Contribution of Side-Chain Chromophores to the Optical Activity of Proteins. Model Compound Studies. III. Alanyl-*p*-hydroxyphenylglycine Diketopiperazine

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Abstract: The optical properties and the conformational energy of the molecule alanyl-*p*-hydroxyphenylglycine diketopiperazine have been calculated as a function of molecular conformation. The results of the theoretical calculations have been compared with experimental circular dichroism spectra to determine the conformation assumed by the molecule in solution and to test the theoretical optical formalism which was used. The results indicate that conformations which are characterized by a nonplanar conformation of the cyclic dipeptide ring and a conformation of the phenolic side chain which can be characterized by values of the dihedral angle χ_1 between 120 to 180° are probably highly populated.

The optical properties of a series of model compounds have been under investigation in this laboratory for several years. The general procedure has been to compute the chiroptical properties and conformational potential energy of simple, sterically hindered, molecules as a function of molecular conformation. Comparison of theoretical predictions with experimental circular dichroism (CD) or optical rotatory dispersion (ORD) data often yields useful information as to the conformations assumed by the molecule in solution and provides a means of testing the theoretical formalisms which were employed for the calculations.

The ultimate goal of these investigations is to utilize the techniques developed, and the results of the model compound studies themselves, to draw inferences regarding the conformational properties of biological macromolecules in solution. Hopefully, it will prove possible to utilize similar techniques to probe the structure and conformation of proteins and other complex molecules.

Prior investigations have been carried out on the amino acids tyrosine[1] and *p*-hydroxyphenylglycine[2] and derivatives thereof. The relationships between the conformation and the optical properties of several diketopiperazines (cyclic dipeptides) have also been studied.[3,4]

The diketopiperazines possess a number of features which make them very useful model compounds. The closed ring implies a very restricted conformational freedom. This simplifies the theoretical calculations, which must be carried out as a function of molecular conformation, with the result that theoretical predictions are more readily correlated with experimental results. Furthermore, the presence of two peptide groups permits the possibility of exciton coupling between the π–π^* transitions of these chromophores and, hence, the splitting of the individual energy levels into two states. Interaction between the side-chain chromophores and the peptide chromophores is also possible.

The cyclic dipeptide alanyl-*p*-hydroxyphenylglycine diketopiperazine (AHPGDKP) possesses an additional feature of interest, namely, the bonding of the phenolic chromophore directly to the α-carbon atom of one of the amino acid residues of the diketopiperazine ring. The electronic transition dipole moments of this chromophore are polarized either perpendicular or collinear with the axis of internal rotation about the C^{α}–C^{β} bond (dihedral angle χ_1). The chiroptical properties of the former transitions should be sensitive to rotation about χ_1, whereas the latter transitions should be relatively insensitive to such rotation. However, Cotton effects arising from the latter transitions should be sensitive to changes in the backbone conformation of the diketopiperazine ring.

The close physical proximity of the chromophores of this molecule suggests that strong coupling might occur between the side chain and peptide chromophores. This coupling should be largely dependent on the degree of fold of the diketopiperazine ring. It is of interest to see whether our theoretical methods can successfully handle the rather difficult problem of predicting the optical properties of this molecule.

Experimental Section

Methods. D-Alanyl-*p*-hydroxy-D-phenylglycine diketopiperazine (D-AHPGDKP) was synthesized from the corresponding dipeptide by the method of Kopple.[5] The dipeptide was synthesized from the corresponding amino acids by standard techniques. The D isomer was synthesized instead of the L because our supply of *p*-hydroxy-D-phenylglycine was much more plentiful. The diketopiperazine was found to be ninhydrin negative and showed a single spot when subjected to thin-layer chromatography on silica gel in butanol–water–acetic acid (4:1:1). Low-resolution mass spectral analysis also gave results which were consistent with the structure of the expected product.

Solutions were prepared with doubly distilled water and spectroquality grade solvents. Triethyl phosphate (TEP) was freshly distilled under reduced pressure. All solutions, except those in TEP, were filtered with a millipore apparatus to remove dust particles immediately prior to spectral measurements. TEP solutions were filtered through sintered glass.

