sium bromide pellets prepared by grinding 1 mg. of sample in 350 mg. of potassium bromide and subjecting the mixture to pressure. Solution spectra were obtained in matched cells ranging in thickness from 0.01 to 0.10 mm. by running the various dioxane solutions against the pure solvent.

Molecular Weight Determinations.—Molecular weight determinations were performed on several of the oligomeric peptides derived from γ-methyl L-glutamate in order to check the molecularity and association of the compounds prepared. Both cryoscopic and isothermal distillation techniques were used for the pentamer and lower homologs. These procedures have been described by Daniels, *et al.*,[40] and Linstead, *et al.*,[41] respectively. The experimental values are listed in Table II.

Details of the equilibrium ultracentrifugation techniques are given in the preceding paper.[1] The results are contained in Table II of this paper and in the previous paper of this series.[1]

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN, BROOKLYN, N. Y.]

Conformational Aspects of Polypeptides. V. Molar Rotational Model Compounds for Poly-γ-methyl L-Glutamate[1]

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This report summarizes the synthesis and use of model compounds which can be employed to explain the optical rotatory properties of oligomeric peptides and polymers of the γ-methyl L-glutamate series. The model compounds serve as a basis for determining the onset of secondary structure in the series of oligomeric peptides. Effects of residue position and neighboring group interactions on optical activity of peptides are elucidated and discussed. The model compound rotations are solvent dependent, and can be used to calculate a "random coil" optical rotation in a variety of solvents. Significant deviations of the optical activity of the oligomeric peptides from the calculated "random coil" rotation are attributed to intramolecular hydrogen bonding in dimethylformamide and *m*-cresol. In dioxane the deviations result from a combination of intra- and intermolecular hydrogen bonding. There are no large differences between calculated and experimental rotations through the undecamer in dichloroacetic acid, a "random coil" solvent. Calculated "random coil" rotations at various temperatures in dimethylformamide were obtained to show that helical effects decrease with increasing temperature.

Introduction

The optical rotatory power of a polypeptide is dependent on many factors.[4-11] To a first approximation, it is composed of the sum of the rotations for the individual asymmetric centers (configurational optical activity).[4] To this must be added the secondary (helical forms) and tertiary structural arrangements of the polymer chain as a whole (conformational optical activity).[4-11] Additional factors include specific interactions between solvent and peptide chain and, where they exist, side chain–main chain interactions[12] and also intermolecular chain–chain interactions (associational optical activity).[13,14]

The contributions of the configurational and conformational aspects are dependent upon the structure of the polypeptide in solution, which in turn is determined by the nature of solvation.[14-16] The type of solvation is related to the strength of the interactions between solvent and the peptide chain, a "random coil" resulting when all chain–chain and intra-chain hydrogen bonds are broken in favor of solvent–chain hydrogen bonds. A mildly interacting solvent does not disrupt the inter- and intramolecular hydrogen bonds, permitting association and/or helix formation.[17]

Optical rotatory properties have been employed in detecting the type of structure existing in solution.[18,19] It has been shown that there is a significant difference in the optical rotation and rotatory dispersion of a polypeptide "random coil" form as compared to the same polymer in a helical form.[20-22]

Results and Discussion

Model Compounds.—As suggested by Brand[23] and Doty[24] and developed by us in this paper, the end groups of a peptide solvate differently from internal residues. Thus their contribution to the optical activity is different from internal residues. As the molecular weight of a polymer decreases, increasing consideration must be given to these end group effects.

(1) (a) Previous paper in this series, M. Goodman, E. E. Schmitt and D. A. Yphantis, *J. Am. Chem. Soc.*, **84**, 1288 (1962). (b) This research was supported by grants from the National Science Foundation (G8514) and the National Institutes of Health (A3868).

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TABLE I

MOLAR ROTATIONS OF MODEL COMPOUNDS[a]

Compound[b]	Mol. wt.	Dioxane		Dimethylformamide		Dichloroacetic acid		*m*-Cresol	
		$[\alpha]^{25}D$	ϕ	$[\alpha]^{25}D$	ϕ	$[\alpha]^{25}D$	ϕ	$[\alpha]^{25}D$	ϕ
I	366	−17.3	−0.633	− 7.2°	−0.264	−12.8°	−0.468	−38.3°	−1.402
II	394.5	+ 7.6°	+ .300	−13.0	− .513	+ 1.2	+ .047	−11.8	−0.466
III	423.5	−19.7	− .835	− 5.9	− .249	−16.0	− .678	−35.5	−1.503
IV	509	−27.1	−1.390	− 9.0	− .460	−22.8	−1.160	−42.6	−2.168
V	537	− 9.6	−0.515	−14.0	− .750	−10.7	−0.575	−10.7	−0.574
VI	566	−24.3	−1.375	−10.5	− .590	−18.8	−1.064	−20.0	−1.132

OMe
|

[a] Note all rotations are in 1% solution. [b] We have synthesized Z-glu-OEt. Its rotatory data are: DMF $[\alpha]^{25}D$ −21.5° (ϕ = −0.694); DCA $[\alpha]^{25}D$ −5.5° (ϕ = −0.178).

We have been studying the optical rotatory properties of polymers and oligomeric peptides derived from γ-methyl L-glutamate.[1,17,25-27]

OMe (OMe) OEt
| | |
Z-glu—(glu—)$_n$—glu-OEt
(a) (b) (c)

Our work suggested that the total configurational optical activity for poly-γ-methyl L-glutamate can be viewed as being related to the sum of the rotations of the following compounds which we synthesized

OMe
|
Z-glu-gly-OMe (I)

OEt
|
Z-gly-glu-OEt (II)

OMe
|
Z-gly-glu-gly-OMe (III)

OMe OMe
| |
Z-glu—glu-gly-OMe (IV)

OMe OEt
| |
Z-gly-glu—glu-OEt (V)

OMe OMe
| |
Z-gly-glu—glu-gly-OMe (VI)

where compounds I, II and III serve as models for the N-terminal (a), C-terminal (c) and internal (b) residues, respectively, in the polymer structure shown above.

