On the Molecular Rotations of Polypeptides of Alanine[*]

By Hideo MURAKAMI

(Received May 18, 1953)

Introduction

Abderhalden and Gohdes observed the specific rotations $[\alpha]_D$ for a series of polypeptides of *l*-alanine.(1) Levene and Yang also observed them for a series of *d*-alanine, and obtained almost equal data concerning their absolute values.(2)

---

[*] This work was reported at the meeting for general discussion of protein structure held on May 20, 1951, in Osaka, under the auspices of the Chemical Society of Japan.

1) E. Abderhalden and W. Gohdes, *Ber.*, **64**, 2070 (1931).

2) P. A. Levene and P. S. Yang, *J. Biol. Chem.*, **99**, 405 (1932).

As was pointed out by Kauzmann and Eyring, the molecular rotation per one alanine residue $[M]_D/n$ (n is the number of residues) increases remarkably as the polypeptide chain becomes longer. This fact indicates that the neighbouring alanine residues are not optically independent and there will be some interactions. Kauzmann and Eyring gave an interesting suggestion for this point based on their consideration for the optical rotations of sugar.[3] Firstly, they noticed that the molecular rotations of ring compounds such as arabinose, xylose, mannose, galactose, glucose, etc. are relatively great (30°~260°) compared with those of corresponding alcohols (less than 10°). They interpreted these differences between ring and chain compounds by considering the statistical free rotation (restricted rotation) around a single bond. That is, in case of open chain compounds, the above-mentioned statistical free rotation is allowed, and the first order effect of optical rotation is almost entirely diminished. On the other hand, in the case of ring compounds, the rotation around the single bond is prevented on account of their rigid structures and the first-order effect of optical rotation can contribute considerably. Taking these facts into account, they suggested that the molecules of polypeptides of alanine may also form a ring involving hydrogen bond or some other type of bond. Based on this suggestion, we try to explain theoretically the observed rotations in Table I by assuming the probable model for the configuration of polypeptide.

TABLE I

OBSERVED VALUES OF SPECIFIC AND MOLECULAR ROTATION

Polypeptides	$[\alpha]_D$ in 2 N HCl		$[M]_D$	$[M]_D/n$ n is the number of residues
	Abderhalden	Levene	Levene	
alanine	+ 14.5°	− 14.5°	− 13°	−13°
alanyl-alanine	− 37.6°	+ 36.5°	+ 58°	+29°
di-alanyl-alanine	− 77.3°	+ 77.5°	+179°	+60°
tri-alanyl-alanine	−115°	+115°	+347°	+87°
tetra-alanyl-alanine	−133°	+132.5°	+494°	+99°

I. MOLECULAR MODEL

Ring Strncture of Polypeptide Chain In order to calculate the molecular rotation theoretically, it is necessary that the detailed structure of the molecule is known. Conversely, if the calculated results for a probable model agree well with experimental results, the assumed model becomes more reliable.

The configurations of the polypeptide chain in protein have been studied extensively, and the numerous probable models for them have been presented by many authors.[4-9] These models are mostly based on the experimental data for crystal, fiber or film of highly polymerized large polypeptide molecules whether they be natural or synthetic.

At present, however, we are researching the configurations of small polypeptide molecules in dilute water solution, so the situations are considerably different from the above-described ones. Moreover, our model must be rigid enough and correspond to the ring structure which was demanded by Kauzmann and Eyring. Therefore, it is required that not only the known bond lengths and bond angles are satisfied approximately, but also many hydrogen bonds are formed to construct the rings. Further, it can be expected that the rigidity of the ring becomes greater as the ring becomes smaller, so long as the geometrical conditions for the stability of hydrogen bonds and internal rotations are satisfied.

Considering these conditions, we picked out (2_7 a) the model of Huggins and (2_7 b) the model of Shimanouchi and Mizushima as the most probable ones. In these models, sevenmembered rings are formed by hydrogen bonds, and concerning internal rotation, the stable configurations of the polypeptide chain are kept. At the present stage, however, the application of these models should be limited to the configurations of the relatively short polypeptide chain in solution. For the configurations of highly polymerized polypeptide chain in protein, some other factors should be taken into account, and their complete determination is a task for future study.

Equilibrium between Isomers Since the above-mentioned models have been presented for the folded α-type keratin, we denote the models (2_7 a) and (2_7 b) as $\alpha_{(1)}$ and $\alpha_{(2)}$ respectively. The only difference between $\alpha_{(1)}$ and $\alpha_{(2)}$ is the relative internal orientation between the side chain and the main chain, that is, in the case of $\alpha_{(1)}$, its orientation exists in G (Gaush) form, and in the case of

---

[3] W. Kauzmann and H. Eyring, *J. Chem. Phys.*, **9**. 41 (1941).