Ultraviolet (uv) absorption spectra were obtained by means of a Cary 118C recording spectrophotometer, which is equipped with the Gary far-uv modification. Nitrogen purging was employed at all wavelengths. All absorption spectra were measured at ambient temperature.

CD spectra were measured on a Cary 60 recording spectropolarimeter equipped with a prototype Model 6003 CD accessory. The CD spectra which were measured for D-AHPGDKP were reflected through zero to obtain curves for L-AHPGDKP, which is the enantiomer for which theoretical calculations were carried out. Fused silica cells with path lengths between 0.1 and 1.0 cm were used. All CD spectra were measured at 27°.

The CD data are reported as molar ellipticity, M_θ, which is defined by

$$\theta = M_\theta Cl/100$$

where θ is the observed ellipticity in degrees, l is the path length in centimeters, and C is the solute concentration in moles per liter.

CD spectra were analyzed by a curve fitting technique based on the steepest descents method due to Fletcher and Powell.[6] The data were fitted to Gaussian band shapes by means of an iterative computer program which minimizes residual mean squares.

All theoretical calculations were carried out on the IBM 360/75 digital computer at the University of California, Santa Barbara Computer Center.

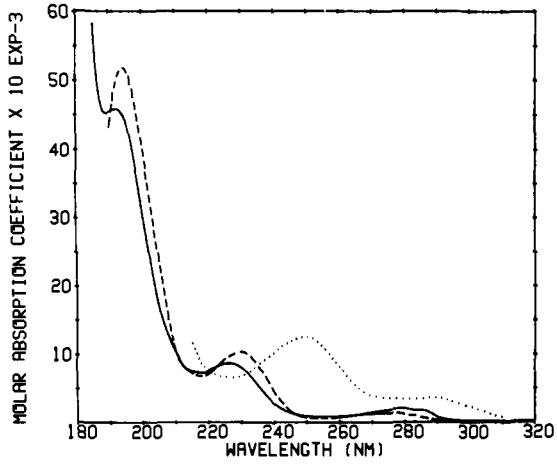

Figure 1. Ultraviolet absorption spectra of alanyl-*p*-hydroxyphenylglycine diketopiperazine: neutral aqueous solution (—); alkaline aqueous solution, pH 10.5 (····); triethyl phosphate solution (- - - -).

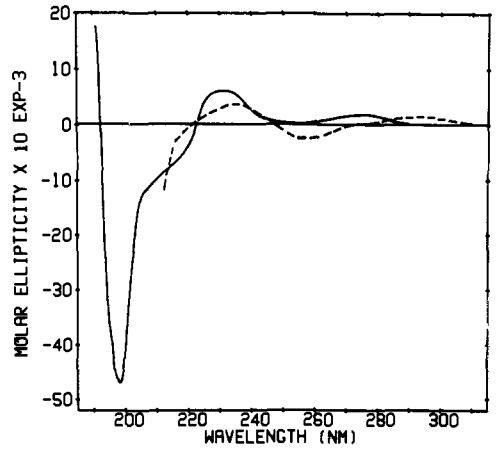

Figure 2. Circular dichroism spectra of L-alanyl-*p*-hydroxy-L-phenylglycine diketopiperazine: neutral aqueous solution (—); alkaline aqueous solution, pH 10.5 (- - - -). These spectra were obtained by reflecting the spectra of the D isomer through zero.

Experimental Results

Figure 1 shows the absorption spectra for AHPGDKP in aqueous solution at pH values of 7 and 10.5, respectively. The two lowest energy bands are the aromatic ^1L_b and ^1L_a bands in Platt's notation,[7] occurring at neutral pH near 273 and 226 nm, respectively. At pH 10 the ^1L_b band is red shifted to 289 nm and the ^1L_a band to 248 nm. These shifts, which occur upon ionization of the phenolic group, are similar to the shifts observed for tyrosine.[8] These spectral shifts are accompanied by an increase in intensity (hyperchromism) in both bands. Figure 1 also shows the absorption spectrum of AHPGDKP in TEP. The ^1L_b band occurs at 279 nm and the ^1L_a band at 230 nm in this solvent.

