

factors α for all four polymers have been calculated according to eq. 3, and they are given in column nine of Table I. The reported uncertainties were estimated from the uncertainties in A_2 , M_n , and $[\eta]$.

Discussion

Values of $\langle r^2 \rangle_0 / n_p l_p^2$ calculated from eq. 2 are presented in the next to the last column of Table I. These results are subject to errors of about $\pm 10\%$; the estimates of M_v constitute the largest source of uncertainty. The similarity of results for the four diverse systems is striking. It strongly suggests that specific interactions involving side chains or solvents exert little influence on the unperturbed dimensions of the polypeptide random coil in the systems examined.

The four polypeptides listed in Table I are characterized by large side chains. They have been investigated in aqueous, phenolic, and acidic solvents. The absence of any measurable dependence of the polypeptide unperturbed dimensions on the amino acid side chains casts doubt on the importance of interactions between *first neighbor* side chains in proteins.

Also worthy of note is the fact that even in dichloroacetic acid the unperturbed dimensions of PBLG are in close agreement with those for the other polymers. If protonation of the amide group occurs in this solvent, as seems likely,²⁶ the planar, *trans* conformation of the peptide group evidently is not destroyed by the proton transfer.

The measured values of the mean square unperturbed end-to-end distance are compared in the final column of Table I with the quantity $\langle r^2 \rangle_{0,f}$ calculated for a polypeptide chain having all amide bonds *trans*, with free rotation prevailing about the other chain bonds.^{12,15} The ratio $\langle r^2 \rangle_0 / \langle r^2 \rangle_{0,f}$ for polypeptides is typical of values found for other polymers.¹³

Theoretical interpretation of these results in terms of polypeptide structure is presented in the following paper.¹⁷

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The Configuration of Random Polypeptide Chains. II. Theory

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The experimentally measured dimensions of polypeptide chains in the unperturbed, random coil form have been successfully correlated with the chain structure using the rotational isomeric state model and statistical mechanical methods applicable to linear systems of interacting subunits. The polypeptide chain with all its amide groups in the trans conformation may be treated as a sequence of virtual bonds of fixed length connecting successive α -carbons; the mutual orientation of a pair of adjoining virtual bonds is determined by the angles of rotation about the single bonds at the intervening α -carbon atom. Contributions to the configuration energy from bond rotation (torsional) potentials and interactions between nonbonded atoms and groups have been assessed and subsequently evaluated using approximate analytical expressions. From the chain geometry and the character of these interactions, it is shown that the mutual orientation of a given pair of adjoining virtual bonds is sensibly independent of the orientations of all other virtual bonds in the chain. Electrostatic interactions between amide groups, heretofore ignored, markedly affect the configuration. Satisfactory agreement between theoretical calculations and experimental results is achieved only by taking account of these dipolar interactions, in addition to the relevant torsional and van der Waals energies. The dependence of the polypeptide unperturbed dimensions on chain length has been investigated also.

Introduction

The unperturbed mean dimensions of a number of linear polymers have now been successfully correlated with their chain structures and associated impedences to bond rotations. The basis for these correlations is provided by the rotational isomeric state model for chain molecules.¹ Mathematical methods applicable to this model are furnished by the well-known theory originally developed for a linear array of interacting magnetic dipoles.^{2,3} They have been adapted to the treatment of the unperturbed mean square end-to-end distance and dipole moment of linear polymer molecules by Birshtein and Ptitsyn,⁴ Lifson,⁵ Nagai,⁶ and Hoeve.⁷ These methods, which take account of the interdependence of nearest-neighbor rotations, have been applied to polyethylene,⁸ poly(dimethylsiloxane),⁹

(1) M. V. Volkenstein, "Configurational Statistics of Polymeric Chains," English Translation, Interscience Publishers, New York, N. Y., 1963.

(2) H. A. Kramers and G. H. Wannier, *Phys. Rev.*, **60**, 252 (1941).

(3) G. F. Newell and E. W. Montroll, *Rev. Mod. Phys.*, **25**, 353 (1953).

(4) T. M. Birshtein and O. B. Ptitsyn, *Zh. Tekhn. Fiz.*, **29**, 1048 (1959); T. M. Birshtein, *Vysokomolekul. Soedin.*, **1**, 798, 1086 (1959); O. B. Ptitsyn, *Usp. Fiz. Nauk*, **49**, 371 (1959).

(5) S. Lifson, *J. Chem. Phys.*, **30**, 964 (1959).

(6) K. Nagai, *ibid.*, **31**, 1169 (1959).

(7) C. A. J. Hoeve, *ibid.*, **32**, 888 (1960).

(8) C. A. J. Hoeve, *ibid.*, **35**, 1266 (1961).

(9) P. J. Flory, V. Crescenzi, and J. E. Mark, *J. Am. Chem. Soc.*, **86**, 146 (1964).

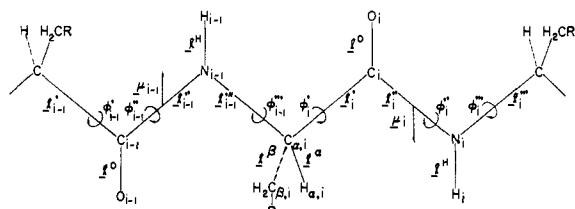


Figure 1. Section of fully extended, *trans* α -L-polypeptide.

polyoxymethylene,¹⁰ and polyoxyethylene.¹¹ Vinyl polymers in which substituents introduce the feature of stereoirregularity may be treated¹² by a recent extension of these methods.¹³

The spatial configuration of the random coil polypeptide chain is obviously a subject of close relevance to protein denaturation and dimensional changes in fibrous proteins. Its interpretation in terms of the molecular structure gains further interest from certain characteristics of the polypeptide chain which set it apart from those chains treated previously. First to be noted is the highly polar character of its chain backbone. Hence, one may expect larger electrostatic contributions to the bond rotational potentials than have been dealt with heretofore.¹⁴ Secondly, the relative structural complexity of the polypeptide chain notwithstanding, the bond rotations within a given amino acid residue of the chain turn out to be very nearly independent of rotations in neighboring residues, as we shall show. This circumstance is primarily a consequence of the planar, *trans* conformation of the amide group. It leads to considerable simplification of the theoretical calculation of the unperturbed dimensions of the polypeptide chain.

In this paper we interpret the experimentally determined unperturbed polypeptide coil dimensions¹⁵ in terms of the polymer chain structure and the factors contributing to the potentials for rotations about the chain bonds.

Theoretical Treatment

Polypeptide Structure. A portion of an α -L-polypeptide molecule in its fully extended, planar configuration is depicted in Figure 1. The sequence of bond vectors l'_i , l''_i , and l'''_i comprises the three-bond repeating unit of the chain. Structural parameters consisting of the lengths of these bonds l' , l'' , and l''' and the supplements of the associated bond angles θ' , θ'' , and θ''' defined in Figure 2 are well established from X-ray crystallographic analysis. Values of these quantities, taken from a recent review by Sasisekharan,¹⁶ are given in Table I. Also included are the lengths of other bonds in the peptide unit defined in Figure 1, and the supplements of the bond angles formed by each of

(10) P. J. Flory and J. E. Mark, *Makromol. Chem.*, **75**, 11 (1964).

(11) J. E. Mark and P. J. Flory, *J. Am. Chem. Soc.*, **87**, 1415 (1965).

(12) J. E. Mark and P. J. Flory, in preparation.

(13) P. J. Flory, *Proc. Natl. Acad. Sci. U. S.*, **51**, 1060 (1964).

(14) The importance for the polypeptide chain configuration of electrostatic interactions between polar groups in the chain backbone has been cited in a recent preliminary communication: D. A. Brant and P. J. Flory, *J. Am. Chem. Soc.*, **87**, 663 (1965). The role of dipolar interactions in two ordered polypeptide configurations has been discussed by R. G. C. Arridge and C. G. Cannon, *Proc. Roy. Soc. (London)*, **A278**, 91 (1964).