The optical rotatory data for benzyloxycarbonyl-γ-methyl-α-ethyl L-glutamate do not correspond to that for the higher oligomers, and therefore this compound cannot serve as a model compound (structure below).

OMe
|
Z-glu-OEt

This observation is reasonable since the compound does not contain a peptide bond. Because of the different type of solvation involved, the optical rotation will deviate drastically from that of the peptide oligomers (see Table I).

Proper use of these model compounds requires the employment of a unit of rotation which measures the contribution of the optically active portion of the molecule only. Contrary to Brand's approach,[23] the use of specific and/or residue rotation is precluded. The most logical basic unit to use is molar rotation defined as

$$\phi = \frac{[\alpha]^{25}_D \times \text{mol. wt.}}{10{,}000} \frac{\text{deg.}}{\text{cm. moles}} \qquad (1)$$

Molar rotations were obtained in a variety of solvents for the compounds listed (Table I) The total molar rotation of a peptide, then, is made up of the sum of end groups plus the internal residue contributions

$$\phi_{total} = \phi_{end} + n\phi_{internal} \qquad (2)$$

where ϕ_{total} is the molar rotation of a peptide, ϕ_{end} is the molar rotation of the N-terminal + C-terminal residues, $\phi_{internal}$ is the molar rotation of an internal residue and n is the number of internal residues.

It is evident from the data in Table I that the molar rotation of a residue is dependent on the position of the residue. In addition, it can be seen that the molar rotation of the N-terminal residue is similar to the molar rotation of the internal residue in each solvent. This can be explained by the similarity in structure between the N-terminal and internal asymmetric carbon atoms; both are adjacent to an amide type bond on either side.

O R O
‖ | ‖
—C—N—C—C—N—
H |* H
H

The N-terminal residue has a urethan link of the benzyloxycarbonyl blocking group rather than a simple amide on one side which may account for the small differences observed. The C-terminal residue, on the other hand, possesses an asymmetric carbon adjacent to an ester group on one side and an amide on the other side, and therefore exhibits entirely different rotations from the other residues.

When ϕ_{total} (calculated from eq. 2) is plotted *vs.* n in various solvents a straight line is obtained, the slope of which is determined by the molar rotational contributions of the internal residues (Fig. 1). The molar rotations in various solvents were obtained from the specific rotations for the series of γ-methyl L-glutamate derived oligomers (n = 0–9, Fig. 1).[1,26,27] Also the molar rotations for the same compounds were calculated (ϕ_{calc}) based on the data of the models (Table I) and use of eq. 2.

To determine the contribution of glutamic acid residues adjacent to each other, model compounds IV-VI (Table I) were prepared. The contribution to the optical activity of the molecule by neighboring optically active residues was determined by measuring the optical activity of compounds IV-VI and comparing the result to that obtained from the corresponding model compounds I-III.

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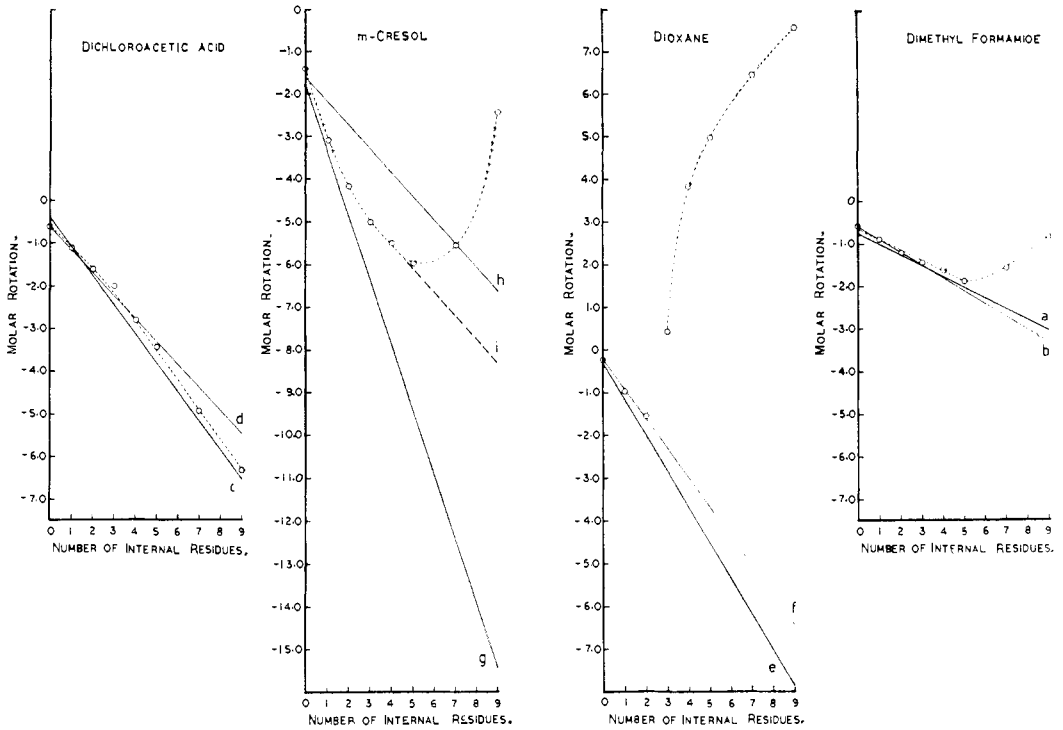

Fig. 1.—Molar rotations based on the model compounds I–III (lines a, c, g, e) and the model compounds IV–VI (lines b, d, f, h) and oligomeric peptides (circles) derived from γ-methyl L-glutamate, in various solvents.

The difference is attributed to neighboring asymmetric residue interactions.

To calculate the molar rotation lines based on these complex model compounds (Fig. 1, lines b,d,f,h), eq. 2 is once again employed. The sum of the molar rotations of compounds IV and V is used to simulate the molar rotation of the oligomeric tetrapeptide, with adjacent glutamyl interactions. To obtain the higher oligomer rotations, half of the molar rotation of compound VI is used as the internal residue molar rotation in eq. 2. Thus a line is constructed commencing with the tetramer by plotting the total molar rotation of a series of peptides *versus* their number of internal residues. The dimer and trimer molar rotations are obtained simply by extrapolation of this line to zero internal residues.