[4] M. L. Huggins, *Chem. Rev.*, **32**, 195 (1943).

[5] T. Shimanouchi and S. Mizushima, *Kagaku*, **17**, 24, 52 (1947), *Kagaku-no-Ryoiki* (*Journal of Japanese Chemistry*) **6**, 311 (1952), S. Mizushima, T. Shimanouchi, M. Tsuboi, T. Sugita and E. Kato. *Nature*, **164**, 918 (1949).

[6] E. J. Ambrose and A. Elliott, *Proc. Roy. Soc.*, **A 205**, 47 (1951).

[7] W. T. Astbury, *Nature*, **164**, 439 (1949).

[8] L. Bragg, J. C. Kendrew and M. F. Perutz, *Proc. Roy Soc.*, **A 203**, 321 (1950).

[9] L. Pauling and R. B. Corey, *Proc. Nat. Acad. Sci.*, **37**, 205, 241 (1951).

$\alpha_{(2)}$, exists in T (Trans) form. Other situations are equal exactly, so $\alpha_{(1)}$ and $\alpha_{(2)}$ can be said to be rotational isomers. If the energy difference between these isomers is small enough, it becomes necessary to consider the equilibrium between them in solution. According to previous experiences, T form is slightly stabler than G form except in special cases. This relation predicts that the equilibrium is shifted towards $\alpha_{(2)}$.

In the case of the polypeptide chain, however, we must take into account not only the above-mentioned interaction between the side chain and the main chain, but also the interaction between the side chain and the C=O group. In $\alpha_{(1)}$, the relative orientation between the side chain and the C=O group comes half way between G and T forms, and in $\alpha_{(2)}$, it exists in C (Cis) form. That is, C=O group takes C form with hydrogen atom on α-carbon atom in the case of $\alpha_{(1)}$, and with side chain (methyl group) in the case of $\alpha_{(2)}$. Consequently, the repulsive energy due to steric hindrance becomes greater in $\alpha_{(2)}$ than in $\alpha_{(1)}$, and $\alpha_{(2)}$ is unstabilized by this amount. Thus this repulsive energy tends to cancel the stabilization energy due to T-type configuration between the side chain and the main chain in $\alpha_{(2)}$. Though one cannot give any conclusion as to whether this cancellation is complete or not, one can expect that the energy difference between $\alpha_{(1)}$ and $\alpha_{(2)}$ is small enough.

In the following calculations, we assume as the idealized limiting case that $\alpha_{(1)}$ and $\alpha_{(2)}$ have the same stability and exist in solution with equal statistical weight. By this assumption, any unknown quantity concerning the equilibrium constant is excluded. Moreover, the main chain of polypeptide comes to have an effective plane of symmetry in the statistical meaning. Consequently, the calculations are exceedingly simplified. Later, some conclusion will be given for a case where the equilibrium is shifted towards $\alpha_{(2)}$ by the analysis of the results of calculation.

Next, we should consider the equilibrium between folded α-type configuration and that of the extended β-type or intermediate ones. In the case of water solution, the stability of the intramolecular hydrogen bond is diminished by some amount, and one can expect and larger contribution of extended or intermediate forms compared with the case in which a non-polar solvent is used. As can be easily seen, the latter ones (β- and intermediate types) have a larger degree of freedom compared with the former one (α-type), and corresponed to the state of larger entropy. Therefore the contribution of latter ones may increase rapidly with the elevation of temperature. Concerning this point, Kauzmann and Eyring suggested that the investigation of the effect of temperature on the molecular rotations of these polypeptidies would be of interest here.

In the actual calculations, the contribution of the latter ones are neglected. So it must be noticed that the calculated results correspond to the experimental results which are observed at sufficientry low temperature. Even if the contribution of latter ones could not be neglected, we might expect that the observed change of molecular rotations in a series of polypeptides would correspond well to the calculated results so long as the equilibrium constant was approximately constant regardless of the length of chains and the molecular rotations of the latter ones were sufficiently small as were inferred by Kauzmann and Eyring.

To simplify the calculations, it is assumed that the interatomic distances in C—C and C—N bonds are all 1.54 Å, and that in C=O and C—H bond is 1.26 Å and 1.1 Å respectively. Further, it is assumed that the valence angles of C and N atoms are tetrahedral angle (109°28′), and C—N bonds (C are not α-carbons) are perpendicular to a dyad axis of symmetry. In the present model, repeat distance is about 5.03 Å. In the actual molecule, true valence angles will make the molecule flatter than the assumed model and the repeat distance slightly larger.