(15) D. A. Brant and P. J. Flory, *J. Am. Chem. Soc.*, **87**, 2788 (1965).

(16) V. Sasisekharan, "Collagen," N. Ramanathan, Ed., John Wiley and Sons, Inc., New York, N. Y., 1962, p. 39.

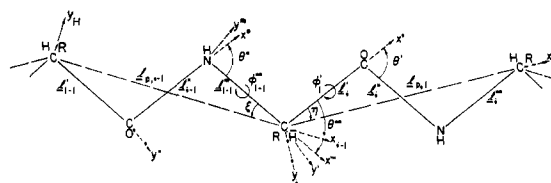


Figure 2. Section of fully extended, *trans* α -L-polypeptide showing virtual bonds (---) connecting α -carbons and coordinate axes (-----) of chain backbone bonds and virtual bonds.

these bonds with the preceding main chain bond.¹⁶ All bond lengths and bond angles are assumed to be fixed. Subscripts indexing the serial order of the repeating peptide units are included in Figures 1 and 2. The angles for rotations about the main chain bonds are denoted by φ'_i , φ''_i , and φ'''_i . We adopt the convention whereby they are assigned values of zero in the planar, *trans* conformation and take on positive values for right-hand rotations of either of the groups joined by a bond with respect to a reference frame attached to the other group.

Table I

Bond	Length, Å.	180° - bond angle
C_α -C	$l' = 1.53$	$\theta' = 66^\circ$
C-N	$l'' = 1.32$	$\theta'' = 57^\circ$
N- C_α	$l''' = 1.47$	$\theta''' = 70^\circ$
C-O	$l^o = 1.24$	$\theta^o = 59^\circ$
N-H	$l^H = 1.00$	$\theta^H = 57^\circ$
C_α - C_β	$l^\beta = 1.54$	$\theta^\beta = 70^\circ$
C_α - H_α	$l^\alpha = 1.00$	$\theta^\alpha = 70^\circ$

That the polypeptide amide group is planar and predominantly in the *trans* conformation is well documented.¹⁶⁻¹⁸ We thus assume in all that follows that the angle for rotation about each amide bond of the chain φ''_i is restricted to the single value zero characteristic of the *trans* conformation. The distance between neighboring α -carbon atoms is consequently independent of the intervening angles φ'_i and φ'''_i , and the chain may be treated as a sequence of n_p virtual bonds of length l_p joining the α -carbons. Virtual bonds are shown in Figure 2 as heavy dashed lines.

The mutual orientation of any two successive virtual bonds is determined by the pair of angles φ_{i-1}''' and φ_i' for rotations about the single bonds adjoining the intervening α -carbon; a given configuration of the chain is completely determined by specifying the values for every such pair of rotation angles in the chain. The average chain configuration thus depends upon the statistical weight assignable to each combination of values which the complete set of rotation angles may assume. This statistical weight will depend on the potentials associated with rotations about all bond pairs l_{i-1}''' and l_i' .

Rotational Potentials. Contributions to the potential functions for rotations about the polypeptide chain

(17) L. A. LaPlanche and M. T. Rogers, *J. Am. Chem. Soc.*, **86**, 337 (1964).

(18) R. J. Kurland and E. B. Wilson, Jr., *J. Chem. Phys.*, **27**, 585 (1957).

bonds may conceivably arise from any or all of the following sources: (1) inherent torsional potentials attributable to the covalent bond apart from interactions of neighboring substituent atoms or groups, (2) van der Waals interactions between nonbonded atoms within or directly connected to the chain backbone, (3) electrostatic interaction of polar groups in the chain backbone, (4) interactions between the respective side chains on two different amino acid units, (5) interactions between an amino acid side chain and the polymer backbone, (6) interactions between polymer and solvent. The similarity of the experimental results reported in the previous paper¹⁵ for four systems differing markedly with respect to solvent and amino acid side chain suggests that interactions of types 4, 5, and 6 do not make significant contributions to the rotational potential functions for these systems. The first three types of contributions have been discussed recently by Scott and Scheraga.¹⁹ It is important to recognize that the potential for rotation about a given bond of a polymer chain depends, in general, not only upon the angle for rotation about that bond but also on the rotation angles of neighboring bonds. The necessity of appropriately accounting for the interdependence of rotations in theoretical treatments of polymer unperturbed dimensions has been adequately demonstrated.⁸⁻¹²

Assessment of the contributions of inherent torsional potentials to the total potentials for rotations about the polypeptide bonds l' and l''' is hampered by the scarcity of information, accessible principally from microwave spectroscopy, on analogous low molecular weight compounds. For the bond l' a partial analogy is afforded by the carbon-carbon bond in molecules of the class CH_3COX . The microwave spectra of compounds of this type with $\text{X} = \text{H, F, Cl, Br, CN, and OH}$ ²⁰ yield internal rotation barriers from 0.5 to 1.3 kcal./mole. Scott and Scheraga¹⁹ estimate 1.1 kcal./mole for the average inherent torsional contribution to these barriers. The observed hindrance potentials are threefold and have minima such that a methyl hydrogen eclipses the carbonyl oxygen in each low energy conformation. The partial double bond character of the amide bond raises the prospect of a contribution of sixfold periodicity to the potential for rotation about bond l' in analogy to the carbon-nitrogen bond in nitromethane.²¹ The heights of all sixfold rotational barriers which have been observed are, however, of the order of a few calories at most,²⁰ and we therefore conclude that any sixfold contribution to the torsional potential for rotation about bond l' may be neglected. The inherent torsional potential for rotation about bond l' may thus be treated as threefold with minima located such that a bond of the α -carbon eclipses the carbonyl oxygen, *i.e.*, at $\varphi' = 0, 120, \text{ and } 240^\circ$, and with a barrier height in the neighborhood of 1 kcal./mole. The torsional potential function for bond l' may therefore be represented by

$$V_t' = (V_0'/2)[1 + \cos 3(\varphi' - 60)] \quad (1)$$

where V_0' is the torsional barrier height.

Microwave spectral data bearing on the internal rotation potential for bonds analogous to l''' are even more sparse. The threefold barrier in methylamine is 1.98 kcal./mole.²⁰ To the extent that the amide bond may be interpreted as a double bond, it is permissible to regard l''' as analogous to the carbon-carbon single bond in propylene. The threefold internal rotation barrier for the propylene bond is 1.98 kcal./mole with minima occurring at eclipse of the double bond by one of the methyl hydrogens.²⁰ The meager evidence available thus suggests that the inherent torsional contribution to the potential for rotation about bond l''' is threefold with a barrier height V_0''' between 1 and 2 kcal./mole. Pauling and Corey²¹ argue from X-ray crystallographic data that the torsional potential minima for bond l''' correspond to conformations in which a bond of the α -carbon is coplanar with the peptide group. Two types of threefold torsional potential functions are consistent with this hypothesis. One of these, represented by

$$V_{t,a}''' = (V_0'''/2)[1 + \cos 3(\varphi''' - 60)] \quad (2)$$

assigns the minima to angles for which a bond of the α -carbon eclipses the amide hydrogens. In the favored conformations of the other, which is given by

$$V_{t,b}''' = (V_0'''/2)[1 + \cos 3\varphi'''] \quad (3)$$

a bond of the α -carbon eclipses the amide bond. It is perhaps significant that the angle for rotation about bond l''' in glycine residues in crystalline lower peptides in which the glycine α -carbon is bonded to an amide nitrogen almost invariably adopts a value corresponding to one of the minima in $V_{t,a}'''$.¹⁶ Inasmuch as steric interactions are greatly reduced in the absence of the β -carbon, this observation may be indicative of the nature of the inherent torsional potential for bond l''' .

van der Waals repulsions between atoms within or connected directly to the chain backbone provide the dominant contributions to the total rotational potential functions. Inspection of molecular scale models suggests that 23 pair interactions involving carbonyl carbon, carbonyl oxygen, amide nitrogen, amide hydrogen, α -hydrogen, and the β -methylene group need to be considered. With the exception of the α -carbon, all subscripted atoms in Figure 1 which are separated by three or more bonds, at least one of which is l' or l''' , are potentially involved in steric conflicts. While interactions involving the i th β -methylene or α -hydrogen depend only upon either φ_{i-1}''' or φ_i' , most of the interactions between atoms of the $(i-1)$ - and i th peptide units depend *simultaneously* upon the pair of angles φ_{i-1}''' and φ_i' . van der Waals contributions to the potential function for rotation about bond l_{i-1}''' thus depend upon φ_i' as well as upon φ_{i-1}''' and *vice versa*. We note, however, that van der Waals interactions which depend *simultaneously* on a pair of rotation angles φ_i' and φ_i''' do not occur. This is due to the separation of atoms and groups of adjoining amino acid residues by the intervening planar, *trans* amide bond.

