Electronic Absorption Spectra of Hydrogen Bonded Amides

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The vacuum ultraviolet absorption spectra of various hydrogen bonded amides were measured at various temperatures and concentrations. It was found that the characteristic absorption bands of amides, which appear at 180—190 mμ, greatly change by hydrogen bonded dimer formation and moreover their changes are sensitive to the relative orientation of the monomers. That is to say, they shift to shorter wavelengths by about 6000 cm⁻¹ by the hydrogen bonded ring dimer formation and shift to longer wavelengths by about 3000 cm⁻¹ by the hydrogen bonded chain dimer formation. The theoretical consideration on the energy level splittings due to hydrogen bonded dimer formation explained satisfactorily these observations. The results seem to be important in connexion with the determination of the configurations of polymers which contain peptide bonds as a unit.

Vakuum-UV-Absorptionsspektren verschiedener Amide, die H-Brücken ausbilden, wurden vermessen. Die charakteristische Bande der Amide bei 180—190 mμ wird durch H-Brücken in Abhängigkeit von der Form des gebildeten Dimeren verschoben: so ist die Verschiebung im Falle der Ringbildung + 6000 cm⁻¹, im Falle linearer Verkettung − 3000 cm⁻¹. Dies Phänomen wird theoretisch erklärt. Die Ergebnisse sind für die Strukturaufklärung von polymeren Amiden von Bedeutung.

Les spectres d'absorption U.V. dans le vide ont été mesurés pour différentes amides formant des liaisons hydrogène. La bande caractéristique des amides vers 180—190 mμ est déplacée lors de la formation d'une liaison hydrogène: pour des dimères ce déplacement est de + 6000 cm⁻¹ lors de la formation d'un composé cyclique et de − 3000 cm⁻¹ lors de la formation d'un composé linéaire. On explique théoriquement ce phénomène. Les résultats ont une signification pour la compréhension de la structure des amides polymères.

Introduction

Hydrogen bond may be regarded as one of the most interesting subjects in the fields of biology and biochemistry as well as of physical chemistry. For example, Watson and Crick's model [1] of DNA indicates that hydrogen bonds play an important role in the formation of the double helix coil. Löwdin [2] suggested that proton transfer between the bases of nucleic acids through hydrogen bond may greatly contribute to mutation. El-Bayoumi and Kasha [3] reported that energy transfer occurs in hydrogen bonded aromatic systems.

Tinoco [4] and Rhodes [5] presented a theory of hypochromism and hyperchromism in polymers. According to their theory, the second order configuration of polymers can be correlated with the intensities of their absorption bands. For instance, the characteristic absorption bands of the bases of nucleic acids, which

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appear in the 260 mµ region, change their intensities by the formation of the hydrogen bonded helical polymers. However, an appreciable shift of the absorption peak due to the polymer formation has never been reported [6].

We undertook to study from both experimental and theoretical points of view the shifts of the absorption spectra of some amides due to the hydrogen bonded dimer formation, for the purpose of obtaining fundamental knowledge about the relation of the band position with the relative orientation of amide in peptide polymers.

Experimental

Measurements of absorption spectra of δ-valerolactam (m.p. 39°), ε-caprolactam* (m.p. 70°) 2,5-diketopiperazine (m.p. 311—312°), and N-methylacetamide (m.p. 28°) — all carefully purified by recrystallizations and vacuum-distillations — were made with a Cary recording spectrophotometer model 14 M and a vacuum ultraviolet spectrophotometer constructed in our laboratory [7]. A quartz cell of 0.01 cm light-path length was used for the measurements of solution spectra. A quartz cell of 2.5 cm long was used for the measurements of gaseous δ-vale-

rolactam, ε-caprolactam and 2,5-diketopiper-azine and quartz cells of 0.1 and 0.01 cm long were used for the measurements of gaseous N-methylacetamide. Since all the samples mentioned above are solid at room temperature, the measurements in gaseous states were made at high temperatures (60°—168°). Furthermore, since the samples are all deliquescent except for 2,5-diketopiperazine, careful treatment is necessary for avoiding moisture. The sample was warmed in vacuo until it was liquified for the purpose of degassing and dehydration and then distilled or sublimated into the optic al cell. A pair of copper plates, a heater and a thermocouple were attached to the cell. This is shown in Fig. 1. Lastly we measured the