II. MOLECULUR ROTATION

Division of Rotatory Parameter According to the electromagnetic theory, molecular rotation $[M]$ is given as follows,

$$[M]=\frac{288\pi^2 N}{\lambda^2}\,\frac{n^2+2}{3}\,\beta \qquad (1)$$

where N is Avogadro's number, λ the wavelength of incident polarized light, and n the refractive index of the solution. Taking into account the variation of vector potential over the molecule, Rosenfeld developed a quantum-mechanical theory of dispersion, and gave the following expression for a rotatory parameter β:

$$\beta=\frac{c}{3\pi h}\sum_b{}'\frac{\mathrm{Im}\{(a\,|\,\vec{\mu}\,|\,b)\;(b\,|\,\vec{m}\,|\,a)\}}{\nu_{ba}{}^2-\nu^2} \qquad (2)$$

Here, $(a\,|\,\vec{\mu}\,|\,b)$ and $(b\,|\,\vec{m}\,|\,a)$ are the matrix components of electric and magnetic moments connecting state a and b; ν_{ba} the frequency of the transition between state a and b, ν the frequency of the incident plane polarized light, c the velocity of light, and h Planck's constant. Then, the theoretical problem of optical activity is merely to calculate Eq. (2) for the actual molecule. In order to proceed with the calculation, Kirkwood introduced a simplifying assumption, that is, electrons were localized in the respective constituent group not only in normal state but also in excited states. By this assumption, one can break up the total electric and magnetic moments into sums over the groups as follows,

$$\vec{\mu}=\sum_i \vec{\mu_i} \tag{3}$$

$$\vec{m}=\sum_k \frac{e}{2mc}\vec{\boldsymbol{R}_k}\times\vec{\boldsymbol{P}}^{(k)}+\vec{\boldsymbol{m}_k} \tag{4}$$

where $\vec{\boldsymbol{\mu}_i}$ is the electric moment of group i, $\vec{\boldsymbol{p}}^{(k)}$ is the electric momentum of group k, and $\vec{\boldsymbol{m}_k}$ is the magnetic moment referred to the center of gravity of group k situated at a point $\vec{\boldsymbol{R}_k}$ from the center of gravity of the molecule as a whole. Substituting these relations in Eq. (2), Kirkwood obtained the following results.

$$\beta=\beta^{(0)}+\beta^{(1)}+\sum_k \beta^{(k)} \tag{5}$$

$$\beta^{(0)}=-\frac{1}{3h}\sum_{i>k}\sum_b \frac{\nu_{ba}}{\nu_{ba}^2-\nu_2} \times Re\{\vec{\boldsymbol{R}_k}[(a\mid\vec{\boldsymbol{\mu}}^{(i)}\mid b)\times b\mid\vec{\boldsymbol{\mu}}^{(k)}\mid a)]\} \tag{6}$$

$$\beta^{(1)}=\frac{c}{3\pi h}\sum_{j>k}\sum_b \frac{\mathrm{Im}\{(a\ \vec{\boldsymbol{\mu}}^{(i)}\ b)(b\ \vec{\boldsymbol{m}}^{(k)}\ a)\}}{\nu_{ba}^2-\nu^2} \tag{7}$$

$$\beta^{(k)}=\frac{c}{3\pi h}\sum_b \frac{\mathrm{Im}\{(a\ \vec{\boldsymbol{\mu}}^{(k)}\ b)(b\ \vec{\boldsymbol{m}}^{(k)}\ a)\}}{\nu_{ba}^2-\nu^2} \tag{8}$$

$\beta^{(0)}$ is the term which was taken up and studied by Kirkwood and he considered that it was originated from the dynamical coupling between two electrons in the different groups i and k.[10,11] Not being studied in detail, the term of $\beta^{(1)}$ has usually been neglected expecting it probably to be small, $\beta^{k)}$ is the term which was studied by Condon, Eyring, Kauzmann, Walter, et al., and they considered that it resulted from the motion of one electron in a dissymmetrical field of each group.[12,13,14]

As is obvious from the above discussion, we should consider both terms of $\beta^{(0)}$ and $\beta^{(k)}$ at least, in the calculations of molecular rotations. Accordingly, we will calculate both terms of $\beta^{(0)}$ and $\beta^{(k)}$, and give our conclusion considering both contributions from one-electron theory and dynamical coupling theory.

One-Electron Effect

a) Partial Rotation of N → E Type Transition When molecular rotation $[M]$ is observed in the visible region, the contribution from partial rotation becomes greater as the frequency of the absorption band (electronic transition), which is responsible for this partial rotation, exists nearer to the frequency of the observed light. If one remembers the factor $1/(\nu_{ba}^2-\nu^2)$ in equation of dispersion, this situation is evident. Consequently, when the special absorption band exists in the longer wavelength region far apart from the other absorptions in the ultraviolet region, most of the observed molecular rotation is determined by the partial rotation which is associated with this special transition.