The rotations of the oligomeric peptides in dimethylformamide (Fig. 1) agree with those calculated from the model compounds (lines a and b), as long as secondary structure does not exist. The values calculated on the basis of the simple model compounds I-III (line a) differ only slightly from the experimental oligomeric values because of the end groups. Large deviations do not appear until the nonapeptide. The complex model compounds IV-VI (line b) eliminate the end group difference and a slight deviation begins at the pentamer, with the large deviation still commencing at the nonamer. The detection of the small deviation at the pentamer stage is in agreement with optical rotatory dispersion data.[17] The appearance of the large deviation at the nonamer may be explained by the stabilization of the helix by the doubly intramolecularly hydrogen bonded residues (residue five in the nonamer chain)[17], and concurs with the calculation by Schellman[28] using thermodynamic data. Evidently, when interactions of adjacent glutamyl residues are built into the model compounds they become more suitable as model structures for poly-γ-methyl L-glutamate in dimethylformamide.

In dichloroacetic acid the oligopeptides show two "random coil" slopes (Fig. 1), a shallow one for the dimer through the tetramer, and a steeper one from the pentamer on. It is interesting to note that the slope based on the simple model compounds I-III (line c) corresponds to the steeper slope of the oligomers, while compounds IV-VI (line d) exhibit a slope equal to that shown by the di- through the tetrapeptide. We interpret these phenomena as resulting from a combination of neighbor–neighbor interactions[29] and end group solvation hindrance. Thus when neighboring L-glutamyl residues are adjacent to the end groups as in the dimer through tetramer, the amides cannot be solvated normally because of the bulkiness of the dichloroacetic acid molecules solvating the end groups. At the pentamer the amides surrounding residue "three" are now free to solvate as in a high polymer because they are not bonded to an end residue.

This change in solvatability of internal residues gives rise to what we term the non-hindered "random coil" slope encountered with the higher oligomers and the simple model compounds. Studies of the situation in trifluoroacetic acid and other acidic solvents are presently under way to test this

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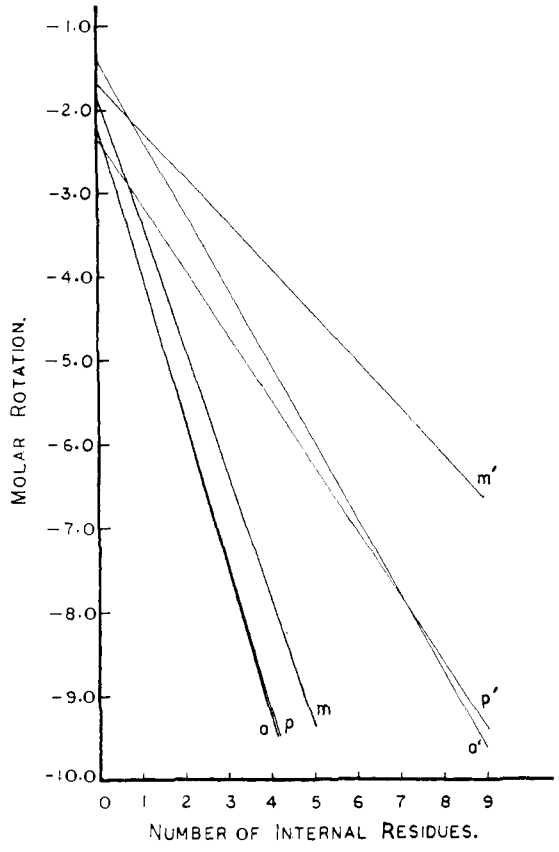

Fig. 2.—Molar rotations based on the model compounds I–III in *ortho* (*o*)-, *meta* (*m*),-and *para* (*p*) cresol; and based on the model compounds IV–VI in *ortho* (*o'*)-, *meta* (*m'*) and *para* (*p'*) cresol.

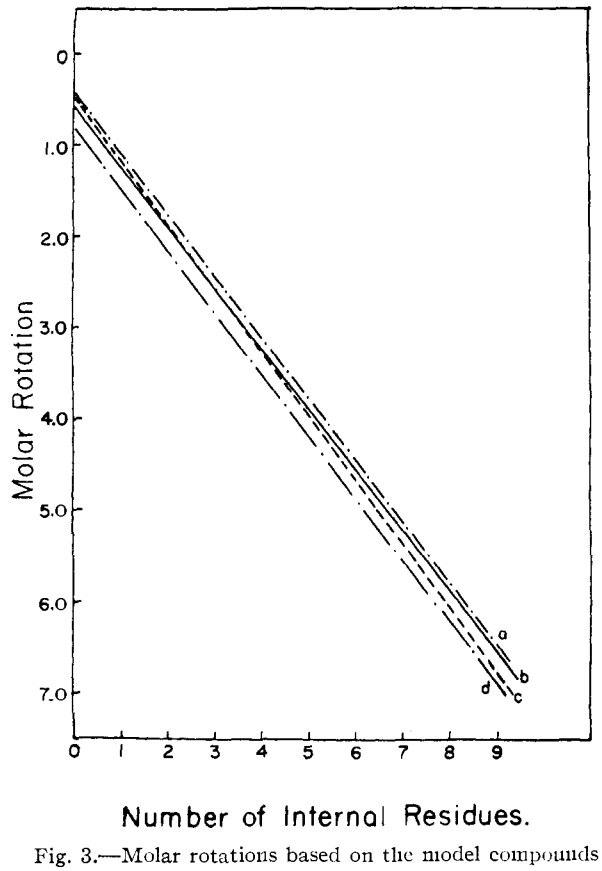

Fig. 3.—Molar rotations based on the model compounds in dichloroacetic acid (line a), trifluoroacetic acid (line b), acetic acid (line c) and formic acid (line d).

hypothesis.[30] At present it is not possible to be more specific concerning the nature of solvation of the peptide chain.