Fig. 1. Experimental apparatus for the optical measurement at high temperature

absorption spectra heating the absorption cell. To avoid decomposition, the heating temperature was restricted below the boiling point of the sample. In this experiment, the vapor pressure of the sample could not be determined accurately. However, from the known molar extinction coefficients of the first $\pi \to \pi^*$ transition bands of the respective samples in aqueous solution, we could roughly estimate their vapor pressures. Concerning δ-valerolactam and ε-caprolactam, the absorption measurements were made at three or four different temperatures up to 160°. As for N-methylacetamide, the measurement was done by changing the vapor pressure of the sample. The temperature and pressure dependence of the absorption spectra was measured for the purpose of obtaining knowledge about the change of the absorption spectrum due to dimer formation.

The results of the measurements are shown in Figs. 2—6. In Figs. 3—5, the ordinates represent the absorbance. The molar extinction coefficients could not be evaluated because of the uncertainties of the vapor pressures. In Fig. 6, the origins of the ordinates which represent the absorbance values in arbitrary scale are different for curves 1, 2 and 3.

Theoretical

In order to estimate the magnitude of the change in the energy levels of the amides due to the dimer or trimer formation, calculations of the DAVYDOV type splitting were made with various types of hydrogen bonded amides; namely ring
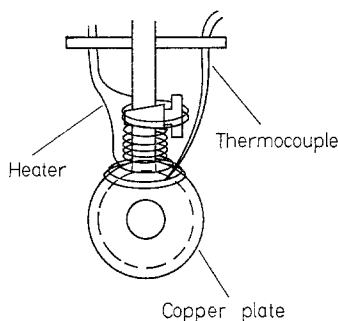
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* The authors should like to thank Dr. Y. KURITA, Basic Research Laboratories, Toyo Rayon Co. Ltd., for his kindness in presenting this sample.

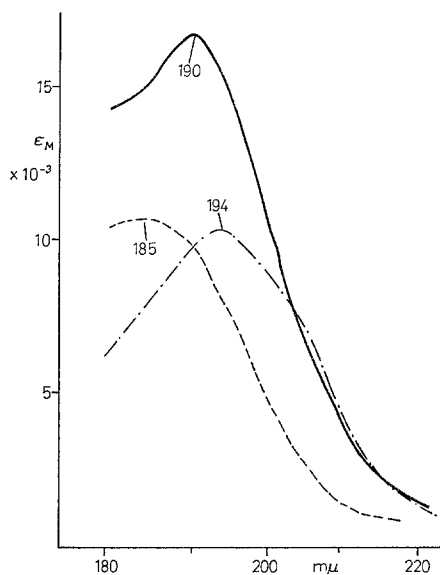Fig. 2. Spectra of the aqueous solutions of 2,5-diketopiperazine (————), δ-valerolactam (—·—·—), and N-methylacetamide (- - - - - -)

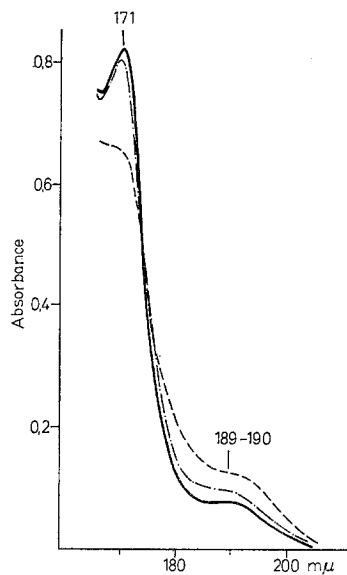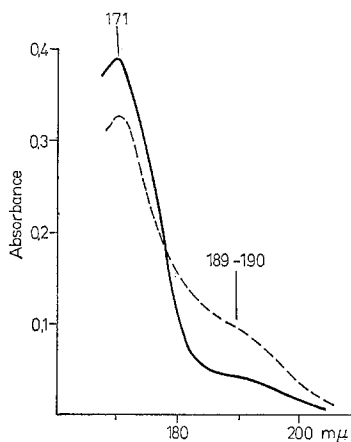Fig. 3. Vacuum ultraviolet absorption spectrum of gaseous δ-valerolactam; ———— 61 °C, —·—· 85 °C, - - - - 168 °C