(19) R. A. Scott and H. A. Scheraga, *J. Chem. Phys.*, **42**, 2209 (1965).

(20) D. R. Hershbach, "Bibliography for Hindered Internal Rotation and Microwave Spectroscopy," Lawrence Radiation Laboratory, University of California, Berkeley, Calif., 1962.

(21) L. Pauling and R. B. Corey, *Proc. Natl. Acad. Sci. U. S.*, **37**, 729 (1951).

It must be emphasized that theoretical treatment of polymer *unperturbed* dimensions necessitates consideration of *short-range* steric interactions only. Longer range volume exclusion is nullified by restricting attention to polymers in their unperturbed state.^{15,22,23} Possible interactions of the subscripted atoms in Figure 1 with atoms beyond the β -carbon in the i th amino acid side chain and possible interactions between nearest neighbor or second neighbor side chains fall within the short-range category. The molecular models indicate, however, that these interactions must play a relatively minor role in determining the configurations available to the chain, and the experimental results of the preceding paper¹⁵ verify these conclusions.

For the purpose of estimating the configurational energy, potential functions for van der Waals interactions between nonbonded atoms have been approximated according to procedures developed by Hendrickson²⁴ and by Scott and Scheraga.¹⁹ The van der Waals energy of the j th pair of atoms was expressed as a function of the separation of their centers d_j by

$$V_{w,j} = a_j \exp(-b_j d_j) - c_j/d_j^6 \quad (4)$$

The parameters c_j have been estimated, using the Slater-Kirkwood equation, from the polarizabilities α of the atoms and the effective number of electrons N_{eff} in the outer electronic subshell as suggested by Pitzer.²⁵ Atomic and group polarizabilities given by Ketelaar²⁶ have been used, account being taken of the enhanced polarizability expected for molecules containing double bonds. The chosen values of α are listed in Table II along with values for N_{eff} . The electronic structure of CH_2 was assumed to be similar to that of oxygen in assigning a value of N_{eff} for this group.

Table II

Atom or group	$\alpha \times 10^{24}$, cm. ³	N_{eff}	r_w , Å.
H	0.42	0.9	1.20
C	1.30	5	1.70
N	1.15	6	1.55
O	0.84	7	1.50
CH_2	1.77	7	1.80-2.00

Inasmuch as the conclusions to follow are insensitive to the details of the attractive part of the potential function, errors in assignment of α and N_{eff} are inconsequential. Reliable values of b_j are not available for most atom pairs. Consequently, for all interactions b_j has been assigned the value 4.60 as suggested by the limited information currently available from the molecular beam scattering studies of Amdur and co-workers.²⁷ The parameter a_j is obtained by requiring $V_{w,j}$ to be a minimum at a distance d_j^0 equal to the sum of the van der Waals radii of the interacting species.

(22) M. Kurata and W. H. Stockmayer, *Fortschr. Hochpolymer. Forsch.*, **3**, 196 (1963).

(23) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953.

(24) J. B. Hendrickson, *J. Am. Chem. Soc.*, **83**, 4537 (1961).

(25) K. S. Pitzer, "Advances in Chemical Physics," Vol. II, I. Prigogine, Ed., Interscience Publishers, Inc., New York, N. Y., 1959, p. 59.

(26) J. Ketelaar, "Chemical Constitution," Elsevier Publishing Co., New York, N. Y., 1958, p. 91.

(27) For a summary see ref. 19.

Atomic van der Waals radii recommended by Bondi²⁸ are listed in Table II. The possibility that interactions of the CH_2 group may depend upon the orientation of this group is recognized by assigning a range of values for the van der Waals radius of CH_2 .¹⁶ Having evaluated parameters a_j , b_j , and c_j for each pair interaction, we obtain the total van der Waals interaction energy as the sum over all pairs: $V_w = \sum_j V_{w,j}$.

For the molecule immersed in a solvent, creation of intramolecular contacts through alteration of the configuration must occur at the expense of intermolecular interactions with the solvent. These latter interactions being necessarily attractive, the effect of including them would be to suppress the attractive branch of the function $V_{w,j}$ occurring at larger distances d_j between members of pair j . The minimum in this function would thus be effectively eliminated. Calculations have therefore been carried out on two bases: first, ignoring solvent interactions by using $V_{w,j}$ given as above, and, second, using the potential function modified as follows in consideration of polymer-solvent interactions

$$\begin{aligned} V_{w,j}^* &= V_{w,j} - V_{w,j}^0 & d_j < d_j^0 \\ V_{w,j}^* &= 0 & d_j \geq d_j^0 \end{aligned} \quad (5)$$

where $V_{w,j}^0$ is the value of $V_{w,j}$ at its minimum, *i.e.*, at $d_j = d_j^0$ (see above).

The occurrence of a large dipole moment in alkylamides²⁹ suggested in the course of this investigation that dipolar interactions, although heretofore ignored, may nevertheless play an important role in influencing the configurations assumed by polypeptide chains.¹⁴ The vector sum of the peptide group bond dipole moments places the resultant group moment very nearly in the plane of the peptide unit, virtually parallel to the N-H and C=O bonds and pointing in the direction from N to H.^{30,31} This orientation is consistent with that established for formamide by spectroscopic studies,¹⁸ if account is taken of the replacement of the amide hydrogen *cis* to the oxygen in formamide by an α -carbon in the peptide unit; the N-C bond moment in the peptide group is much smaller than the N-H moment in formamide.³⁰ We have accordingly approximated the electrical asymmetry of the peptide group by ascribing to it a point dipole moment, shown as \mathbf{u} in Figure 1, situated in the plane of the peptide group at the midpoint of the amide bond so as to form an angle of 56° with this bond. (See Table I for the orientation of the C=O and N-H bonds with respect to the amide bond.) A magnitude of 3.7 D. has been assigned to the group moment on the basis of closely agreeing values for various alkylamides.²⁹

The mutual potential energy of two such dipoles \mathbf{u}_i and \mathbf{u}_j separated by the vector \mathbf{a} was calculated according to the expression for interaction of two point dipoles

$$V_d = (1/\epsilon)[\mathbf{u}_i \cdot \mathbf{u}_j/a^3 - 3(\mathbf{u}_i \cdot \mathbf{a})(\mathbf{u}_j \cdot \mathbf{a})/a^5] \quad (6)$$

(28) A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964).

(29) R. M. Meighan and R. H. Cole, *ibid.*, **68**, 503 (1964).

(30) C. P. Smyth, "Dielectric Behavior and Structure," McGraw-Hill Book Co., Inc., New York, N. Y., 1955, pp. 244, 245.