Complications arise in dioxane. The optical rotations of the oligomeric peptides are approximated quite well by the calculations based on the simple model compounds I-III (line e) while the complex model compounds IV-VI give essentially exact agreement (line f), from the dimer through the tetramer. Commencing with the pentamer huge deviations from both of the calculated lines are observed. These deviations are a result of a combined effect of association and folding of the peptide chain. We have put forth the idea[27] that the association is based upon prior formation of intramolecular hydrogen bonds. In fact we have suggested[1,27] that association is a means for the intramolecular hydrogen bonding to be stabilized.

An alternate explanation involves the hindrance to association by the end groups. At the pentamer, this effect is sufficiently diminished to allow association to commence. The rotatory dispersion data in dioxane[1,27] for the pentamer through the undecamer show increasingly positive values for b_0. Wada[14] has interpreted a positive b_0 as being related to the β-structure. Blout,[31] on the other hand, believes that the β-structure for poly-γ-esters of L-glutamates would have a b_0 close to zero.

Multiple effects exist also in the case of *m*-cresol (Fig. 1). The oligomers through the heptamer have two distinct slopes, a steep slopef rom dimer to tetramer and a more shallow slope from the pentamer through the heptamer. As with dichloroacetic acid, the slope based on the simple model compounds I-III (line g) corresponds to the steep slope of the oligomers, while the slope based on the complex model compounds IV-VI (line h) corresponds to the shallow slope.

In contrast to dichloroacetic acid, there is no end group steric hindrance to solvation since calculations based on the simple model compounds and the oligomers (di-through tetramer) exhibit essentially the same slope. However, when an internal glutamyl residue is adjacent to another glutamyl residue, interactions (most probably of side chain nature) appear which give rise to the shallow slope both for the oligomers (penta-through heptamer) and the complex model compounds.

At the nonamer, the oligomeric peptides begin to show intramolecular hydrogen bonding of the helical type since large deviations from the shallow "random coil" (line i) are encountered. Preliminary rotatory dispersion data[30] and preliminary temperature dependence studies[30] support these conclusions. Measurements in *o*- and *p*-cresol reveal similar effects for the model compounds to

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TABLE II

MOLAR ROTATIONS OF MODEL COMPOUNDS IN ACIDIC SOLVENTS

Compound	Dichloroacetic acid $[\alpha]^{25}D$	Dichloroacetic acid ϕ	Trifluoroacetic acid $[\alpha]^{25}D$	Trifluoroacetic acid ϕ	Acetic acid $[\alpha]^{25}D$	Acetic acid ϕ	Formic acid $[\alpha]^{25}D$	Formic acid ϕ
I	−12.8	−0.468	−14.7°	−0.538	−13.1°	−0.479	−12.7°	−0.465
II	+ 1.2°	+ .047	− 1.1	− .043	+ 0.5	+ .020	− 8.7	− .343
III	−16.0	− .678	−15.6	− .661	−16.6	− .703	−15.9	− .673

those found for the *m*- isomer solvent (Fig. 2). This implies that in these solvents the phenolic groups and not structural variations among the isomers determine a large part of the solvation effects.

We have examined the molar rotations of the simple model compounds I-III in several acidic solvents (dichloroacetic, trifluoroacetic, acetic and formic acids). Table II and Fig. 3 summarize the studies in these solvents. The data show that the molar rotations of the internal and N-terminal residues are independent of the acid used. This implies that the optical activity of a polymer derived from γ-methyl L-glutamate would have essentially the same rotation in each acid. This situation may not obtain with the complex model compounds IV-VI or oligomers or polymers. At present we are extending our investigation to cover these types of compounds.[30]

The specific rotation of any polymer of known molecular weight in a random coil solvent can be calculated from the models by use of eq. 3

$$[\alpha][(n + 2)R] = \phi_{end} + n\phi_{internal} \qquad (3)$$

where $[\alpha]$ = specific rotation of a polymer of $\overline{DP}$ $n+2$ and R = mean residue weight including the additional weight of the blocking groups at the end. The use of eq. 3 may be limited by polydispersity effects. Unless the molecular weight distribution is completely elucidated, the contribution of low molecular weight material to the total optical activity would be obscure.

The applicability of the employment of model compounds for peptides was tested further by using the data obtained by Brand and co-workers in the lysine and alanine series.[32-34] Molar rotations for compounds which may serve as models were calculated from specific rotations in 0.5 *N* hydrochloric acid and are shown in Table III. The rotations

TABLE III[32-36]

ALANINE AND LYSINE MODEL COMPOUNDS

Compound	$[\alpha]D$	ϕ
H-Gly-Ala-OH	−59.3°	−0.867
H-Ala-Gly-OH	+22.6	+0.330
H-Gly-Ala-Gly-OH	−65.3	−1.320
H-Lys-Gly-OH	+40.7	+0.826
H-Gly-Lys-OH	−12.8	− .259
H-Gly-Lys-Gly-OH	−32.1	− .835

of oligomeric peptides in this series to the hexapeptide in the alanine series and tetrapeptide in the lysine series were obtained.[32-38] The comparison of the molar rotations of the oligomers and the molar rotations calculated from these model compounds and eq. 2 seem to agree fairly well. These data are shown in Table IV. Data for the lysine series are insufficient to draw any conclusions and were not plotted.

TABLE IV

ALANINE OLIGOMER MOLAR ROTATIONS[32-36]

Peptide of alanine	$[\alpha]D$ in 0.5 *N* HCl	ϕ_{expt}	ϕ_{calc}
Dimer	− 37.3	−0.59	−0.537
Trimer	− 85.4	−1.97	−1.857
Tetramer	−131	−3.96	−3.177
Pentamer	−147	−5.52	−4.497
Hexamer	−156.6	−6.95	−5.817

Peptides of the general type gly-amino acid-gly, gly-amino acid, and amino acid-gly (where amino acid is optically active) have been synthesized, but there is very little information about optical activities. Of greater importance is the fact that oligomers have not been synthesized.