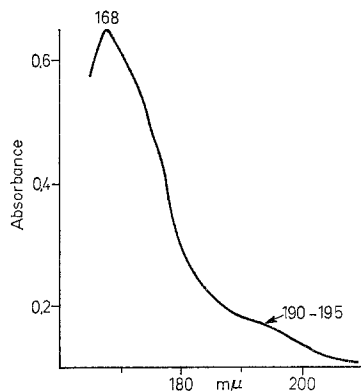Fig. 4. Vacuum ultraviolet absorption spectrum of gaseous ε-caprolactam. ———— 102 °C, - - - - - - 155 °C

Fig. 5. Vacuum ultraviolet absorption spectrum of gaseous 2,5-diketopiperazine at 90 °C

dimer, chain dimer and chain trimer. In addition to them, 2,5-diketopiperazine was taken as an example in which two amide groups are separated by two methylene groups and the DAVYDOV type interaction may be expected between them. Actual calculations were made by the transition density operator method developed by LONGUET-HIGGINS [8].
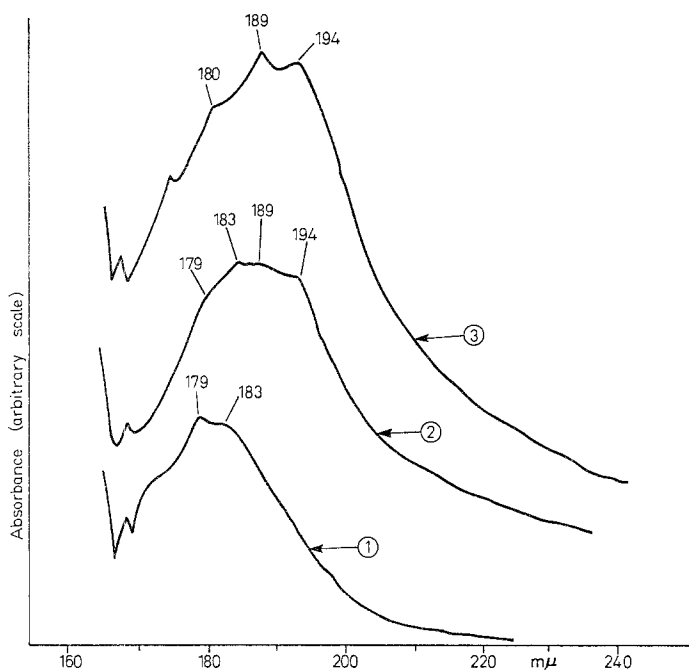
Fig. 6. Vacuum ultraviolet absorption spectrum of gaseous N-methylacetamide at various pressures. Curve 1: 2.5 cm light path length (lowest pressure, $<$ 1 mm Hg); curve 2: 0.1 cm light path length (medium pressure, $\sim$ 10 mm Hg); curve 3: 0.01 cm light path length (highest pressure, $\sim$ 100 mm Hg)

Let us denote the component systems as s and s'. The Hamiltonian (H) of a dimer (which is composed of s and s') can be represented as follows:

$$H = H_s + H_{s'} + V_{ss'} .\tag{1}$$

Here H_s and $H_{s'}$ are the zeroth order Hamiltonian of the component systems s and s'; $V_{ss'}$ is the interaction term between them.

The eigenfunctions (ψ) and the eigenvalues (E) of the total electronic Hamiltonian (H) for the ground and the excited states of the dimer are represented as follows:

$$\psi_G = \varphi_g \varphi_{g'}\tag{3}$$

$$\psi_E = \frac{1}{\sqrt{2}} (\varphi_g \varphi_{e'} \pm \varphi_e \varphi_{g'})\tag{4}$$

$$E_G = \varepsilon_g + \varepsilon_{g'} + \langle \varphi_g \varphi_{g'} \mid V_{ss'} \mid \varphi_g \varphi_{g'} \rangle\tag{5}$$

$$E_E = \tfrac{1}{2}(\varepsilon_g + \varepsilon_{g'} + \varepsilon_e + \varepsilon_{e'}) \pm \langle \varphi_g \varphi_{e'} \mid V_{ss'} \mid \varphi_e \varphi_{g'} \rangle + \langle \varphi_e \varphi_{g'} \mid V_{ss'} \mid \varphi_e \varphi_g \rangle\tag{6}$$

with

$$H_s \varphi_g = \varepsilon_g \varphi_{g'} , H_{s'} \varphi_{g'} = \varepsilon_{g'} \varphi_{g'} , H_s \varphi_e = \varepsilon_e \varphi_{e'} , H_{s'} \varphi_{e'} = \varepsilon_{e'} \varphi_{e'} .$$

In our case, $\varepsilon_g = \varepsilon_{g'}, \varepsilon_e = \varepsilon_{e'}$.

