandescent lamp, in order to accelerate the attainment of photochemical equilibrium. During this time the light was prevented from impinging upon the photocell. The rotating shutter was then placed in position[10] and intermittent irradiation continued until equilibrium was reached, that is, until no further changes in the absorption spectra occurred. Irradiation of the solution was maintained during the actual measurement of the absorption spectrum.

(1) Presented at the XIIth International Congress of Pure and Applied Chemistry, New York, N. Y., September, 1951.

(2) G. M. Wyman and W. R. Brode, THIS JOURNAL, **73**, 1487 (1951).

(3) W. R. Brode and G. M. Wyman, *J. Research Natl. Bur. Standards*, **47**, 170 (1951).

(4) E. I. Stearns, *J. Optical Soc. Am.*, **32**, 282 (1942).

(5) G. S. Hartley, *Nature*, **140**, 281 (1937).

(6) G. S. Hartley, *J. Chem. Soc.*, 633 (1938).

(7) A. H. Cook, *ibid.*, 876 (1938).

(8) A. H. Cook and D. G. Jones, *ibid.*, 1309 (1939).

(9) The intensity of the measuring beam is too low to cause any photochemical effects.

(10) For a detailed description of the rotating shutter, *cf.* J. H. Gould and W. R. Brode, *J. Optical Soc. Am.*, **42**, 380 (1952).