In polypeptides, we are now investigating, $N\rightarrow E$ type transition in carbonyl group exists in the longest wavelength region. The calculations of the partial rotation associated with this transition have been carried out by Eyring et al. for 3-methylcyclopentanone[15] and benzoin.[16] Following Mulliken's usage, we define the coordinate of C=O group as follows. That is, we attribute the direction of π-orbitals of C and O to x-axis, that of non-bonding orbital of O to y-axis, and that of C—O bond to z-axis. In the first approximation, Eyring et al. assumed that the electron was localized in the oxygen atom throughout the process of $N\rightarrow E$ type transition, and they attributed it to the transition $2p_y\rightarrow 2p_z$. According to the study by McMurry, however, it should be attributed to the transition $2p_y\rightarrow 2p_x$ in the same approximation.[17] In this transition, the electric moment is zero and the magnetic moment is relatively large. (Even if the contribution of π-orbital of C atom is taken into account, this transition is still impossible). Then in order to obtain the non-zero value of $\beta^{(k)}$, the electric moment must have z-component, that is, one must consider the contribution of $3d_{yz}$-orbital at least in the excited state. This requirement is satisfied by using the following wave function.

$$\begin{aligned}\Psi_a&=\Psi 2p_y\\ \Psi_b&=\Psi_{2px}'+C\ \Psi_{3dyz}\end{aligned} \tag{9}$$

Substituting these wave functions in Eq. (8), one obtains

$$\beta^{(\mathrm{k})}=\frac{c}{3\pi h}\frac{C}{\nu_{ba}^2-\nu^2}\mathrm{Im}\{(\Psi_{2py}\mid\mu_z\mid\Psi_{3dyz})(\Psi_{2px}'\mid m_z\mid\Psi_{2py})\} \tag{10}$$

where, C is given by first-order perturbation theory as follows,

$$C=\frac{(\Psi_{2px}'\mid\sum_i V_i\mid\Psi_{3dyz})}{E_{2px}'-E_{3dyz}} =\frac{1}{E_{2px}'-E_{3dyz}}\sum_i \gamma_x{}^{(i)}\gamma_y{}^{(i)}\gamma_z{}^{(i)}U_i \tag{11}$$

$$U_i=\int\Psi_{2p}'V_i\Psi_{3d}(Z^{13}{}_{(i)}-3X^{12}{}_{(i)}Z_{(i)}')d\tau$$

Here, V_i is the electrostatic perturbing potential from the atom i except the carbonyl group. (In practice, it is sufficient to consider the contribution from the atoms by which the center or plane of symmetry is lost). In the case of neutral atoms, these potentials originate from the incomplete screening of the nuclear charge on account of the extension of electron clouds, and fall off exponentially with the increase of distances from nuclei. Non-diagonal elements U_i of perturbing potentials

---

[10] J. G. Kirkwood, *J. Chem. Phys.*, **5**, 479 (1937).

[11] W. W. Wood, W. Fickett and J. G. Kirkwood, *J. Chem. Phys.*, **20**, 561 (1952).

[12] E. U. Condon, *Rev. Mod. Phys.*, **9**, 432 (1937).

[13] E. U. Condon, W. Altar and H. Eying, *J. Chem. Phys.*, **5**, 753 (1937).

[14] E. Gorin, J. Walter and H. Eyring, *J. Chem. Phys.*, **6**, 824 (1938).

[15] W. J. Kauzmann, J. E. Walter and H. Eyring, *Chem. Rev.*, **26**, 339 (1940).

[16] T. C. Kwoh and H. Eyring, *J. Chem. Phys.*, **18**, 1186 (1950).

[17] H. L. McMurry, *J. Chem. Phys.*, **9**, 231 (1941).

V_i are correlated to D_i by the following relation

$$D_i = -\frac{32}{5}\frac{a_0}{e^2}U_i$$

where the values of D_i are given by Eyring et al. as the function of distance for the cases of hydrogen and carbon atoms, and we use them in the present calculation. (In Eyring's calculation, U_i is approximated by $\int \Psi_{2p}' V_i \Psi_{3d} Z'^3{}_{(i)} d\tau$. Quantitatively, this approximation gives probably too great values). The evaluation of two center integral $(\Psi_{2px}' \mid V_i \mid \Psi_{3dyz})$ requires a rotation of the coordinate system in which the new Z-axis coincides with the line joining the center of the perturbing atom and the carbonyl oxygen atom. The terms γ_x, γ_y and γ_z in Eq. (11) are the direction cosines of the above-mentioned line in the old-coordinate system. The values of γ_x, γ_y and γ_z are calculated using the molecular model which is discribed in Part I.