By the use of the above equations, the change in the transition energy $(\varDelta E)$ due to the dimer formation can be written as follows:

$$\varDelta E = (E_E - E_G) - (\varepsilon_e - \varepsilon_g)$$
$$= \langle \varphi_e \varphi_{g'} \mid V_{ss'} \mid \varphi_e \varphi_{g'} \rangle \pm \langle \varphi_e \varphi_{g'} \mid V_{ss'} \mid \varphi_g \varphi_{e'} \rangle - \langle \varphi_g \varphi_{g'} \mid V_{ss'} \mid \varphi_g \varphi_{g'} \rangle .\tag{7}$$

This quantity is then evaluated by the transition density operator method of LONGUET-HIGGINS [8].
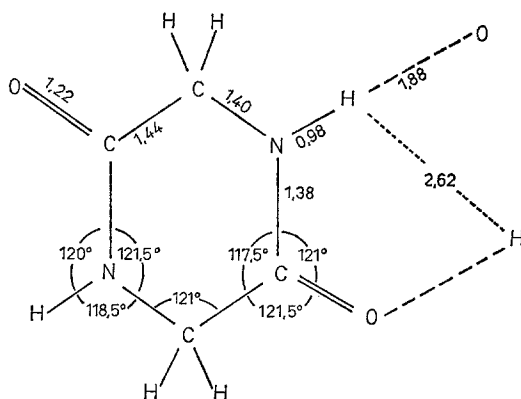
Fig. 7. Geometrical structure of 2,5-diketopiperazine (distances in Å)

Here let us explain the geometrical parameters adopted in the present calculations. The structure of 2,5-diketopiperazine was determined by the electron diffraction experiment by VAINSHTEIN [9] as is shown in Fig. 7. From this figure, it may be inferred that the lone pair orbital of the oxygen atom which takes part directly in hydrogen bond formation is in sp^2 hybridized state. Hydrogen bonded δ-valerolactam and ε-caprolactam whose N–H and C=O bonds are located in cis position with respect to C–N bond are known to form a ring dimer [10, 11], and on the other hand hydrogen bonded N-methylacetamide whose N–H and C=O bonds are in trans position [12] forms the chain dimer or trimer. However, their geometrical configurations have never been determined accurately. Therefore, we assumed them as is shown in Figs. 8 and 9 by an analogy with 2,5-diketopiperazine.

As for the zeroth order wavefunctions of the π-electron systems of the isolated amide, we took the wavefunctions of formamide calculated by NAGAKURA [13]. These wavefunctions explain satisfactorily the characters of the ground and excited states of formamide [14—16]. The evaluated transition densities $\langle \alpha \mid \varrho\,(r) \mid \beta \rangle$ are given in the Table. By the aid of these values, the energies of the
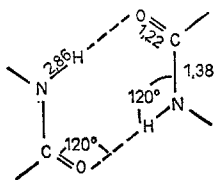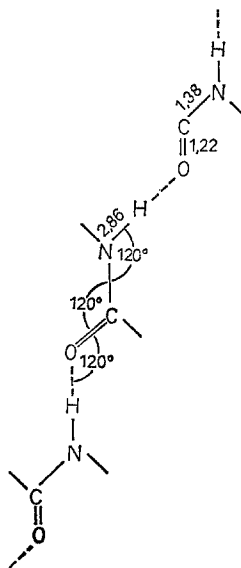

Fig. 8

Fig. 9

Fig. 8. Assumed geometrical configuration of ring dimer (distances in Å)

Fig. 9. Assumed geometrical configuration of chain polymer (distances in Å)

Table. *Values of the Transition Densities*[a]