The aromatic ^1B_b and ^1B_a bands and the peptide $\pi-\pi^*$ bands absorb strongly in the region below the ^1L_a band. These bands account for the extremum at 192.5 nm in neutral aqueous solution and the increase in absorption further to the blue.

Figure 2 gives the CD spectra of L-AHPGDKP in aqueous solution at neutral and alkaline pH. The positive Cotton effect centered at 276 nm in the neutral solution spectrum obviously arises from the aromatic ^1L_b transition. There is another positive Cotton effect with an apparent extremum at 230 nm. In addition, there is a negative shoulder to the red of an intense negative band at 199 nm and an unresolved positive Cotton effect below 190 nm. The close proximity of several transitions in this wavelength region makes the assignments of these Cotton effects a difficult task. This problem is greatly clarified by the CD spectrum in aqueous solution at pH 10.5. In this spectrum the Cotton effect arising from the ^1L_b transition is evident as before, exhibiting the expected red shift of several nanometers relative to neutral pH. The red shift of the ^1L_a band has demonstrated that the Cotton effect arising from this band is negative. The apparent shift to 258 nm has undoubtedly been enhanced by the presence of the positive Cotton effect at 235 nm. In retrospect, the shoulder to the red of the intense negative band at 199 nm in the neutral aqueous solution spectrum is probably due at least partly to a negative Cotton effect arising from the ^1L_a transition. The full resolution of this Cotton effect is prevented by the presence of positive and negative Cotton effects immediately to the red and blue, respectively.

The positive Cotton effects with apparent maxima at 235 and 230 nm in aqueous solution at basic and neutral pH, respectively, may now be assigned to the peptide $n-\pi^*$ transi-

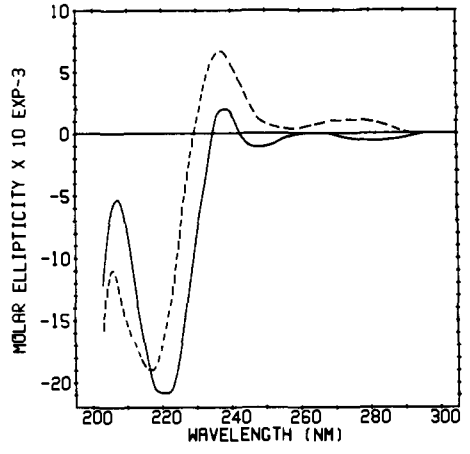

Figure 3. Circular dichroism spectra of L-alanyl-*p*-hydroxy-L-phenylglycine diketopiperazine: methanol solution (- - - -); triethyl phosphate solution (—). These spectra were obtained by reflecting the spectra of the D isomer through zero.

tions of the diketopiperazine ring with some certainty. This band occurs several nanometers further to the red than one would normally expect for a peptide $n-\pi^*$ Cotton effect in a simple amide, but similar behavior has been noted for other diketopiperazines.[3]

These assignments are substantiated by the CD spectrum of L-AHPGDKP in methanol, as given in Figure 3. The effect of the less polar solvent is to red shift the $n-\pi^*$ transition and blue shift the $\pi-\pi^*$ transition to a lesser extent. As a result of these solvent shifts, the ^1L_a transition is more clearly resolved in methanol. This Cotton effect has an apparent extremum at 218 nm in this solvent system.

The CD spectrum of L-AHPGDKP in TEP is also shown in Figure 3. In this solvent, the Cotton effect due to the ^1L_b band has changed sign. This is probably due to a solvent-induced conformational change. Since the polarization of the ^1L_b transition dipole moment is perpendicular to the axis of internal rotation about χ_1, this Cotton effect would be expected to be particularly sensitive to a small change in this dihedral angle. The Cotton effect arising from the $n-\pi^*$ transition of the DKP ring is again positive, with an apparent extremum at 237 nm. The ^1L_a Cotton effect is negative with a minimum at 220 nm.