As the value of electric moment, we assume $(\Psi_{2py} \mid \mu_z \mid \Psi_{3dyz}) = 0.22\ e$. This value is obtained by using the experimental absolute intensity ($f \sim 0.03$) of Rydberg series in ethylene.[18] (For carbonyl group, there is no quantitative data concerning intensity). Strictly speaking, as the value of electric moment one should take the smaller value because the above-mentioned intensity corresponds to the total intensity of a few lines in Rydberg series. For magnetic moment, we assume tentatively

$$(\Psi_{2px}' \mid m_z \mid \Psi_{2py}) = \frac{eh}{4\pi mci}(\Psi_{2px}' \mid l \mid \Psi_{2px})$$

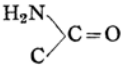
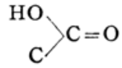
$$= \frac{eh}{4\pi mci}0.91$$

If one considers the contribution of π-orbital of carbon atom in the excited state, this value may probably become smaller. Using these values and $n_D = 1.33$, $\nu = 0.509 \times 10^{15}\ \text{sec}^{-1}$ (D line of Na), one can describe the molecular rotation as follows,

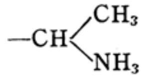
$$[M]_D = 45.87 \times 10^3 \frac{0.259}{\nu_{ba}^2 \times 10^{-30} - 0.259}$$

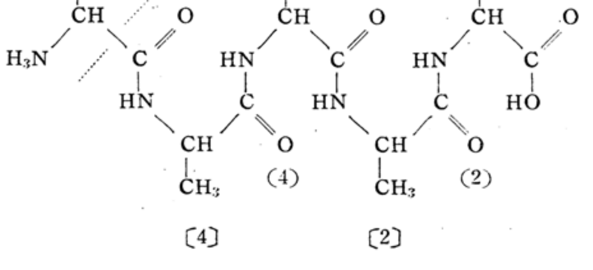
$$\frac{1}{E_{2px}' - E_{3dyz}} \sum_i \gamma_x{}^{(i)} \gamma_y{}^{(i)} \gamma_z{}^{(i)} D_i \qquad (12)$$

where ν_{ba} and E_{2px}' are dependent on the nature of the atom which combines with carbonyl carbon, and use the following values:

	$\nu_{ba} \times 10^{-15}\ \text{sec}^{-1}$	$E_{2px}' - E_{3dyz}$ (ev)
 $H_2N(C)C{=}O$	1.68	−0.58
 $HO(C)C{=}O$	1.44	−1.58

Of course, these values are not conclusive and may probably become to require some revision in the future study.

b) Method of Calculation We assume the molecular model which is described in Part I. In this model, the rotational isomers $\alpha_{(1)}$ and $\alpha_{(2)}$ have the same stability, and exist in solution with equal statistical weight. Then, the main chain of polypeptide comes to have effective plane of symmetry in the statistical meaning. This situation makes the calculations exceedingly simple, and in order to obtain the partial rotation associated with $N \rightarrow E$ type transition, it is sufficient for it by considering the perturbations from the side chain (methyl group) and the hydrogen atom which is attached to α-carbon together with the side chain. (In the following, we say that the term of methyl group is including this hydrogen atom). As a subsidiary assumption, we consider that end group COOH contributes to the formation of seven-membered ring, and another end group  $-CH(CH_3)(NH_3)$ exists in the effective plane of symmetry on account of their statistical free rotation around the single bond. Consequently, in case of tetra-alanyl-alanine, the molecular model becomes as follows:



---

[18] J. R. Platt, H. B. Klevens and W. C. Price, *J. Chem. Phys.* **17**, 466 (1949).

As is shown in the foregoing figure, we number carbonyl oxygens as (1), (2), (3), (4), (5), and methyl groups as [1], [2], [3], [4]. Then, we can divide the perturbations (or partial rotations due to these perturbations) into three classes according to the relative positions between carbonyl oxygen and methyl group, that is:

A: {(1) [1]} {(2) [2]} {(3) [3]} {(4) [4]}
B: {(2) [1]} {(3) [2]} {(4) [3]} {(5) [4]}
C: {(3) [1]} {(4) [2]} {(5) [3]}

As described previously, interaction function D_i decreases very rapidly with the increase of r, and we neglect the interactions at intervals of more than 5 Å (for example {(2) [3]} {(5) [2]} etc.).