The use of model compounds enables one to determine the theoretical "random coil" rotations in helical solvents. The difference in rotation of an oligomer or polymer from this "random coil" value may be thought of as the optical rotatory contribution resulting from conformational effects. If any peptide could be obtained in an entirely helical form in a given solvent and with the knowledge of the theoretical random coil rotation from the model compound data, the degree of helicity of the given peptide could be determined.

Temperature Dependence.—With many solvents, as the temperature is lowered a more completely helical structure is attained.[39-43] Conversely, with an increase in temperature the tendency is to destroy the helical structure. As the chain length is increased, a sharper helix-coil transition is noted.[22,43] In lower oligomeric peptides the transition is broad.[1,27] It is therefore essential to study the temperature dependence of optical activity in helix-forming solvents of the model compounds. This may serve as a basis for the complete random coil at any given temperature. Furthermore, if the oligomers are compared, it would be possible to note the approach to an entirely "random coil" structure at high temperature, and completely helical structure at low temperature.

Studies have been carried out in dimethylformamide at temperatures between 0–70°. Optical rotations for the model compounds and the oligomers were measured at temperatures of approximately 5°, 25° and 70° (Table V). The

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TABLE V

MOLAR ROTATIONS OF MODEL COMPOUNDS AT VARIOUS TEMPERATURES IN DIMETHYLFORMAMIDE

Compound	70°				25°				5°			
	$[\alpha]_D$	ϕ_D	$[\alpha]_{546}$	ϕ_{546}	$[\alpha]_D$	ϕ_D	$[\alpha]_{546}$	ϕ_{546}	$[\alpha]_D$	ϕ_D	$[\alpha]_{546}$	ϕ_{546}
I	− 4.6	−0.168	− 5.9	−0.216	− 7.2	−0.264	− 8.0	−0.293	− 8.3	−0.304	− 7.2	−0.263
II	−10.2	− .403	−11.9	− .470	−13.0	− .513	−14.4	− .568	−16.0	− .631	−16.1	− .635
III	− 6.8	− .296	− 8.5	− .360	− 5.9	− .249	− 6.9	− .292	− 5.6	− .236	− 6.8	− .287
IV	−10.4	− .529	−11.9	− .606	− 9.0	− .460	−11.5	− .585	− 8.8	− .448	−11.2	− .570
V	−13.7	− .736	−16.3	− .875	−14.0	− .750	−15.7	− .843	−13.4	− .720	−15.3	− .822
VI	−12.1	− .685	−14.4	− .815	−10.8	− .611	−13.3	− .753	−10.6	− .600	−12.9	− .730

variation of rotation with temperature for the terminal group model compounds is such that the rotation decreases as the temperature increases (Table V). This fact is also evident for the dipeptide where only the terminal group effect exists. The internal model compound, however, shows an increase in rotation with increasing temperature. This effect is also noticeable with the higher oligomers prior to the appearance of secondary structure.

The molar rotations were calculated from the model compounds (and eq. 2) and compared to the rotations of the oligomers at these temperatures (Fig. 4). As the temperature is raised, the deviations from the calculated values decrease. Once again the complex model compounds IV-VI (lines b,d,f) explain the oligomeric peptide data better than the simple model compounds I-III (lines a,c,e). At 70° both sets of model compounds give values coincident with the dipeptide

$$\left(\begin{array}{c} \text{OMe OEt} \\ | \quad | \\ \text{Z–glu—glu-OEt} \end{array}\right),$$

thus eliminating end group deviations completely. This is not surprising since differences derived from neighbor–neighbor interactions should be eliminated as solvation becomes more complete at the higher temperatures (Fig. 4).

The temperature studies are of particular interest when secondary structure appears. The deviations from the calculated lines for the penta-, hexa- and heptapeptides disappear at temperatures close to 70°. With the nonamer, however, the deviation, although decreasing as above, does not disappear entirely at 70°. These facts are consistent with the interpretation that secondary structure is gradually disrupted as the temperature is raised. It is highly desirable to know the limiting temperature of maximum helical content and also the initial temperature where secondary structure is destroyed completely. With these data the "precentage helicity" of any compound can be determined at a given temperature. The thermodynamics of the helix–random coil transition can then be calculated.

Summary.—A general method for the investigation of the structure of optically active polypeptides in solution was presented in this paper. Phenomena such as steric hindrance to solvation and optically-active side chain interactions in specific solvents have been studied readily by comparing the rotations of sets of model compounds to related oligomers. Effects such as intramolecular and intermolecular hydrogen bonding were also measured by a comparison of the molar rotation of a peptide to its predicted value calculated from the rotations of model compounds. In solvents where hydrogen bonding occurs it can be detected as large deviations from the calculated molar rotations. The model compounds, in addition, afford a convenient basis for the examination of the effect of temperature on the formation and disruption of intramolecular hydrogen bonds. In dimethylformamide, the helical character of the oligopeptides decreases with increasing temperature. We are presently extending this general approach to the study of other problems related to peptide stereochemistry and to polypeptides containing other amino acid residues.

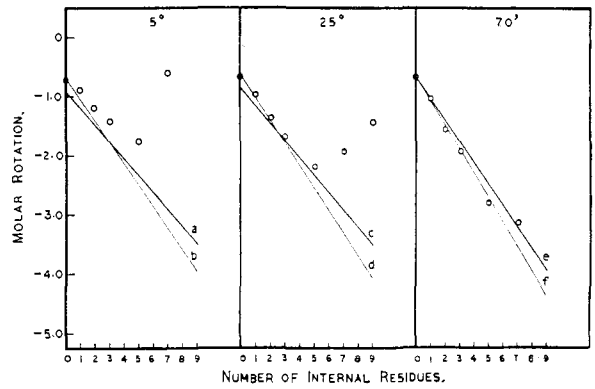


Fig. 4.—Molar rotations (measured at 546 mμ) based on the model compounds I-III (lines a, c, e), the model compounds IV–VI (lines b, d, f) and the oligomeric peptides (circles) derived from γ-methyl L-glutamate at 5°, 25° and 70° in dimethylformamide.