There is an additional negative band at 247 nm in the TEP spectrum that does not appear in any of the other sol-

Table I. Curve Fitting Parameters that Characterize CD Spectrum of L-Alanyl-*p*-hydroxy-L-phenylglycine Diketopiperazine

Transition	Rotatory strength[a]		Wavelength[b]		Bandwidth[c]	
	Water	Methanol	Water	Methanol	Water	Methanol
^1L_b	0.0089	0.0095	273	270	11.2	17.2
^1L_a	−0.29	−0.58	222	226	17.6	16.9
n−π^*	0.25	0.47	226	230	14.6	14.4

[a] Rotatory strengths are expressed in units of Debye–Bohr magnetons. [b] Wavelengths are in nanometers. [c] Bandwidths are half-widths in nanometers at $1/e$ of maximum ellipticity.

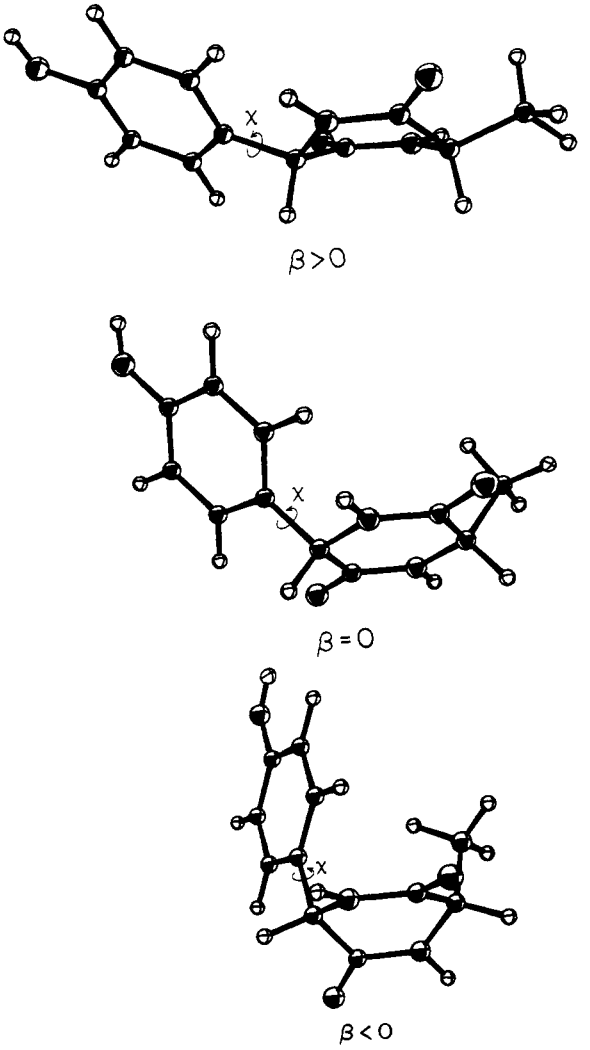

Figure 4. Conformational angles χ_1 and β as defined for L-alanyl-*p*-hydroxy-L-phenylglycine diketopiperazine. β is a measure of the degree of folding of the diketopiperazine ring. It is the angle that the plane of the atoms $-C_a''-C'-N-C_b''-$ makes relative to the plane in which these same atoms are found when the diketopiperazine ring is in its planar conformation. $\beta = 0°$ corresponds to the planar conformation.

vents. A negative shoulder has been observed in the CD spectrum of L-alanyl-L-alanine diketopiperazine (L-AADKP) in solvents of low dielectric constant,[3] but it occurs near 220 nm. The occurrence of the negative band in the CD spectrum of L-AHPGDKP more than 25 nm to the red of the negative shoulder which is found for L-AADKP would seem to preclude the possibility of these bands having similar origins. It seems more likely that the apparent Cotton effect that appears near 247 nm is, in reality, only the low-energy tail of the ^1L_a Cotton effect, which is centered to the blue of the n−π^* Cotton effect. Thus, the differences in the methanol and TEP spectra between 210 and 260 nm could be explained as follows. In methanol, the ^1L_a band lies slightly to the blue of the n−π^* band; the ^1L_a band gives a negative Cotton effect, whereas the n−π^* Cotton effect is positive. In TEP solution the overlap of the two bands is increased, and the intensity of the ^1L_a Cotton effect is increased, with the result that regions of negative ellipticity from the ^1L_a transition appear to both the red and blue of the n−π^* Cotton effect. This obviously requires a red shift of the ^1L_a band, because the n−π^* must certainly red shift in TEP, and the bandwidth of the ^1L_a must be considerably broader than that of the n−π^*.