In each class of A, B and C, the relative orientations of the carbonyl group and the methyl group are different with the situations whether the configuration of the polypeptide chain exists in $\alpha_{(1)}$ or in $\alpha_{(2)}$. To indicate this distinction, we use the superscripts (1) and (2). In the following, we consider that the above-defined notation, for example $A^{(1)}$, corresponds to the partial rotation due to the perturbation from the methyl group to the carbonyl oxygen which exists in the relative position of class A and configuration of $\alpha_{(1)}$. So long as we assume that $\alpha_{(1)}$ and $\alpha_{(2)}$ are equally probable statistically, the mean value, for example,

$$A=(A^{(1)}+A^{(2)}/2) \tag{13}$$

corresponds to the observed value. In the present case, the frequency of $N\rightarrow E$ type transition, consequently, the magnitude of the partial rotation depends on the atom, whether it is oxygen or nitrogen, which combines with carbonyl carbon. To distinguish these two cases, we use the subscripts O and N. Then the molecular rotations of a series of polypeptides are given as follows:

$$\left.\begin{array}{ll} \text{alanyl-alanine} & A_O+B_N \\ \text{di-alanyl-alanine} & A_O+A_N+2B_N+C_N \\ \text{tri-alanyl-alanine} & A_O+2A_N+3B_N+2C_N \\ \text{tetra-alanyl-alanine} & A_O+3A_N+4B_N+3C_N \end{array}\right\} \tag{14}$$

The calculated values of A_O, A_N, B_N and C_N are given in Table II. Perturbations from one carbon atom and three hydrogen atoms in methyl group and one hydrogen atom attached to α-carbon together with methyl group are all taken into account. Using Eq. (14) and the values in Table II, we can easily calculate the molecular rotations of a series of polypeptides. These calculated values are shown in Table III together with the observed values. As is seen in Table III, the calculated values agree well with experimental ones. It is interesting to see that each of A_0, A_N, B_N and C_N has the same sign, and this is the main reason which enables us to obtain the above-described agreement. If they had different signs, they might cancel with each other in Eq. (14) and give us much smaller molecular rotations.

TABLE II

$A_O^{(1)}=110.0°$	$A_O^{(2)}=-22.4°$	$A_O=43.8°$
$A_N^{(1)}=211.5°$	$A_N^{(2)}=-43.1°$	$A_N=84.2°$
$B_N^{(1)}=89.4°$	$B_N^{(2)}=-45.5°$	$B_N=21.9°$
$C_N^{(1)}=44.7°$	$C_N^{(2)}=36.7°$	$C_N=40.7°$

TABLE III

CALCULATED MOLECULAR ROTATIONS $[M]_D$ DUE TO ONE-ELECTRON EFFECT

Polypeptides	Calculated values*	Observed values*
alanine	0°	0°
alanyl-alanine	66°	71°
di-alanyl-alanine	213°	192°
tri-alanyl-alanine	359°	360°
tetra-alanyl-alanine	506°	507°

* In the calculated values, $[M]_D$ of alanine is taken as the origin, and corresponding to this situation, observed values are corrected by amount of $[M]_D$ of alanine (13°).

We must emphasize, however, that the above-obtained quantitative agreement is only an apparent one. Firstly, as is described in the last section, the values of matrix elements U_i, $(\psi_{2py}|\mu_z|\psi_{3dyz})$ and $(\psi_{2px}'|m_z|\psi_{2py})$ belong to rather upper limits. Secondary, we can expect the appearance of the firist member of Rydberg series ($2p\rightarrow 3d$) in the slightly shorter wavelength region than the position of $N\rightarrow E$ type transition. And the theoretical consideration predicts that the partial rotation associated with this Rydberg transition has the opposite sign to that of $N\rightarrow E$ type transition, and tends to cancel it. Considering this fact, the calculated values become smaller than 20% of the observed values. (Even if we consider the contribution of $4d$, $5d$,......functions, analogous situations are probable). The above situation is rather inferable, if one remembers that the wavelength of $N\rightarrow E$ type transition is shifted considerably towards the shorter side in case of polypeptide. In the present treatment, the partial rotation associated with the transition of the non-bonding electron in the hydroxyl group or nitrogen atoms in the main chain is not considered. The positions of these transitions, however, exist in the shorter side than that of $N\rightarrow E$ type transition, and the degree of cancellation due to the opposite partial rotation associated with Rydberg series may become greater.

At any rate, it is interesting that the theoretical and observed results agree well at least in qualitative tendency, and the quantitative comparison will be discussed in the next section considering the dynamical coupling effect.

Dynamical Coupling Effect

a) Theoretical Foamula.—Assuming a dynamical coupling between two electrons in groups i and k (that is, a van der Waals type interaction between groups i and k), Kirkwood applied the first order perturbation calculation to Eq. (6). After some algebraic simplification, he obtained the simple expression for $\beta^{(0)}$ as follows[10,11]*:

$$\beta^{(0)}=\frac{1}{6}\sum_{i>k}^{N}\alpha_i\alpha_k\delta_i\delta_k\, G_{ik}\,\vec{R}_{ik}\,(\vec{b}_i\times\vec{b}_k) \tag{15}$$

$$G_{ik}=\frac{1}{R_{ik}^3}\left[\vec{b}_i\vec{b}_k-3\frac{(\vec{b}_i\cdot\vec{R}_{ik})(\vec{b}_k\cdot\vec{R}_{ik})}{R_{ik}^2}\right]$$