Synthesis of Peptides.—A combination of the mixed anhydride and active ester techniques have been employed in the synthesis of the peptides.[44–49] Benzyloxycarbonylamino acids and amino acid esters were coupled *via* the classical mixed anhydride technique[44–46] forming the blocked dipeptide ester. Where amino acid active esters were used,[47,48] the blocked dipeptide active ester was allowed to react without isolation with an amino acid ester hydrochloride to form the corresponding tripeptide derivative.[49]

The tetrapeptide (compound VI, Table I) was prepared by removal of the benzyloxycarbonyl group from the tripeptide (compound IV) by sol-

(44) J. R. Vaughan and R. L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).
(45) T. Wieland and H. Bernhard, *Ann.*, **572**, 190 (1951).
(46) J. M. Kenner, *Chemistry & Industry*, 15 (1951).
(47) M. Bodansky, *Nature*, **175**, 685 (1955).
(48) B. Iselin, W. Rittel, P. Sieber and R. Schwyzer, *Helv. Chim. Acta*, **40**, 373 (1957).
(49) M. Goodman and K. C. Stueben, *J. Am. Chem. Soc.*, **81**, 3980 (1959).

volysis using saturated hydrogen bromide in glacial acetic acid. The tripeptide hydrobromide was then allowed to react with carbobenzoxyglycine *via* the mixed anhydride method.

Experimental[50]

Amino Acid Derivatives.—The amino acid esters were prepared *via* Fischer esterifications, benzyloxycarbonylamino acids *via* the method of Bergmann and Zervas.[51] The following γ-methyl L-glutamate derivatives were synthesized *via* the procedure described by Goodman, Schmitt and Yphantis[26]: 1, benzyloxycarbonyl-γ-methyl L-glutamate; 2, benzyloxycarbonyl-γ-methyl-α-*p*-nitrophenyl L-glutamate; 3, diethyl L-glutamate hydrochloride; 4, γ-Methyl α-*p*-nitrophenyl L-glutamate hydrobromide; 5, benzyloxycarbonyl-γ-methyl-L-glutamyl γ-methyl-α-*p*-nitrophenyl L-glutamate.

Benzyloxycarbonylglycyl Diethyl L-Glutamate (I).[52]—A solution of benzyloxycarbonylglycine (2.09 g., 0.01 mole) in 75 ml. of ethyl acetate was cooled to 0°. Isobutyl chloroformate (1.4 ml., 0.01 mole) followed by triethylamine (1.4 ml., 0.01 mole) were added and the reaction was allowed to proceed for 20 minutes. Diethyl L-glutamate hydrochloride (2.36 g., 0.01 mole) was then added, followed by slow addition of triethylamine (1.4 ml., 0.01 mole). The reaction proceeded for 4 hours, at which time the reaction mixture was diluted with 125 ml. of ethyl acetate. The solution was extracted with hydrochloric acid (2 *N*), aqueous potassium chloride and aqueous soldium bicarbonate solutions. The solution was dried over magnesium sulfate, and the solvent removed under reduced pressure. The oil which formed was crystallized from ethyl acetate–ether–petroleum ether. Recrystallizations were carried out from ethyl acetate–petroleum ether to give needles, 2.8 g. (71%), m.p. 57°.

Anal. Calcd. for $C_{19}H_{26}N_2O_7$: C, 57.87; H, 6.60; N, 7.11. Found: C, 57.95; H, 6.73; N, 7.37.

Benzyloxycarbonyl-γ-methyl-L-glutamylglycine Methyl Ester (II).—Benzyloxycarbonyl-γ-methyl L-glutamate (2.95 g., 0.01 mole) was dissolved in 75 ml. of chloroform and cooled to 0°. Isobutyl chloroformate (1.4 ml., 0.01 mole) and triethylamine (1.4 ml., 0.01 mole) were added. The reaction was stirred for 20 minutes. Glycine methyl ester hydrochloride (1.25 g., 0.01 mole) was added, followed by slow addition of triethylamine (1.4 ml., 0.01 mole). The reaction proceeded for 4 hours, when chloroform (125 ml.) was added as a diluent. The reaction mixture was extracted with 2 *N* hydrochloric acid, aqueous potassium chloride, and aqueous sodium bicarbonate solutions. The organic solution was dried over magnesium sulfate, and solvent evaporated under reduced pressure. The oil which formed was crystallized from ether. The product was recrystallized from chloroform–ether yielding 2.1 g. (58%), m.p. 109–110°.

Anal. Calcd. for $C_{17}H_{22}N_2O_7$: C, 55.80; H, 6.01; N, 7.65. Found: C, 56.01; H, 5.96; N, 7.82.

Benzyloxycarbonylglycyl-γ-methyl-L-glutamylglycine Methyl Ester (III).—Into a dried round-bottom flask containing benzyloxycarbonyl-γ-methyl-L-glutamylglycine methyl ester (1.2 g., 0.003 mole) was added 1.5 ml. of hydrogen bromide (33%) in glacial acetic acid. The compound dissolved with evolution of carbon dioxide. After 30 minutes the hydrobromide was precipitated from solution with ether. The oil which formed was triturated with ether several times, dissolved in methanol, and reprecipitated with ether. The oil was triturated once again with ether and dried under vacuum to yield 0.8 g. (80%) of product which was used directly in the following reaction.

Benzyloxycarbonylglycine (0.52 g., 0.0025 mole) dissolved in 50 ml. of ethyl acetate was cooled to 0°. Isobutyl chloroformate (0.35 ml., 0.0025 mole) and triethylamine (0.35 ml., 0.0025 mole) were added and the reaction proceeded for 20 minutes. The hydrobromide oil, prepared above (0.8 g., 0.0027 mole), dissolved in ethyl acetate was added followed by triethylamine (0.35 ml., 0.0025 mole). The reaction proceeded for 4 hours, when the reaction mixture was diluted with ethyl acetate, and extracted with 2 *N* hydrochloric acid, aqueous potassium chloride and aqueous sodium bicarbonate solutions. The organic layer was dried over magnesium sulfate and evaporated under induced pressure. An oil formed, which was crystallized with difficulty from ethyl acetate–ether several times before a constant melting point of 108–110° was obtained; 0.54 g. (52%).