One serious problem with the above scenario is that one would not really anticipate the ^1L_a transition to undergo such a red shift. However, the uv absorption spectra (Figure 1) do indicate a significant red shift and increase in intensity in both the 230 and 270 nm regions for TEP relative to neutral aqueous solution.

Curve fitting spectra such as these, in which several Cotton effects of different signs occur very close together, is difficult. Under such conditions, the parameters which characterize a given Gaussian band will not all be independent. However, in an attempt to resolve the question as to the signs of the ^1L_a and n−π^* Cotton effects, the data were subjected to curve fitting analysis. The aqueous solution data were fit first, and a reasonable set of parameters was obtained. The CD spectrum of L-AHPGDKP in methanol was then subjected to the same curve fitting procedure, using the aqueous solution parameters as initial parameters. The results of this procedure, which are presented in Table I, indicate that the ^1L_a band is considerably broader than the n−π^* band and also show that the relative magnitude of the ^1L_a Cotton effect has increased in methanol. The absolute magnitudes of the rotatory strengths of the ^1L_a and n−π^* bands are probably unreliable because the bands occur within less than one-half bandwidth of each other. However, the effect of decreasing the dielectric constant of the solvent appears to produce changes similar to those which would be required to obtain a CD spectrum resembling that observed in TEP. Nevertheless, it should be pointed out that it was not possible to obtain a really good fit of the TEP data in the 240-nm region without resorting to physically unrealistic parameters. Notwithstanding, it is possible to generate CD curves with the appearance of the TEP data by assuming parameters similar to those shown for methanol in Table I. Thus, the difficulty which was experienced in attempting to fit the CD spectrum of L-AHPGDKP in TEP may be a result of deficiencies in the curve fitting procedure itself.

It is possible that the anomaly which is observed near 240 nm in the TEP spectrum may, in fact, arise from an additional electronic transition which is obscured by other bands in the aqueous and methanolic spectra. For example, an n−σ^* transition might be expected to behave in a fashion similar to that observed, and it is possible that an n−σ^* transition could occur near the wavelength region in question. However, regardless of the origin of the band, the important point is that the data are consistent with a negative ^1L_a Cotton effect and a positive n−π^* Cotton effect in all of the solvents which were used. This is certainly reasonable, since one would expect the n−π^* and ^1L_a Cotton effects to be much less sensitive to minor conformational changes than the ^1L_b band.

The higher energy Cotton effects are more difficult to assign. The intense negative Cotton effect at 199 nm in neutral aqueous solution may arise from the ^1B_b or ^1B_a transitions of the phenolic chromophore, or it may be the negative lobe of a negative π−π^* couplet of the diketopiperazine

ring. The best procedure regarding this band, and the positive Cotton effect to the blue, is to base any assignment upon the results of the theoretical calculations.

Theoretical Methods

The conformational energy calculations which were carried out involved the calculation of the contributions of nonbonded, electrostatic, and torsional interactions to the total conformational potential energy. The calculations were based on the techniques which have been developed by Flory,[9] Scheraga et al.,[10,11] and Ramachandran[12] and have been described in detail elsewhere.[1,2]

The atomic coordinates for L-AHPGDKP were based upon the planar diketopiperazine ring of glycylglycine diketopiperazine.[13] The methyl and phenolic side chains were added assuming standard bond lengths for carbon–carbon bonds.[14] The coordinates for the phenolic group were based upon the X-ray crystal structure data for tyrosine[15] but were modified to obtain perfect C_{2v} symmetry.

The conformation of the amino acid side chains has been defined by means of the most recent standard dihedral angle conventions.[16] Since it has been assumed that only symmetrical folding of the diketopiperazine backbone is possible, the conformation of this group has been defined by a single conformational angle, β. This angle is defined in Figure 4.