(31) A. Wada, "Polyamino Acids, Polypeptides and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, p. 131.

where ϵ is the dielectric constant and a is the scalar magnitude of \mathbf{a} . High frequency dielectric constant measurements on solid amides and polyamides,³² for which contributions from the orientation of permanent dipoles are small, suggest the appropriate value of ϵ for nearest-neighbor interactions to be in the range 2.5 to 4.5. For example, the dielectric constant for nylon is 3.4 to 4.0 at 10^6 c.p.s. while that for solid acetamide at 10^8 c.p.s. is 4.0. On the other hand, the high frequency dielectric constant has been estimated at 2.5 from refractive index data for a number of amides.³³

The contribution from nearest-neighbor dipole-dipole interactions, which depend upon the pair of rotation angles φ_{i-1}'''' and φ_i' , has been included in the rotational potential function. The inverse cube dependence of V_d on the distance and the intervention of the coil promoting solvents of relatively high dielectric constant cause average interactions of second neighbor dipoles to be smaller than those of first neighbors by at least an order of magnitude. The effect on the unperturbed dimensions of interactions of second neighbor and more distant dipoles, which depend upon sequences of more than two rotation angles, are sufficiently small to be neglected according to calculations not included here.

The total potential function for rotations governing the mutual orientation of virtual bonds i and $i - 1$ is taken to be the sum of the various contributions discussed above. The foregoing considerations indicate that all of the contributing potential functions, *i.e.*, eq. 1-6, depend on one or both of the angles φ_{i-1}'''' and φ_i' but on no other rotation angles. Thus, while rotations about bond l_{i-1}'''' and l_i' are indeed interdependent, the total potential for rotations about this pair of bonds may be treated as independent of all other rotation angles of the chain, and consequently the mutual orientation of virtual bonds i and $i - 1$ is independent of the orientations of all other virtual bonds. The rotational potential function for the chain as a whole may be expressed as the sum of the potentials for all successive pairs of virtual bonds. The separability of the potential for the molecule as a whole into terms, each of which depends only on an isolated pair of angles φ_{i-1}'''' and φ_i' , permits factoring of the chain partition function into independent terms each depending only on this pair of angles. This circumstance greatly simplifies the theoretical treatment of the polypeptide unperturbed dimensions as we shall presently show.

Calculation of Intrachain Distances. The dependence of the van der Waals and the dipole-dipole interaction energies on φ_{i-1}'''' and φ_i' is implicit rather than explicit in the expressions given above. Calculation of the necessary intrachain distances as a function of these angles is accomplished most easily by matrix methods,³⁴ and for this purpose let a right-handed Cartesian coordinate system be defined for each bond of the chain skeleton in the usual way. That is, we take the x -axis in the direction of the given bond and the y -axis in the

plane defined by this bond and the preceding one; the positive direction of the y -axis is required to make an acute angle with the preceding bond. These coordinate systems are shown in Figure 2.

Orthogonal matrices $T(\theta, \varphi)$ of the form

$$T(\theta, \varphi) = \begin{bmatrix} \cos \theta & \sin \theta & 0 \\ \sin \theta \cos \varphi & -\cos \theta \cos \varphi & \sin \varphi \\ \sin \theta \sin \varphi & -\cos \theta \sin \varphi & -\cos \varphi \end{bmatrix}$$

express the transformation of a vector from the coordinate system of a given bond to the coordinate system of the preceding bond in the chain backbone. The matrices are functions of θ , the supplement to the fixed valence angle between these two bonds, and the angle φ of rotation about the latter of them. Let $T'(\theta', \varphi')$ represent the transformation from the coordinate system of a bond l'' to the coordinate system of the adjacent (preceding) bond l' . Similarly, $T''(\theta'', \varphi'')$ will designate the transformation from the coordinate system of a bond l''' to the reference frame of the adjacent bond l'' , and $T'''(\theta''', \varphi''')$ the transformation from the coordinate system of a bond l' to that of the adjacent bond l''' . A bond connecting a pendant atom or group with the main chain, *i.e.*, l^0 , l^N , l^β , and l^α , may be expressed unambiguously as a vector in the reference frame which has its origin on the same skeletal atom to which the pendant atom or group is attached. Hence, the foregoing orthogonal matrices suffice for transforming the representation of any bond vector of interest here to the reference frame of any other bond. The sum of bond vectors connecting any pair of atoms is easily written as a function of the rotation angles for the intervening bonds, and from the vector sum the interatomic distance may be calculated. For example, the vector sum \mathbf{h} of bonds connecting C_{i-1} and N_i is given by

$$\mathbf{h} = l' + T'l'' + T''T'''l' + T''T'''T'l''$$

and the interatomic distance is $(\mathbf{h} \cdot \mathbf{h})^{1/2}$. Distances between peptide group dipoles may be calculated in similar fashion.

Mean Square Unperturbed Dimensions. For the polypeptide chain comprising n_p virtual bonds of length l_p , the characteristic dimensionless ratio $\langle r^2 \rangle_0 / n_p l_p^2$ of the mean square end-to-end length $\langle r^2 \rangle_0$ may be expressed as

$$\langle r^2 \rangle_0 / n_p l_p^2 = 1 + 2 \sum_{k=1}^{n_p-1} (1 - k/n_p) P_k \quad (7)$$

where P_k is the average projection on a given virtual bond of a unit vector lying along the k th succeeding virtual bond in the chain.³⁵ This average projection will depend only on the number $k - 1$ of intervening bonds and not otherwise on the location of the bonds in the chain sequence for a chain of bonds in which the mutual orientation of a given pair of adjoining bonds is independent of the orientation of all other bonds in the chain. The polypeptide chain of virtual bonds satisfies this condition in excellent approximation, as indicated above.

Let a right-handed Cartesian coordinate system be affixed to each virtual bond, with axis x_i in the direction of the i th virtual bond and with the y_i axis in the plane

(32) W. O. Baker and W. A. Yager, *J. Am. Chem. Soc.*, **64**, 2171 (1942).

(33) S. J. Bass, W. I. Nathan, R. M. Meighan, and R. H. Cole, *J. Phys. Chem.*, **68**, 509 (1964).

(34) Methods for calculating polypeptide intrachain distances which are equivalent to the methods presented have been given by G. Nemethy and H. A. Scheraga, *Biopolymers*, **3**, 155 (1965).

(35) P. J. Flory, *Brookhaven Symp. Biol.*, **13**, 89 (1960).

of the i th peptide unit so as to make an acute angle with bond l_i'' . If \hat{T}_{i-1} is the orthogonal matrix which transforms a vector presented in the coordinate system associated with virtual bond i to the coordinate system of virtual bond $i - 1$, then $P_k = (\langle \hat{T}^k \rangle)_{11}$ where \hat{T}^k is the product of k successive matrices of the type \hat{T}_{i-1} . The angle brackets denote the statistical mechanical average of the matrix product, and the subscript 11 identifies the one-one element of the averaged product. Subscripts indexing the ordinal placement of peptide units are rendered unnecessary for reasons cited above.

The matrix

$$T^\eta \equiv T(-\eta, \varphi' + \pi) = \begin{bmatrix} \cos \eta & -\sin \eta & 0 \\ \sin \eta \cos \varphi' & \cos \eta \cos \varphi' & -\sin \varphi' \\ \sin \eta \sin \varphi' & \cos \eta \sin \varphi' & \cos \varphi' \end{bmatrix}$$

effects the transformation of a vector from the coordinate system of virtual bond i to the coordinate system of chemical bond l_i' while the matrix

$$T^\xi \equiv T(\xi, \pi) = \begin{bmatrix} \cos \xi & \sin \xi & 0 \\ -\sin \xi & \cos \xi & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

transforms a vector given in the coordinate system of chemical bond l_{i-1}'''' to that of virtual bond $i - 1$. All relevant angles and coordinate systems are shown in Figure 2. The matrix \hat{T}_{i-1} may be expressed as the product

$$\hat{T}_{i-1} = T^\xi(\xi)T''''(\theta_{i-1}''', \varphi_{i-1}''')T^\eta(\eta, \varphi_i)$$

and is seen to be a function of the pair of rotation angles φ_{i-1}'''' and φ_i' only. Independence of this pair of rotations from neighboring rotations renders each \hat{T} in the product \hat{T}^k independent so that $\langle \hat{T}^k \rangle$ may be replaced in P_k by $\langle \hat{T} \rangle^k$. This substitution results in the simplification of the treatment referred to above, whereby, the summation appearing in eq. 7 takes the form of a finite geometric series in the averaged matrix $\langle \hat{T} \rangle$

$$\langle r^2 \rangle_0 / n_p l_p^2 = 1 + 2 \sum_{k=1}^{n_p-1} (1 - k/n_p) \langle \hat{T} \rangle_{11}^k \quad (8)$$

The summation in eq. 8 is easily evaluated, with the result

$$\langle r^2 \rangle_0 / n_p l_p^2 = \left\{ (E + \langle \hat{T} \rangle)(E - \langle \hat{T} \rangle)^{-1} - \left(\frac{2}{n_p} \right) \langle \hat{T} \rangle (E - \langle \hat{T} \rangle^{n_p})(E - \langle \hat{T} \rangle)^{-2} \right\}_{11} \quad (9)$$

where E is the identity matrix of order 3. Comparison of eq. 9 with the general result for finite polymer chains with interdependent rotations¹³ shows the extent of the simplification permissible for polypeptide chains with *trans* peptide units.