An alternate procedure was also employed: Benzyloxycarbonylglycine (1.05 g., 0.005 mole) was dissolved in 50 ml. of ethyl acetate and cooled to 0°. Isobutyl chloroformate (0.7 ml., 0.005 mole) and triethylamine (0.7 ml., 0.005 mole) were added and the reaction proceeded for 20 minutes. γ-Methyl-α-*p*-nitrophenyl L-glutamate hydrobromide (1.8 g., 0.005 mole) was then added, followed by triethylamine (0.7 ml., 0.005 mole) slowly. After 4 hours glycine methyl ester hydrochloride (0.62 g., 0.005 mole) was added followed by triethylamine (0.7 ml., 0.005 mole) slowly. The reaction proceeded for 12 hours, was diluted with ethyl acetate and extracted with 2 *N* hydrochloric acid, aqueous potassium chloride and saturated aqueous sodium carbonate solutions until the yellow *p*-nitrophenoxide color disappeared. The organic layer was dried over magnesium sulfate, and the solvent removed under reduced pressure. A solid formed which was recrystallized from ethyl acetate–ether solution. After two recrystallizations, 1.04 g. (49%), m.p. 110–111°, was obtained.

Anal. Calcd. for $C_{19}H_{25}N_3O_8$: C, 53.90; H, 5.80; N, 10.00. Found: C, 54.10; H, 5.64; N, 10.30.

Benzyloxycarbonyl-di-(γ-methyl-L-glutamyl)-glycine Methyl Ester (IV).—Benzyloxycarbonyl-γ-methyl-L-glutamyl-γ-methyl *p*-α-nitrophenyl L-glutamate (2.8 g., 0.005 mole) was dissolved in a 1:1 mixture of dimethylformamide–chloroform and cooled to 15°. Glycine methyl ester hydrochloride (0.62 g., 0.005 mole) was added followed by triethylamine (0.7 ml., 0.005 mole) slowly, in the rapidly stirred solution. The reaction proceeded for 14 hours at which time the volume was reduced under vacuum, and a large excess of ethyl acetate (200 ml.) was added. The solution was extracted with 2 *N* hydrochloric acid, aqueous potassium chloride and aqueous sodium carbonate solutions until colorless. The organic layer was dried over magnesium sulfate and evaporated under reduced pressure. Solid particles formed which were recrystallized twice from hot ethyl acetate, yielding the desired product, 1.8 g. (72%), m.p. 149°.

Anal. Calcd. for $C_{23}H_{31}N_3O_{10}$: C, 54.22; H, 6.09; N, 8.25. Found: C, 54.21; H, 6.06; N, 8.56.

Benzyloxycarbonylglycyl-γ-methyl-L-glutamyl Diethyl L-Glutamate (V).—Benzyloxycarbonylglycine (1.05 g., 0.005 mole) was dissolved in 1:1 ethyl acetate–dimethylformamide mixture and cooled to 0°. Isobutyl chloroformate (0.7 ml., 0.005 mole) and triethylamine (0.7 ml., 0.005 mole) were added and the reaction proceeded for 20 minutes. γ-Methyl-α-*p*-nitrophenyl L-glutamate hydrobromide (1.8 g., 0.005 mole) followed by triethylamine (0.7 ml., 0.005 mole) were then added and the reaction was allowed to proceed for 4 hours. Diethyl L-glutamate hydrochloride (1.18 g., 0.005 mole) was then added followed by triethylamine (0.7 ml., 0.005 mole) which was added slowly. The reaction was allowed to continue for an additional 12 hours. The reaction mixture was diluted with ethyl acetate (200 ml.) and extracted with 2 *N* hydrochloric acid, aqueous potassium chloride and aqueous sodium carbonate solutions until colorless. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The compound was recrystallized from ethyl acetate–ether–petroleum ether three times to give needles, 1.5 g. (85%), m.p. 84–85°.

Anal. Calcd. for $C_{25}H_{35}N_3O_{10}$: C, 55.86; H, 6.52; N, 7.82. Found: C, 55.94; H, 6.40; N, 7.84.

Benzylcarbonylglycyl-di-(γ-methyl-L-glutamyl)-glycine Methyl Ester (VI).—Into a dried round-bottom flask containing benzyloxycarbonyldi(γ-methyl-L-glutamyl)-glycine methyl ester (compound IV prepared above; 1.3 g., 0.0025 mole) was added 1.5 ml. of hydrogen bromide (33%) in glacial acetic acid. The compound dissolved with evolution of carbon dioxide. After 30 minutes the hydrobromide was precipitated with ether. The oil which formed was triturated with ether several times, dissolved in methanol and reprecipitated with ether. The oil was triturated once again with ether and dried under vacuum to yield 1.1 g. of oil which was used directly in the following reaction.

(50) All melting points are corrected. Analyses were carried out by Schwarzkopf Laboratories, Woodside, Long Island, N. Y.

(51) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(52) B. Helferich, P. Schellenberg and J. Ulbrich, *Chem. Ber.*, **90**, 700 (1957).

Benzyloxycarbonylglycine dissolved in dimethylformamide was chilled to 0°. Isobutyl chloroformate (0.35 ml., 0.0025 mole) and triethylamine (0.35 ml., 0.0025 mole) were added, and the reaction proceeded for 20 minutes. The hydrobromide oil (1.1 g., 0.0025 mole) produced above was then added, followed by triethylamine (0.35 ml., 0.0025 mole) slowly. The reaction proceeded for 4 hours, then was diluted with ethyl acetate (200 ml.) and extracted with 2 *N* hydrochloric acid, aqueous potassium chloride and aqueous sodium bicarbonate solutions. The organic layer was dried over magnesium sulfate and evaporated under reduced pressure. The solid which formed was recrystallized from warm ethyl acetate twice to give 0.5 g. of product, m.p. 136–139°.