The atomic coordinates for L-AHPGDKP in the zero dihedral angle conformation are presented in Table II. The zero dihedral angle conformation is defined as the conformation in which the diketopiperazine ring is planar (β = 0°), and the methyl group of the alanine residue and the phenolic group of the *p*-hydroxyphenylglycine residue are each eclipsed with respect to their C^{α}–N bonds. Insofar as the side-chain conformational angles are concerned, the zero dihedral angle conformation has been defined according to the standard conventions.[16]

The static charges required to determine electrostatic interactions were determined by the method of Del Re,[17] as modified by Poland and Scheraga,[18] and are listed in Table II. Parameters used in the determination of nonbonded interactions were those of Ooi et al.[11] Parameters which were used to compute torsional energies were taken from the same source.[11]

All interactions within the diketopiperazine ring have been excluded in our calculations. These interactions would only affect the relative stability for conformations with different values of β. Karplus and Lifson[19] have determined that for glycylglycine diketopiperazine these interactions remain relatively constant for a wide variation in angle of fold, so their exclusion is justified since we are not interested in absolute quantities.

The choice of a value of the effective dielectric constant can influence the outcome of the electrostatic potential energy calculations. Determination of this parameter is a difficult and uncertain task, and values ranging from 1 to 5 are commonly used.[11,12,20] Since electrostatic energies are inversely proportional to this parameter, it is possible that regions of minimum total conformational energy could be affected by the value chosen. In the case of L-AHPGDKP, varying the effective dielectric constant from one to five produced no significant differences in the positions of the total conformational energy minima, because the nonbonded interactions are dominant. The conformational energy results, which are presented herein, represent values which were calculated with an effective dielectric constant of unity.

The optical calculations are based on a matrix approach described in detail elsewhere for simple dipeptides[21] and for tyrosine.[1] This formalism is based on the configuration interaction of excited states and implicitly includes all of the specialized interaction mechanisms which are known to be of importance insofar as the generation of optical rotatory power is concerned. That is, the coupled oscillator mechanism,[22-24] the μ–m mechanism,[21,25-28] and the one-electron mechanism[29] are inherent in this theoretical procedure. All of the required optical parameters have been reported previously.[1,21]

Table II

Atom	Coordinates, Å			Static charge[a]
	x	*y*	*z*	
O'^1	3.465	−1.000	0.0	−2.024
C'^1	2.227	−1.073	0.0	1.525
N^1	1.449	0.0	0.0	−0.961
H^1	1.857	0.913	0.0	0.981
$C\alpha_1$	0.0	0.0	0.0	0.226
$H\alpha_1$	−0.359	0.581	0.860	0.277
C'^2	−0.636	−1.357	0.0	1.531
O'^2	−1.873	−1.432	0.0	−2.023
N^2	0.143	−2.429	−0.001	−0.967
H^2	−0.263	−3.342	−0.001	0.979
$H\alpha_2$	1.950	−3.008	0.860	0.219
$C\alpha_2$	1.591	−2.428	0.0	0.222
$C\beta_1$	−0.496	0.805	−1.189	−0.141
$H\delta_1$	0.674	2.597	−3.834	0.258
$H\gamma_1$	1.483	1.282	−1.892	0.254
$H\gamma_2$	−2.577	0.495	−0.731	0.254
$H\delta_2$	−3.387	1.810	−2.673	0.258
$H\zeta$	−1.351	3.402	−5.024	1.458
$O\zeta$	−1.856	3.015	−4.452	−1.664
$C\delta_1$	−0.036	2.138	−3.156	−0.455
$C\epsilon$	−1.408	2.286	−3.376	0.666
$C\gamma_2$	−1.868	0.954	−1.409	−0.232
$C\delta_2$	−2.324	1.695	−2.502	−0.455
$C\gamma_1$	0.420	1.397	−2.063	−0.232
$C\beta_2$	2.096	−3.247	−1.208	−0.527
$H\beta_1$	3.188	−3.360	−1.144	0.191
$H\beta_2$	1.835	−2.724	−2.140	0.191
$H\beta_3$	1.625	−4.240	−1.201	0.191

[a] The units of charge are 10^{-10} esu; i.e., the charge of the electron is −4.8 in these units.