	N	C	O
$\langle 0 \mid \varrho\,(r) \mid 0 \rangle$	+0.2089	+0.3316	−0.5405
$\langle 0 \mid \varrho\,(r) \mid 1 \rangle$	+0.3458	−0.0184	−0.3364
$\langle 1 \mid \varrho\,(r) \mid 1 \rangle$	+0.6025	−0.3617	−0.2408

[a] 0 and 1 indicate the π ground and the first π^* excited state of amide, respectively.

interaction between the isolated systems were evaluated. The results of the calculations are schematically shown in Fig. 10. In this figure, E^+ and E^- correspond to the energies of the excited states of the dimer, the wavefunctions of which are $1/\sqrt{2}(\varphi_g\varphi_{e'} + \varphi_e\varphi_{g'})$ and $1/\sqrt{2}(\varphi_g\varphi_{e'} - \varphi_e\varphi_{g'})$, respectively. In the parallel orientation $E_G \rightarrow E_{E^-}$ is the allowed transition and in the head to tail orientation, $E_G \rightarrow E_{E^+}$ becomes allowed. From these results, it is expected that the first $\pi \rightarrow \pi^*$ transition band shifts to shorter wavelengths by the formation of the ring dimer and shifts to longer wavelengths in the case of the chain dimer or trimer.
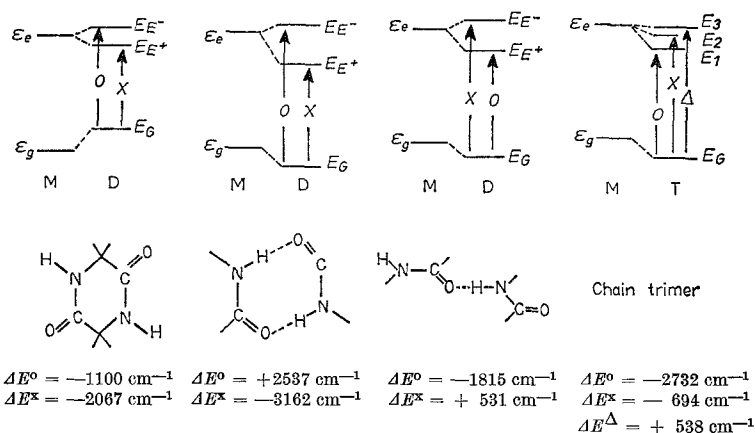

$\Delta E^\circ = -1100 \text{ cm}^{-1}$ $\Delta E^\circ = +2537 \text{ cm}^{-1}$ $\Delta E^\circ = -1815 \text{ cm}^{-1}$ $\Delta E^\circ = -2732 \text{ cm}^{-1}$
$\Delta E^x = -2067 \text{ cm}^{-1}$ $\Delta E^x = -3162 \text{ cm}^{-1}$ $\Delta E^x = + 531 \text{ cm}^{-1}$ $\Delta E^x = - 694 \text{ cm}^{-1}$
                                                                                                                        $\Delta E^\Delta = + 538 \text{ cm}^{-1}$

Fig. 10. Calculated energy levels of dimer and trimer. o allowed transition, × forbidden transition, ⊿ nearly forbidden transition

Results and Discussion

As is clearly seen in Fig. 2, the aqueous solutions of δ-valerolactam, 2,5-diketopiperazine and N-methylacetamide show strong absorption bands whose peaks are located at 194, 190 and 185 mμ, respectively. This experimental result coincides well with that by HAM and PLATT [17]. According to the investigation by KLOTZ and FRANZEN [18], N-methylacetamide molecules do not form hydrogen bonds with each other in aqueous solution of the concentration region of $10^{-2} - 10^{-3}$ Mol/l. The absorption spectra of δ-valerolactam and 2,5-diketopiperazine in aqueous solution show no change in the spectral positions and the intensities by changing the concentration in the range of $10^{-2} - 10^{-3}$ Mol/l. This probably means that these compounds exist in a monomer state as is similar with N-methylacetamide. Therefore, the above spectra may be ascribed to the monomer (of course, hydrogen

bonds may be formed between amides and water). Moreover, the oscillator strengths of these absorption bands are 0.308, 0.468 and 0.338 for δ-valerolactam, 2,5-diketopiperazine and N-methylacetamide, respectively. From the strong intensities of these bands, we can safely conclude that they correspond to the first $\pi \rightarrow \pi^*$ transition band characteristic of amides. Furthermore, from an analogy with the spectrum of formamide [13], they may be regarded as the intramolecular charge-transfer band accompanying an electron transfer from the amino group to the carbonyl group.