The mean square unperturbed radius of gyration $\langle s^2 \rangle_0$ of a chain of n identical bonds of length l , the chain being one in which the mutual orientation of any pair of connecting bonds is independent of all other chain bonds, is given by³⁶

(36) Equation 10 follows from eq. C-9 on p. 430 of ref. 23 after appropriate modification to make n count the number of bonds in the chain rather than the number of atoms.

$$\langle s^2 \rangle_0 = nl^2 / (n + 1)^2 + \frac{1}{(n + 1)^2} \sum_{k=1}^{n-1} (n - k) \langle r^2 \rangle_{0,k} \quad (10)$$

where $\langle r^2 \rangle_{0,k}$ is the mean square unperturbed distance between pairs of chain atoms separated by $k + 1$ intervening bonds. Because of the independence of pairs of angles φ_{i-1}'''' and φ_i' from all other rotation angles of the molecule, the polypeptide chain satisfies the conditions for applicability of eq. 10, *i.e.*, the mutual orientation of a given pair of virtual bonds is uncorrelated with the orientations of other virtual bonds of the chain. Equation 9 with n_p replaced by $k + 1$ thus provides the appropriate expression for $\langle r^2 \rangle_{0,k}$ for chains of polypeptide virtual bonds. Substitution of this expression for $\langle r^2 \rangle_{0,k}$ into eq. 10 and subsequent evaluation of the summation yields

$$\begin{aligned} \langle s^2 \rangle_0 / n_p l_p^2 &= \frac{1}{(n_p + 1)^2} + \\ &\frac{(n_p - 1)(n_p + 4)}{6(n_p + 1)^2} \{ (E + \langle \hat{T} \rangle)(E - \langle \hat{T} \rangle)^{-1} \}_{11} - \\ &\frac{(n_p - 1)}{(n_p + 1)^2} \{ \langle \hat{T} \rangle (E - \langle \hat{T} \rangle)^{-2} \}_{11} + \\ &\frac{2}{(n_p + 1)^2} \left\{ \langle \hat{T} \rangle^3 - \langle \hat{T} \rangle^{n_p+2} (E - \langle \hat{T} \rangle)^{-3} - \right. \\ &\left. \frac{1}{n_p} [\langle \hat{T} \rangle^3 - n_p \langle \hat{T} \rangle^{n_p+2} (E - \langle \hat{T} \rangle)^{-3} + \langle \hat{T} \rangle^4 - \right. \\ &\left. \left. \langle \hat{T} \rangle^{n_p+3} (E - \langle \hat{T} \rangle)^{-4} \right] \right\}_{11} \quad (11) \end{aligned}$$

In the limit $n_p \rightarrow \infty$ eq. 11 reduces to the general result $\langle s^2 \rangle_0 = \langle r^2 \rangle_0 / 6$.³⁷

Evaluation of $\langle \hat{T} \rangle$. Calculation of the polypeptide chain dimensions as expressed by $\langle r^2 \rangle_0 / n_p l_p^2$ or $\langle s^2 \rangle_0 / n_p l_p^2$ requires evaluation of the average matrix $\langle \hat{T} \rangle$. Averaging of \hat{T} implies the averaging of each of its elements, $\hat{T}_{vw}(\varphi_{i-1}''', \varphi_i')$. If the potential $V(\varphi_{i-1}''', \varphi_i')$ expresses the total energy for rotation about the pair of bonds l_{i-1}'''' and l_i' , then the partition function for each such pair of bonds is

$$z = \int_{\varphi_{i-1}''''=0}^{2\pi} \int_{\varphi_i'=0}^{2\pi} \exp(-V/RT) d\varphi_{i-1}'''' d\varphi_i' \quad (12)$$

while the average of an element of \hat{T} is given by

$$\langle \hat{T}_{vw} \rangle = z^{-1} \int_{\varphi_{i-1}''''=0}^{2\pi} \int_{\varphi_i'=0}^{2\pi} \hat{T}_{vw} \times \exp(-V/RT) d\varphi_{i-1}'''' d\varphi_i' \quad (13)$$

In order to facilitate numerical calculations it is convenient to replace the integration over the continuously varying angles φ_{i-1}'''' and φ_i' by summation over r discrete values for each angle.⁵ Thus

$$z = \sum_{\varphi_{i-1}''''} \sum_{\varphi_i'} \exp(-V/RT) \quad (14)$$

and

$$\langle \hat{T}_{vw} \rangle = z^{-1} \sum_{\varphi_{i-1}''''} \sum_{\varphi_i'} \hat{T}_{vw} \exp(-V/RT) \quad (15)$$

The approximation involved in thus replacing the integral by a sum over discrete values of the integrand

(37) Reference 23, p. 428 ff.

is readily reduced below the limits of uncertainty in the rotational potential function V by choice of a sufficient number of terms in the sum.

Since T^k is independent of the rotation angles, $\langle \hat{T} \rangle = T^k \langle T''' T^n \rangle$. In terms of the sets of discrete values for the rotation angles, a convenient expression for the average matrix product $\langle T''' T^n \rangle$ may be developed following earlier treatments.^{5,6,9} We define for this purpose an $r \times r$ matrix U with rows indexed by the r discrete values of φ_{i-1}''' and columns indexed by the r discrete values of φ_i' . The elements of U are Boltzmann factors, $\exp(-V/RT)$, evaluated for each of the r^2 pairs of values accessible to the angles φ_{i-1}''' and φ_i' . If J is the $r \times 1$ column matrix with all elements unity and J^T is the transpose of J , the configuration partition function for the pair of bonds l_{i-1}''' and l_i' is given by $z = J^T U J$. From U a new matrix \mathfrak{u} of order $3r$ may be constructed by taking the direct product (denoted by \times) of the identity matrix E of order 3 with U . Thus

$$\mathfrak{u} = E \times U = \begin{bmatrix} U & 0 & 0 \\ 0 & U & 0 \\ 0 & 0 & U \end{bmatrix}$$

where 0 represents the null matrix of order r . Additionally, a diagonal matrix T_{vs}''' of order r is defined with elements given by the vs element of T''' evaluated for each of the r discrete values of φ_{i-1}''' . Nine such matrices are constructed, one for each element of T''' . In like fashion, matrices T_{sw}^n are constructed from the nine elements of T^n , these being evaluated for the r discrete values of φ_i' . Finally, let matrices T_{vs}''' and T_{sw}^n , respectively, supply elements for the matrices $\mathfrak{T}''' = [T_{vs}''']$ and $\mathfrak{T}^n = [T_{sw}^n]$ of order $3r$. If \mathfrak{g} is given by $\mathfrak{g} = E \times J$ and \mathfrak{g}^T is given by $\mathfrak{g}^T = E \times J^T$, then

$$\langle T \rangle = z^{-1} T^k \mathfrak{g}^T \mathfrak{T}''' \mathfrak{u} \mathfrak{T}^n \mathfrak{g} \quad (16)$$