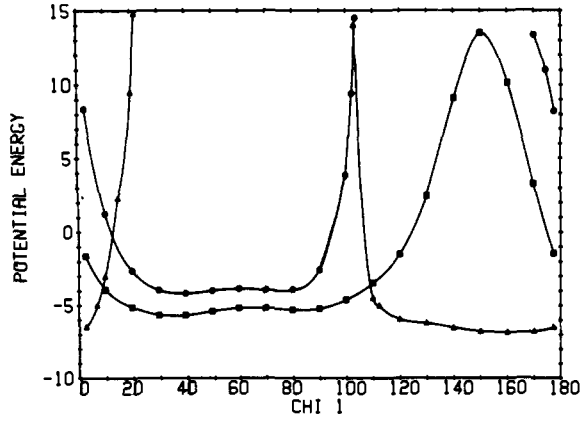

Figure 5. Total conformational potential energy (kcal/mol) of L-alanyl-*p*-hydroxy-L-phenylglycine diketopiperazine as a function of χ_1: triangles, $\beta = -20°$; squares, $\beta = 0°$; circles, $\beta = +20°$.

Theoretical Results and Discussion

The results of the conformational energy calculations are given in Figure 5. The ordinate is total potential energy, which is taken to be the sum of nonbonded, electrostatic, and torsional energies. The abscissa is the dihedral angle χ_1, which defines internal rotation of the phenyl group about the C^{α}–C^{β} bond. This angle is varied from only 0 to

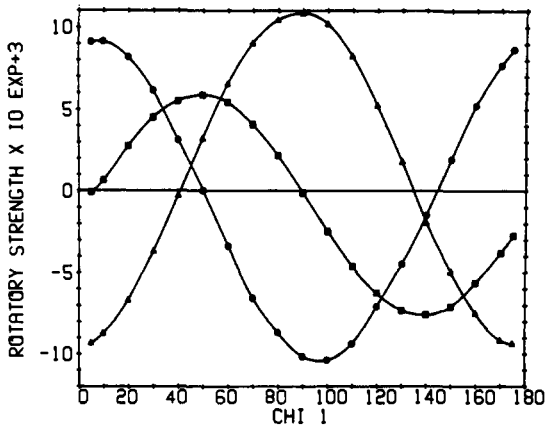

Figure 6. Calculated rotatory strength (DM) for ^1L_b transition as a function of χ_1: triangles, $\beta = 20°$; squares, $\beta = 0°$; circles, $\beta = +20°$.

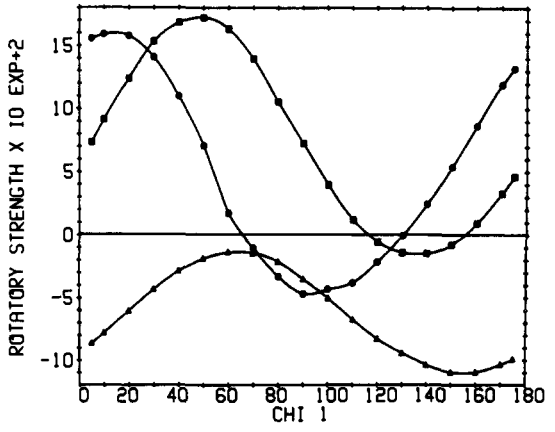

Figure 7. Calculated rotatory strength (DM) for ^1L_a transition as a function of χ_1: triangles, $\beta = -20°$; squares, $\beta = 0°$; circles, $\beta = +20°$.

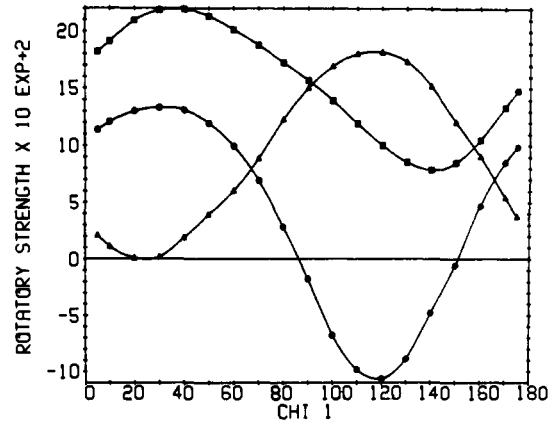

Figure 8. Sum of rotatory strengths (DM) calculated for the peptide n-π^* transitions: triangles, $\beta = -20°$; squares, $\beta = 0°$; circles, $\beta = +20°$.