The absorption spectra of gaseous δ-valerolactam, ε-caprolactam and 2,5-diketopiperazine differ appreciably from the spectra in aqueous solution. These are shown in Figs. 3—5.

δ-valerolactam shows two absorption bands, one of them being located in the region of 189 ∼ 190 mμ and the other at 171 mμ. The latter is five times stronger than the former. In view of the peak wavelength (194 mμ) of the monomer observed with the aqueous solution, the first band at 189 − 190 mμ of gaseous δ-valerolactam may safely be ascribed to the monomer in gaseous state. In this connexion, it may be added that the first $\pi \rightarrow \pi^*$ transition band of amides at about 190 mμ shows only a small solvent shift. (That is to say, the 185 mμ band of N-methylacetamide in aqueous solution appears at about 180 mμ in gaseous state. As for this molecule, it is possible to measure the absorption spectrum of the pure monomer in gaseous state).

The theoretical consideration shown in Fig. 10 concludes that the 190 mμ band of amide shifts toward shorter wavelengths by about 2500 cm⁻¹ as the result of ring dimer formation. From this result it may be inferred that the second observed band at 171 mμ can be ascribed to the ring dimer of δ-valerolactam, although the observed wave number shift (5500 ∼ 6000 cm⁻¹) is larger than the theoretical value.

In order to support this interpretation, we measured the temperature dependence of the absorption spectrum of gaseous δ-valerolactam in the range of 65° to 168°. As is shown in Fig. 3, the intensity of the first band increases by the elevation of the temperature, while second band decreases its intensity.

In the spectrum of *ε-caprolactam*, the similar phenomenon was observed (see Fig. 4). In this case, the monomer-dimer shift amounts to 6000 cm⁻¹. Thus we concluded that monomer-ring dimer equilibrium exists for δ-valerolactam and ε-caprolactam and that the longer wavelength band may be assigned to the first $\pi \rightarrow \pi^*$ transition of the monomer and the shorter wavelength one may be due to the hydrogen bonded ring dimer.

*2,5-diketopiperazine** contains two peptide bonds within a molecule and has a possibility to form ring dimer by hydrogen bond formation. By an analogy with the above two lactams, the $\pi \rightarrow \pi^*$ transition band may be expected to shift

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* The calculated result of the energy level splitting of 2,5-diketopiperazine given in Fig. 10 is concerned with the intramolecular interaction between the two peptide bonds. According to the calculation, the first $\pi \rightarrow \pi^*$ transition band of 2,5-diketopiperazine appears at longer wavelengths by 1100 cm⁻¹ compared with that of single peptide bond. This theoretical expectation may correspond to the fact that the observed peak positions shown in Fig. 2 are 190 and 185 mμ for the aqueous solution of 2,5-diketopiperazine and N-methylacetamide.

toward shorter wavelengths, though quantitative theoretical consideration has not been performed for this case. Thus the spectrum of 2,5-diketopiperazine at 90° shown in Fig. 5 may be interpreted as the mixture of the dimer and monomer spectra. The strong band at 168 mμ may conceivably be due to the dimer and the weak and broad one at 190—195 mμ due to the monomer ($\Delta E \sim 7500$ cm^{-1}).

Now, let us turn our attention to the absorption spectra of N-*methylacetamide*. The results of the pressure dependence of the vacuum ultraviolet absorption of N-methylacetamide are shown in Fig. 6. The experiment by the use of the optical cell of 2.5 cm length and at low pressure of the sample resulted in the observation of the absorption band at 183 and 179 mμ. The doublet may be due to a vibrational structure. In the experiment of the high pressure of the sample by the use of 0.01 cm optical cell, the absorption peaks appear at 194, 189 and 180 mμ in the wavelength region longer than 180 mμ. The experiment of the medium pressure using a cell 0.1 cm long, the absorption band becomes broad and the peaks or shoulders appear at 194, 189, 183 and 179 mμ. That is to say, two forms stable at the high and the low pressures are found to coexist in the medium pressure. It is reasonable to assume that the stable form at the high pressure is a dimer. From the above experimental results, it may be concluded that the $\pi \to \pi^*$ transition band of N-methylacetamide shifts to longer wavelengths by 10 mμ (~ 3000 cm^{-1}) by the chain dimer formation.