Anal. Calcd. for $C_{25}H_{34}N_4O_{11}$: C, 53.00; H, 6.01; N, 9.89. Found: C, 53.18; H, 6.12; N, 10.00.

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE 39, MASS.]

The Isolation, Characterization and Synthesis of *erythro*-β-Hydroxy-L-leucine, a New Amino Acid from the Antibiotic Telomycin[1]

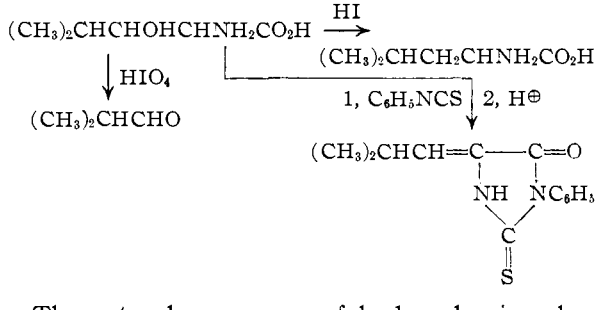
BY JOHN C. SHEEHAN, K. MAEDA, A. K. SEN AND J. A. STOCK

RECEIVED DECEMBER 8, 1961

Among the acidic hydrolysis products of the antibiotic Telomycin is an amino acid not previously encountered in nature. Degradation of the pure, isolated amino acid with periodate led to isobutyraldehyde, and reaction with hydriodic acid produced leucine. Optical rotation data, enzymatic evidence and comparison with authentic *threo*- and *erythro*-β-hydroxy-DL-leucine samples established the structure as *erythro*-β-hydroxy-L-leucine. Enzymatic resolution of synthetic N-acetyl-*erythro*-β-hydroxy-L-leucine afforded synthetic *erythro*-β-hydroxy-L-leucine, identical to the amino acid from Telomycin.

The acid hydrolysis of Telomycin, an antibiotic isolated from an unidentified *Streptomyces*,[2] yields a number of ninhydrin-positive components.[3] Several of these have been identified as known amino acids, but among the novel substances present is one with paper chromatographic behavior similar to that of valine. Isolation by preparative paper chromatography and subsequent purification gave a crystalline substance, m.p. 218–222°. Elementary analysis indicated the empirical formula $C_6H_{13}NO_3$, and the reaction with periodate suggested a β-hydroxy-amino acid.[4] Periodate degradation gave isobutyraldehyde, identified as the 2,4-dinitrophenylhydrazone. This result is in accord with the Kuhn–Roth determination which ascribed one C-methyl group per mole, based on the molecular formula $C_6H_{13}NO_3$. The conclusion that the substance was a β-hydroxyleucine was reinforced by the finding that reduction with red phosphorus, and hydrogen iodide gave leucine. Quantitative ninhydrin degradation[5] gave a value for the carboxyl group close to that expected for hydroxyleucine. Since the optical rotation of the amino acid showed a positive shift in passing from water to aqueous hydrochloric acid solution, the L-configuration was assigned (Clough–Lutz–Jirgensons rule[6]).

Enzymatic evidence confirmed this assignment (see Experimental). Kenner and his co-workers[7] have recently synthesized the *threo* and *erythro* forms of β-hydroxyleucine and have established that Wieland's one-stage synthesis[8] gives predominantly the *threo* isomer. A comparison by paper chromatography of the β-hydroxyleucine isolated from Telomycin with that synthesized by Wieland's method, and later with samples of the racemic *erythro* and *threo* forms obtained from Professor G. W. Kenner (University of Liverpool, England) and from Professor S. Akabori (Osaka University, Japan), enabled us to assign the natural amino acid to the *erythro* series. That the compound isolated from Telomycin is *erythro*-β-hydroxy-L-leucine was finally confirmed by synthesis, including enzymatic resolution of the intermediate N-acetyl-*erythro*-β-hydroxy-DL-leucine, and by comparison of optical rotations and infrared spectra. The melting points and infrared spectra of the dinitrophenyl derivatives of the natural and synthetic L-amino acids were identical. Reaction of the amino acid with phenyl isothiocyanate led to the 5-isobutylidene derivative of phenylthiohydantoin, the result of a not-unexpected[9] β-hydroxyl elimination.



The natural occurrence of hydroxyleucines has been reported by two other groups. Kenner[7,10] has shown that the antibiotic I.C.I. No. 13959 gave, on hydrolysis, *threo*-β-hydroxy-L-leucine. The isolation of a hydroxyleucine, very probably a β-

(1) The presence of a β-hydroxyleucine in Telomycin was reported (J. C. S.) at the Seventeenth International Congress for Pure and Applied Chemistry, held in Munich, Germany, August 30 to September 6, 1959; Meeting Abstracts, p. 108.

(2) M. Misiek, O. B. Fardig, A. Gourevitch, D. L. Johnson, I. R. Hooper and J. Lein, *Antibiotics Ann.*, 852 (1957–1958).

(3) J. C. Sheehan, P. E. Drummond, J. N. Gardner, K. Maeda, D. Mania, A. K. Sen and J. A. Stock, in preparation.

(4) *Cf.* (a) R. Criegee in "Newer Methods of Preparative Organic Chemistry," Interscience Publishers, Inc., New York, N. Y., 1948, p. 20; (b) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York, N. Y., 1961, p. 2210.

(5) A. Steyermark in "Organic Analysis," Interscience Publishers, Inc., New York, N. Y., Vol. II, 1954, p. 7.

(6) Reference 4b, p. 83.

(7) S. Dalby, G. W. Kenner and R. C. Sheppard, *J. Chem. Soc.*, 968 (1960).

(8) T. Wieland, H. Cords and E. Keck, *Chem. Ber.*, **87**, 1312 (1954).

(9) H. Fraenkel-Conrat in "Methods of Biochemical Analysis," Interscience Publishers, Inc., New York, N. Y., Vol. II, 1955, p. 386.

(10) G. W. Kenner and R. C. Sheppard, *Nature*, **181**, 48 (1958).