Numerical Results

Values of the characteristic ratio $\langle r^2 \rangle_0 / n_p l_p^2$ in the limit of large n_p have been calculated using eq. 9 and 16. A temperature $T = 310^\circ\text{K}$. was used throughout to evaluate the elements of U . Because of uncertainties in several of the parameters entering into the rotational potential function V , these have been varied over the reasonable ranges suggested above. Those varied are the torsional barrier heights V_0''' and V_0' , the dielectric constant ϵ , and the van der Waals radius of the β -methylene group r_{CH_2} . In addition, calculations have been carried out using the van der Waals potential functions $V_{w,j}^*$ excluding the attractive branch of the potential as well as with van der Waals functions $V_{w,j}$ containing both repulsions and attractions. The effect of replacing torsional potential function $V_{t,a}'''$ (minima at 0, 120, and 240°) by $V_{t,b}'''$ (minima at 60, 180, 300°) was also investigated. In every case $\langle \hat{T} \rangle$ has been evaluated from eq. 16 with $r = 12$ such that φ_{i-1}''' and φ_i' take the discrete values 0, 30, 60, 90, ..., 330°. These discrete values have been chosen at equal intervals in order to give equivalent representation to all regions of the potential function. The substantial reduction in matrix manipulations resulting from the independence of rotations about l_{i-1}''' and l_i' from all other rotations allows

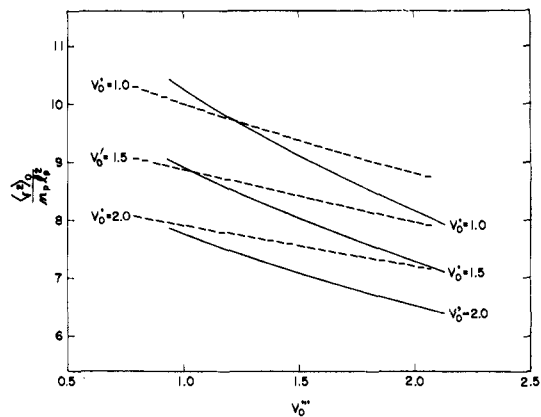


Figure 3. Plots of $\langle r^2 \rangle_0 / n_p l_p^2$ vs. V_0''' for the indicated values of V_0' using torsional potentials V_t' and $V_{t,a}'''$. Results are shown for van der Waals potentials $V_{w,j}^*$ with the attractive branch eliminated (solid lines) and for van der Waals potentials $V_{w,j}$ including repulsions and attractions (dashed lines); $r_{\text{CH}_2} = 1.85 \text{ \AA}$. and $\epsilon = 3.5$ in all cases shown.

us to choose a sufficient number of discrete values for φ_{i-1}''' and φ_i' to approximate closely the continuous rotational potential function. This procedure departs from that of previous treatments of the unperturbed coil dimensions of polymer chains⁸⁻¹² for which the discrete rotation angles were chosen to correspond to significant minima of the rotational potential function. (Computations carried out with $r = 6$, φ_{i-1}''' and φ_i' taking on values 0°, 60°, etc., yielded results within 10% of those reported below for $r = 12$.)

Figure 3 summarizes results of calculations carried out on the basis of threefold torsional potentials V_t' (eq. 1) and $V_{t,a}'''$ (eq. 2), both having minima at 0, 120, and 240°. The characteristic ratio $\langle r^2 \rangle_0 / n_p l_p^2$ is plotted against the barrier height V_0''' for the several values of V_0' indicated beside the curves. Solid lines refer to van der Waals pair potentials $V_{w,j}^*$ with the attractive branch eliminated in accordance with eq. 5; dashed lines refer to the unaltered potential $V_{w,j}$ (eq. 4). The van der Waals radius r_{CH_2} was chosen for these calculations to be 1.85 Å. First neighbor dipole interactions were taken into account using eq. 6, with ϵ assigned the value 3.5. The experimental results,¹⁵ 9.0 ± 0.5 , for the characteristic ratio are reproduced in Figure 3 for reasonable values of the variable parameters. The similarity of results for the two forms of the van der Waals potentials supports our earlier statement regarding the insensitivity of the conclusions to the details of the attractive part of the van der Waals potential functions. In the presence of solvent the van der Waals potentials $V_{w,j}^*$ probably are the more realistic for reasons already discussed.

The curves in Figure 3 are representative of numerous additional results, not included, for other reasonable choices of r_{CH_2} and ϵ , except for displacement along the ordinate axis. The dependence of $\langle r^2 \rangle_0 / n_p l_p^2$ on r_{CH_2} is presented in Figure 4 for both kinds of van der Waals potentials. The curves shown are for $\epsilon = 3.5$; the torsional potential functions V_t' and $V_{t,a}'''$ have again been used with V_0' and V_0''' equal to 1.0 and 1.5 kcal./mole, respectively. The sensitivity of $\langle r^2 \rangle_0 / n_p l_p^2$ to r_{CH_2} reflects the importance of con-

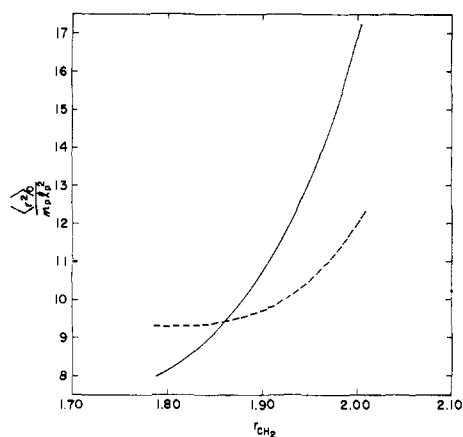


Figure 4. Plots of $\langle r^2 \rangle_0 / n_p l_p^2$ vs. r_{CH_2} for van der Waals potentials $V_{w,j}^*$ excluding the attractive branch (solid line) and for van der Waals potentials $V_{w,j}$ including repulsions and attractions (dashed line). Torsional potentials were $V_{t'}$ and $V_{t,a}'''$ with $V_{0'}$ and $V_{0'''}$ equal to 1.0 and 1.5 kcal./mole, respectively; $\epsilon = 3.5$.

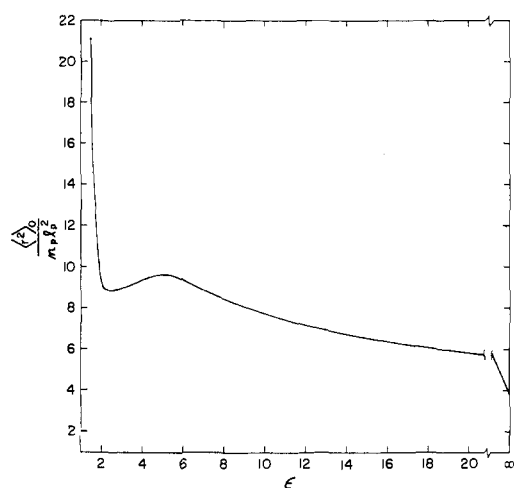


Figure 5. Plot of $\langle r^2 \rangle_0 / n_p l_p^2$ vs. ϵ using van der Waals potentials $V_{w,j}^*$ excluding the attractive branch and $r_{\text{CH}_2} = 1.85 \text{ \AA}$. Torsional potentials were $V_{t'}$ and $V_{t,a}'''$ with $V_{0'}$ and $V_{0'''}$ equal to 1.0 and 1.5 kcal./mole, respectively.

tributions to the total rotational potential from van der Waals potential functions for interactions involving the β -methylene group. The importance of steric repulsions by the β -methylene group in determining stable polypeptide configurations has been stressed by Sasisekharan.¹⁶ His conclusions regarding minimum permissible contact distances for van der Waals interactions involving the β -methylene group are compatible with the energies for these interactions calculated from our van der Waals potential functions for $r_{\text{CH}_2} = 1.80\text{--}1.85 \text{ \AA}$. Our calculated repulsive energies for the other sorts of nonbonded contacts also are consistent with Sasisekharan's conclusions from X-ray crystallographic data regarding minimum permissible contact distances.