$$\vec{R}_{ik}=\vec{R}_k-\vec{R}_i$$

where α_i is the mean polarizability of group i, δ_i is anisotropic factor of α_i and b_i is a unit vector defining the optical axis of symmetry of group i. Application of Eq. (15) is limited in the case that each group has a optical axis of symmetry, and δ_i is defined as follows:

$$\delta_i=(\alpha_{11}^{(i)}-\alpha_{22}^{(i)})/\alpha_i \tag{16}$$

where $\alpha_{11}^{(i)}$ and $\alpha_{22}^{(i)}$ are the components of α_i parallel and perpendicular to $\vec{b}_i$. The above result obtained by Kirkwood can be considered as a quantum mechanical expression for the classical coupled-oscillator theory of Born and Kuhn. The disappearance of Planck's constant h in Eq. (15) indicates the classical character of it.

b) Method of Calculation.—The molecular model is the same as before. Since the main chain has an effective plane of symmetry in a statistical meaning, the most important factor is the term due to the interaction between methyl group and carbonyl group. In the case of dynamical coupling effect, the transition in methyl group can also contribute to the molecular rotation, and the interaction between the main chain and the side chain should also be taken into account. In the present calculation, however, we neglect this term, and the appropriateness for this omission is discussed in the last part of this paper.

Van der Waals type of interaction decreases more gradually than the interaction which is assumed in one-electron theory, so we take into account the classes of D and E together with those of A, B and C.

D: $\{(1)[2]\}\ \{(2)[3]\}\ \{(3)[4]\}$
E: $\{(4)[1]\}\ \{(5)[2]\}$

In the present case, there may be no necessity to distinguish between A_{O} and A_{N}, and the molecular rotations of a series of polypeptides are given as follows:

$$\left.\begin{array}{ll}\text{alanyl-alanine} & A+B\\ \text{di-alanyl-alanine} & 2A+2B+C+D+\\ \text{tri-alanyl-alanine} & 3A+3B+2C+2D+E\\ \text{tetra-alanyl-alanine} & 4A+4B+3C+3D+2E\end{array}\right\} \tag{17}$$

In order to calculate the values of A, B, C, D and E using Eq. (15), it is necessary to know the values of $\alpha\delta$ of carbonyl group and methyl group. As the value of $\alpha\delta$, Kirkwood used that of respective group as a whole, however, we used that of respective bond, which was obtained by Wang as a result of careful analysis of Stuart's experimental value.[19] The values obtained by Wang are shown in Table IV (Carbonyl group is assumed to have an optical axis of symmetry in the first approximation). As is seen in Table IV, $\alpha\delta$ of C—H bond is much smaller than those of C—C and C=O bonds, so the contribution of it to molecular rotation can be neglected. Consequently, we consider only interactions between C=O bond and C—C bonds which combine methyl groups with the main chain, and $\vec{R}_{ik}$ is considered as a vector which combines the center of gravity of respective bond. The results of calculation are shown in Table V. It is rather surprising that each of A, B, C, D and E has the same sign in Table V. The calculated molecular rotations obtained by

TABLE IV

LONGITUDINAL AND TRANSVERSAL POLARIZABILITIES OF BONDS (10^{25} cc)

Bonds	α_{11}	α_{22}	$\alpha\delta=\alpha_{11}-\alpha_{22}$
C—H	7.2	6.2	1.0
C—C	18.2	0.2	18.0
C=O	19.4	8.0	11.4

TABLE V

$A^{(i)}=177.2^\circ$ $A^{(2)}=-0.1^\circ$ $A=88.6^\circ$
$B^{(1)}=160.2^\circ$ $B^{(2)}=-0.02^\circ$ $B=80.1^\circ$
$C^{(1)}=49.95^\circ$ $C^{(2)}=4.53^\circ$ $C=27.2^\circ$
$D^{(1)}=0.024^\circ$ $D^{(2)}=30.69^\circ$ $D=15.4^\circ$
$E^{(1)}=17.16^\circ$ $E^{(2)}=17.23^\circ$ $E=17.2^\circ$

TABLE VI

CALCULATED MOLECULAR ROTATIONS $[M]_{\mathrm{D}}$ DUE TO DYNAMICAL COUPLING EFFECT

Polypeptides	Calculated values	Calculated values × 0.591	Observed values*
alanine	0°	0°	0°
alanyl-alanine	169°	100°	71°
di-alanyl-alanine	380°	225°	192°
tri-alanyl-alanine	609°	360°	360°
tetra-alanyl-alanine	837°	495°	507°

* The observed values are corrected by the same way as in Table III.

---

* The error of signs in reference (10) is corrected in reference (11).