From these experimental results, we may safely conclude that the first $\pi \to \pi^*$ transition band shifts to shorter wavelengths by about 6000 cm^{-1} by the hydrogen bonded ring dimer formation and shifts to longer wavelengths by about 3000 cm^{-1} by the formation of the hydrogen bonded chain dimer. This result at least qualitatively coincides with the theoretical conclusion shown in Fig. 10.

We found theoretically that the first $\pi \to \pi^*$ transition band shifts to shorter wavelengths by 2500 cm^{-1} by the ring dimer formation and to longer wavelengths by 1800 cm^{-1} by the chain dimer formation*. The calculated values are generally smaller than the corresponding observed shifts. In the above calculations, we disregarded exchange interactions between the isolated systems of the dimer or trimer. This might be one of the reasons for the discrepancy between the theoretical and observed values.

Recently, RHODES [5] and TINOCO [4] and others [19, 20] proposed the theory of hypochromism and hyperchromism and many workers [6, 21] adopted it for the purpose of correlating the intensity of the absorption band of a polymer with its second order configuration. However, with respect to the relation of the spectral position of a polymer with the configuration, there has been only few examples. In our study, the position of the characteristic strong absorption band of amides which appears in the vacuum ultraviolet region was found to be sensitive to the orientation of the monomers in the polymer. The observation of its position may give some useful information in discussing the configuration of the polymer which contains peptide bonds as a unit.

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* There is a possibility of chain trimer formation for N-methylacetamide. However, we disregarded the possibility, because in the concentration of 10^{-2}—10^{-3} Mol/l, corresponding to our experimental conditions, the trimer formation cannot be expected [10].

References

[1] WATSON, J. D., and F. H. C. CRICK: Nature 171, 737 (1953).
[2] LÖWDIN, P. O.: Advances chem. Physics 8, 177 (1965).
[3] EL-BAYOUMI, M. A., and M. KASHA: J. chem. Physics 34, 2181 (1961).
[4] TINOCO, I., Jr.: J. chem. Physics 34, 1067 (1961).
[5] RHODES, W.: J. Amer. chem. Soc. 83, 3609 (1961).
[6] HOSOYA, H., J. TANAKA, and S. NAGAKURA: J. molecular Spectroscopy 8, 257 (1962).
[7] TSUBOMURA, H., K. KIMURA, K. KAYA, J. TANAKA, and S. NAGAKURA: Bull. chem. Soc. Japan 37, 417 (1964).
[8] LONGUET-HIGGINS, H. C.: Proc. Roy. Soc. (London) A235, 537 (1956).
[9] VAINSHTEIN, B. K.: J. physic. Chem. U.S.S.R. 29, 327 (1956).
[10] TSUBOI, M.: Bull. chem. Soc. Japan 22, 215 and 255 (1949).
[11] — Bull. chem. Soc. Japan 24, 75 (1951).
[12] MIZUSHIMA, S., T. SHIMANOUCHI, S. NAGAKURA, K. KURATANI, M. TSUBOI, H. BABA, and O. FUJIOKA: J. Amer. chem. Soc. 72, 3490 (1950).
[13] NAGAKURA, S.: Molecular Physics 3, 105 (1960).
[14] KURLAND, R. J., and E. B. WILSON, Jr.: J. chem. Physics 27, 585 (1959).
[15] HUNT, H. D., and W. T. SIMPSON: J. Amer. chem. Soc. 75, 4540 (1953).
[16] PETERSON, D. L., and W. T. SIMPSON: J. Amer. chem. Soc. 79, 2375 (1957).
[17] HAM, J. S., and J. R. PLATT: J. chem. Physics 20, 335 (1952).
[18] KLOTZ, I. M., and J. S. FRANZEN: J. Amer. chem. Soc. 84, 3461 (1962).
[19] MCLACHLAN, A. D., and M. A. BALL: Molecular Physics 8, 581 (1964).
[20] NESBET, K.: Molecular Physics 7, 221 (1964).
[21] RICH, A., and M. KASHA: J. Amer. chem. Soc. 82, 6197 (1960).

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