That variations of the dielectric constant within the range 2.5 to 4.5 have only a small effect upon $\langle r^2 \rangle_0 / n_p l_p^2$ is illustrated in Figure 5. The calculations shown were carried out using the van der Waals potentials $V_{w,j}^*$ with the attractive branch eliminated, torsional potentials $V_{t'}$ and $V_{t,a}'''$ with $V_{0'} = 1.0$ kcal./mole and $V_{0'''} = 1.5$ kcal./mole, and $r_{\text{CH}_2} =$

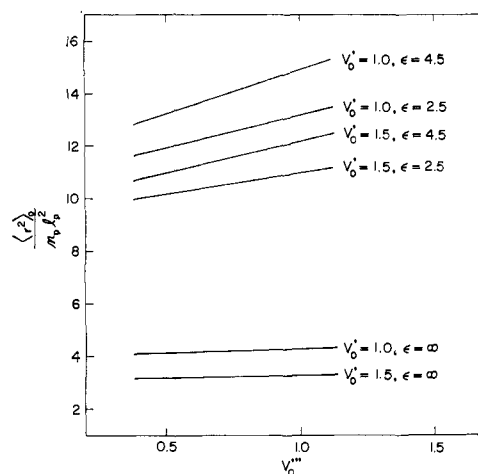


Figure 6. Plots of $\langle r^2 \rangle_0 / n_p l_p^2$ vs. $V_{0'''}$ for the indicated values of $V_{0'}$ and ϵ using torsional potentials $V_{t'}$ and $V_{t,b}'''$. van der Waals potentials $V_{w,j}^*$ with the attractive branch eliminated were used with $r_{\text{CH}_2} = 1.80 \text{ \AA}$.

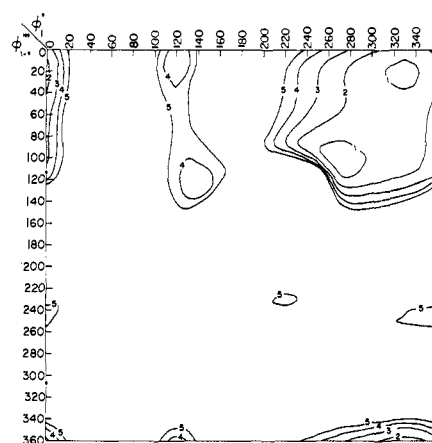


Figure 7. Contour diagram of total potential function V at 1-kcal./mole interval for torsional potentials $V_{t'}$ and $V_{t,b}'''$ with $V_{0'}$ and $V_{0'''} = 1.0$ and 1.5 kcal./mole, respectively; van der Waals potentials $V_{w,j}^*$ with $r_{\text{CH}_2} = 1.85 \text{ \AA}$; $\epsilon = 3.5$. On the basis of this potential surface $\langle r^2 \rangle_0 / n_p l_p^2 = 9.13$.

1.85 \AA . The importance of including peptide dipole-dipole interactions in the total rotational potential function is evident from Figure 5 by comparison of results for $\epsilon = 2.5$ to 4.5 with the result for $\epsilon = \infty$ shown on the right. As reported previously,¹⁴ omission of dipole-dipole interactions yields calculated values of $\langle r^2 \rangle_0 / n_p l_p^2$ which are much smaller than those observed by experiment.

The effect of replacing $V_{t,a}'''$ by the potential $V_{t,b}'''$, having minima at $\phi_{i-1}''' = 60, 180,$ and 300° , is illustrated by comparison of Figure 6 with 3. In Figure 6, $\langle r^2 \rangle_0 / n_p l_p^2$ is plotted against $V_{0'''}$ for several combinations of $V_{0'}$ and ϵ ; torsional potentials $V_{t'}$ and $V_{t,b}'''$ have been used in combination with van der Waals potentials from which the attractive branch has been eliminated; $r_{\text{CH}_2} = 1.80 \text{ \AA}$. Replacement of $V_{t,a}'''$ by $V_{t,b}'''$ is seen to raise the calculated characteristic ratio somewhat and to reverse the dependence of this ratio on $V_{0'''}$. Calculated values of the unperturbed dimensions are again too small if dipole-dipole interactions are ignored.

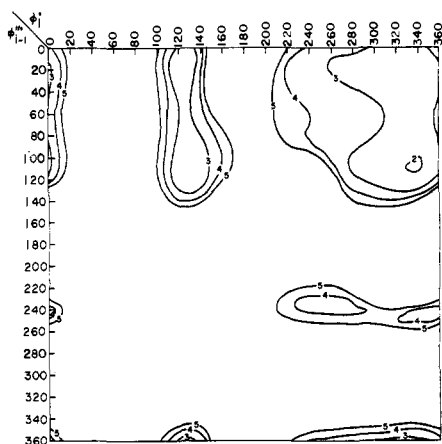


Figure 8. Total potential function V at intervals of 1 kcal./mole using the same values of the parameters as in Figure 7, but ignoring the dipole-dipole energy (i.e., $\epsilon = \infty$). This potential surface yields $\langle r^2 \rangle_0 / n_p l_p^2 = 3.95$.

Figure 7 shows a contour surface for the total rotational potential function of bond rotations φ_{i-1}' and φ_i' for a choice of parameters giving $\langle r^2 \rangle_0 / n_p l_p^2 = 9.13$. Torsional potentials $V_{t'}$ and $V_{t,a}''$ with V_0' and V_0'' equal to 1.0 and 1.5 kcal./mole, respectively, were used along with van der Waals potentials $V_{w,j}^*$ excluding the attractive branch, and with $r_{\text{CH}_2} = 1.85 \text{ \AA}$. and $\epsilon = 3.5$. The contour interval is 1 kcal./mole; all contour lines of 5 kcal./mole or less are shown. Regions of the diagram outside the 5-kcal./mole contour line are effectively excluded to the chain for any temperatures at which the chain is chemically stable.

The foregoing choice of parameters is not unique in yielding a value for the characteristic ratio in the neighborhood of 9, the experimental value. The latitude in the choice of dielectric constant and torsional barrier height will be apparent from Figures 3, 5, and 6; only the value of r_{CH_2} is critical (Figure 4). A number of calculations of the potential surface have been carried out for acceptable variation of the parameters. The main features of Figure 7 are preserved with only minor changes. The striking similarity of the contour map in Figure 7 to the diagram of acceptable polypeptide rotation angles given by Ramachandran, Ramakrishnan, and Sasisekharan³⁸ and based solely on steric considerations reflects the dominant role of van der Waals repulsions in determining the net rotational potential function V . The broad region of high potential energy lying between $\varphi_i' = 20^\circ$ and $\varphi_i' = 100^\circ$ arises primarily from interactions of N_i and H_i with the i th β -methylene group. Interactions between this methylene group and the carbonyl group of peptide unit $i - 1$ lead to the region of high energy between $\varphi_{i-1}''' = 260^\circ$ and $\varphi_{i-1}''' = 340^\circ$. The belt of high energy centered around $\varphi_{i-1}''' = 180^\circ$ derives largely from severe steric conflicts between the $(i - 1)$ - and the i th carbonyl groups while the region of the map near $\varphi_i' = 180^\circ$ is excluded owing to interactions of neighboring N-H groups. In the center of the map near $\varphi_{i-1}''' = \varphi_i' = 180^\circ$, a number of additional intolerable steric conflicts arise.

(38) G. N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan, *J. Mol. Biol.*, **7**, 95 (1963).