19) Sheng-Nien Wang, *J. Chem. Phys.*, **7**, 1012 (1939).

using Eq. (17) and the values in Table V are given in Table VI together with the observed values. The calculated values are too great compared with the observed values. But the calculated values multiplied by 0.591 agree relatively well with experimental ones. That is, the calculated results agree with experimental results, at least in qualitative tendency.

c) **Discussion on the Results.**—In the case of dynamical coupling effect, the calculated values are twice as large as the observed ones. As the main causes of this situation, we can consider the following three factors:

(1) The values of parameters, such as $\alpha\delta$, are too large.

(2) The assumption concerning the equilibrium between two rotational isomers $\alpha_{(1)}$ and $\alpha_{(2)}$ is not appropriate. In the actual calculation, it is assumed that $\alpha_{(1)}$ and $\alpha_{(2)}$ are equally probable, however, one can consider the case in which the equilibrium is shifted towards one side.

(3) In the present calculation, only α-type configurations are taken into account, however, one can also expect the cases in which the contribution of β-and intermediate-type configurations are not negligible.

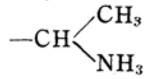
Whether the factor (1) is real or not can be determined by the systematic study for many simple molecules, and at the present stage, it is difficult to give the decided conclusion.

Factor (2) may probably be the most important one, and we will give rather detailed consideration. Observing Tables 2 and 5, one finds that the values of A, B and C are exceedingly larger in $\alpha_{(1)}$ than in $\alpha_{(2)}$. Moreover, the sign of these quantities are definite in $\alpha_{(1)}$, on the contrary, plus and minus signs are mixed in $\alpha_{(2)}$, and tend to cancel each other. The main cause of this situation may probably exist in the fact that the side chains (methyl groups) are approximately perpendicular to the plane of main chain in the case of $\alpha_{(1)}$, on the other hand, side chains are approximately lying in that plane in the case of $\alpha_{(2)}$. If this statement is correct, molecular rotation as a whole considering not only the interaction between side chain and carbonyl group but also all the other interactions in the molecule may be sufficiently smaller in the case of $\alpha_{(2)}$ compared with the case of $\alpha_{(1)}$. Then, the shift of equilibrium towards $\alpha_{(2)}$, makes the molecular rotation of polypeptides smaller. That is, too large theoretical values suggest that the equilibrium is shifted towards $\alpha_{(2)}$ under the observed conditions. This suggestion accords with the expectation from the internal rotation. It is difficult, however, to explain the observed rotations if one neglects the contribution of $\alpha_{(1)}$ completely.

Factor (3) should also be considered, and to know the degree of contribution of this factor, it is very desirable to observe the temperature effect on the molecular rotation.

Factors (2) and (3) diminish not only the molecular rotation due to dynamical coupling effect but also due to one-electron effect as well. Since both effects predict the same sign of molecular rotations (See Table III and VI), that situation has special importance.

Lastly, we consider the degree of approximation of the present calculation. As is described in the last section, only the interaction between the side chain and the carbonyl group is taken into account, and the other interactions, for example, between the main chain and the side chain, are neglected. In order to allow this approximation, it is required that C—C bond and C—N bond (N atom has non-bonding electrons!) have the same $\alpha\delta$. As far as we know, there are no experimental results which are sufficient to verify the above-requirement. We can infer, however, that there will be no large difference between $\alpha\delta$ of C—C and C—N bonds considering the almost equal nature of both bonds. Even if the above-requirement is satisfied, it remains the contribution from the interaction between the side chain and the main chain in the case of di-alanyl-alanine and tetra-alanyl-alanine. We can expect, however, that this contribution may be small if we remember the relatively small values of D and E.

In every case, the influence of the end group  $-\mathrm{CH}{<}^{\mathrm{CH_3}}_{\mathrm{NH_3}}$ is neglected. Strictly speaking, these omissions may not be reasonable. If we take out the differences of molecular rotations between successive polypeptide molecules, however, the influence due to the end group may mostly be subtracted. We can expect an analogous tendency for the part due to interaction between the side chain and the main chain.

Summary

Molecular rotations of a series of polypeptides of alanine have been calculated theoretically. We have assumed the molecular model which is constructed by seven-membered rings due to intra-molecular hy-

drogen bonds (α-type configuration). Calculations are carried out for the both effects of one-electron theory and dynamical coupling theory, and the results correspond relatively well to experimental results. This fact probably indicates the appropriateness of the assumed model. In order to obtain a decisive conclusion, however, it is necessary to proceed with the calculations for other models and compare the results quantitatively. This is a task for future study.

The author wishes to express his sincere thanks to Prof. S. Akabori for his kind guidance and encouragement throughout this work.

Department of Chemistry, Faculty of Science, Osaka University, Osaka