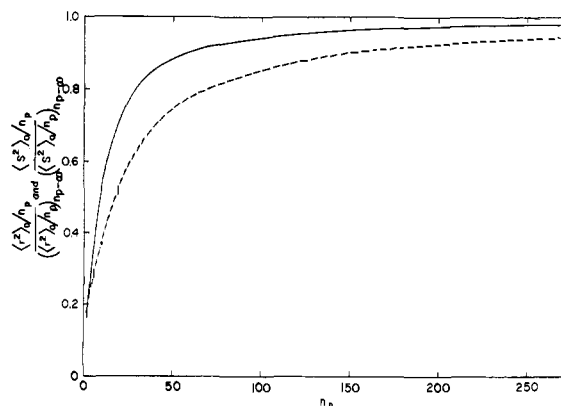


Figure 9. The characteristic ratios $\langle r^2 \rangle_0 / n_p l_p^2$ and $\langle s^2 \rangle_0 / n_p l_p^2$ of the mean square end-to-end length and radius of gyration, respectively, plotted as fractions of their respective asymptotic limits $(\langle r^2 \rangle_0 / n_p) / [(\langle r^2 \rangle_0 / n_p)_{n_p \rightarrow \infty}]$ (solid line) and $(\langle s^2 \rangle_0 / n_p) / [(\langle s^2 \rangle_0 / n_p)_{n_p \rightarrow \infty}]$ (dashed line) against n_p . Parameters used for these calculations are the same as in Figure 7.

The contribution to the rotational potential function from dipole-dipole interactions is seen by comparison of Figure 7 with 8, the latter having been calculated omitting the dipole-dipole energy but otherwise using the same parameters as in Figure 7. Figure 8 presents three regions of low energy: a main one in the neighborhood of $\varphi_{i-1}''' = 30^\circ$; $\varphi_i' = 330^\circ$, and two subsidiary minima located, respectively, along the line for $\varphi_i' = 120^\circ$ and that for $\varphi_{i-1}''' = 240^\circ$. The energies of the latter two are raised considerably by dipole-dipole interactions (compare Figure 7); that of the main minimum is lowered somewhat. This minimum, represented by the basin in the upper right-hand corner of the diagram, is thus rendered the dominant one by dipolar interactions to the virtual exclusion of other conformations. These interactions, heretofore ignored, greatly reduce the rotational freedom in the sense of restricting the favorable ranges of angle. In consequence thereof, the calculated unperturbed dimensions are increased over those computed on the basis of Figure 8.

The apparently anomalous decrease of $\langle r^2 \rangle_0 / n_p l_p^2$ with increasing potential barriers, which is evident in Figure 3, is similarly explained. Increase of the barriers for torsional potentials $V_{t'}$ and $V_{t,a}''$ tends to decrease the energy difference between the various minima of the total potential function. Hence, a wider range of rotational angles becomes available to the chain as V_0' and V_0'' are increased, and the calculated unperturbed dimensions decrease.

The characteristic ratios $\langle r^2 \rangle_0 / n_p l_p^2$ and $\langle s^2 \rangle_0 / n_p l_p^2$ have also been calculated for finite n_p from eq. 9 and 11, respectively, using the same values of various parameters from which the potential of Figure 7 was computed. The results, plotted as fractions of the asymptotic limits, are shown in Figure 9. As expected, the characteristic ratio of the mean square unperturbed radius of gyration converges more slowly than the characteristic ratio of the mean square unperturbed end-to-end distance.³⁹ Moreover, characteristic ratios of the unperturbed dimensions of the relatively stiff polypeptide chain are more slowly con-

(39) P. J. Flory and R. L. Jernigan, in preparation.

vergent than are those of polyethylene and polydimethylsiloxane.³⁹

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Kinetics of the Exchange of Chlorine Atoms between Hydrogen Chloride and Benzyl Chlorides. Solvent Effects^{1,2}

Warren Bruce, Milton Kahn, and Joseph A. Leary

Contribution from the University of New Mexico, Albuquerque, New Mexico, and the University of California Los Alamos Scientific Laboratory, Los Alamos, New Mexico. Received February 8, 1965

The rate of exchange of chlorine atoms between hydrogen chloride and p-nitrobenzyl chloride in 82% dioxane-18% water by weight solution is represented by the rate law: $R_e = 2.04 \times 10^{11} e^{-22,600/RT} \alpha(HCl)(PNBC) + 2.76 \times 10^8 e^{-19,600/RT} (1 - \alpha)(HCl)(PNBC)$, where α is the degree of dissociation of the hydrogen chloride and R_e is the rate of exchange in mole l.⁻¹ sec.⁻¹. The reaction mechanism is believed to be an example of a bimolecular process in which both the chloride ion and the hydrogen chloride ion pair participate. Ordinary fluorescent light has no detectable effect on the rate of exchange, and the over-all rate of exchange is decreased by added lithium perchlorate. Analytical expressions were developed which allow for the hydrolysis of the organic halide during the exchange. No exchange was detected between hydrogen chloride and p-nitrobenzyl chloride in anhydrous benzene after 363 hr. at 60.00°. Similarly, no exchange was observed with benzyl chloride in benzene and acetone. The exchange with benzyl chloride proceeds at a faster rate in ethanol than in dioxane. In dioxane, p-nitrobenzyl chloride exchanges at a larger rate than benzyl chloride.

Introduction

The work presented here is primarily concerned with the exchange of chlorine atoms between hydrogen chloride and p-nitrobenzyl chloride in 82% dioxane-18% water by weight solutions. This system is of particular interest since the thermodynamic data for hydrogen chloride in dioxane-water mixtures have been extensively investigated³; furthermore, this system affords an opportunity to study an exchange in a medium of low dielectric constant and relatively high solvating power.

The mechanism of certain nucleophilic substitution

reactions has been the subject of some controversy; i.e., some reactions are considered to take place either via a single intermediate mechanism having both unimolecular (SN1) and bimolecular (SN2) properties or by concurrent unimolecular and bimolecular processes. Bird, Hughes, and Ingold⁴ have proposed a theory in support of the idea that the mechanisms merge into each other, and the interpretation by Bunsley and Kohnstam⁵ of the solvolysis of benzyl chloride in 50% by volume aqueous acetone lends support to this point of view. Gold⁶ criticized this argument on the theoretical ground that the postulation of fractional reaction orders is incompatible with the current formulation of the laws of thermodynamics. Hill and Fry⁷ have studied chlorine isotope effects in the reactions of substituted benzyl chlorides with cyanide and thiosulfate ions in 80% dioxane-20% water by weight solutions and concluded that there is a smooth transition in the reaction mechanism from SN1 to SN2 as the electron-donating power of the substituent increases. Pocker⁸ takes the position that there is scope for both concurrent as well as intermediate SN1-SN2 mechanisms, the concurrent mechanisms being predominant in nonsolvolytic media where very short-lived intermediates cannot be irreversibly captured by a solvent molecule in the solvation cage. Casapieri and Swart⁹ have reported that the exchange of radioactive chloride between diphenylmethyl chloride and lithium chloride in dimethylformamide is an unambiguous example of concurrent first- and second-order nucleophilic substitution.

Arguments exist in the literature to substantiate the position that ion pairs are not effective as nucleophilic reagents; on the other hand, compelling evidence has been presented to show that both ions and ion pairs are reactive. The second-order rate coefficient for the exchange between lithium radiochloride and benzyl chloride in dimethylformamide decreased as the concentration of the salt increased; however, Casapieri and Swart⁹ have shown that a constant value is obtained on the assumption that only the chloride ion is reactive.

(1) This paper is a portion of the dissertation presented by W. Bruce in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the University of New Mexico, June 1962; presented before the Physical Chemistry Division, 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., Sept. 1962.

(2) Under Contract W-7405-Eng-36 with the U. S. Atomic Energy Commission.

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(6) V. Gold, *ibid.*, 4633 (1956).

(7) J. W. Hill and A. Fry, *J. Am. Chem. Soc.*, **84**, 2763 (1962).

(8) Y. Pocker, *Progr. Reaction Kinetics*, **1**, 218 (1961).

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