180° because of the presence of a twofold symmetry axis coincident with this rotation axis. The methyl group is assumed to be in the staggered conformation with respect to the C^α-N_2 bond ($\chi_2 = 60°$) because of the relatively large barrier to internal rotation which would be expected. Total energy curves are given for $\beta = +20$, -20, and 0°. It can be seen that the energy curves are fairly flat in the region of χ_1 = 20–100° for $\beta = 0°$, 30–80° for $\beta = +20°$, and 120–180° for $\beta = -20°$. The energy curves are discontinued in the region χ_1 = 100–170° degrees for $\beta = +20°$ and 20–100° for $\beta = -20°$ due to the large repulsive interactions that occur when the molecule is in these conformations. The global minimum for the conformational energy appears to occur for negative values of β.

Table III. Averaged Rotatory Strengths[a]

Transition	Assignment	
1	^1L_b	−0.00
2	^1L_a	−0.047
3	n-π^*	0.095
4	^1B_b	−1.172
5	^1B_a	−0.929
6	π-π^*	2.185
7	π-π^*	−0.047

[a] Rotatory strengths in units of Debye–Bohr magnetons.

Figures 6–8 give the results of the optical calculations for the aromatic ^1L_b, ^1L_a, and n-π^* Cotton effects, respectively. Rotatory strengths in Debye-Bohr magnetons are plotted as a function of rotation about χ_1 for β values of +20, −20, and 0°. The oscillatory behavior of the rotatory strengths about zero (Figure 6) illustrates the previously anticipated sensitivity of the sign of the ^1L_b Cotton effect to rotation about χ_1. The sign of the ^1L_a Cotton effect, on the other hand, is not as sensitive to rotation about χ_1 (Figure 7). This behavior is expected since the ^1L_a transition dipole moment is polarized along the rotation axis. The magnitudes of the rotatory strengths for the ^1L_a band are seen to vary with rotation about χ_1, because the charge monopoles of the transition dipole swing around the C^α-C^β axis. As a result, the magnitude of the interaction with the monopoles of the peptide transition dipoles changes, thereby causing this variance.

The total energy curves in Figure 5 suggest the need to average the rotatory strengths over a partition function because of the relatively flat areas which occur at the bottom of the conformational energy wells. Table III gives the results of averaging the rotatory strengths of all of the electronic transitions of interest over the potential energy surface for β values of ±20, ±10, and 0°. Transitions 1, 2, and 3 are the ^1L_b, ^1L_a, and n-π^* Cotton effects, respectively. The rotatory strengths of the two n-π^* transitions have been added together because energy splittings of no more than a few tenths of a nanometer were predicted to occur. Transitions 4, 5, 6, and 7 are the aromatic ^1B_b, ^1B_a, and the two peptide π-π^* Cotton effects, respectively. The aromatic transitions are predicted to occur near 197 and 195 nm for all conformations. Transitions 6 and 7, the peptide π-π^* transitions, are predicted to occur near 190 and 180 nm for most conformations. However, the exciton splitting of these π-π^* transitions depends to a large extent on the degree of interaction with the transitions of the phenolic chromophore and, hence, on the value of β.

The sign of the Cotton effect is the important quantity insofar as the comparison of experiment with theory is concerned. It can readily be seen that the experimental and theoretical results for the ^1L_a and n-π^* transitions are in excellent agreement.

The experimental results indicate that a positive ^1L_b Cotton effect occurs in aqueous and methanolic solutions (Figures 2 and 3), whereas the sign is inverted in triethyl phosphate (Figure 3). This behavior is not surprising. If the predominant conformers are those with negative values of β and χ_1 values from 120 to 180°, as the results of the conformational energy calculations seem to indicate (Figure 5), then it is obvious from the theoretical optical results, which are presented in Figure 6, that the sign of the ^1L_b Cotton effect might be altered by very mild perturbations. Furthermore, since the ^1L_b Cotton effects exhibit a strong oscillato-

ry behavior for all values of β, the averaging of the rotatory strength for this band would require particularly accurate values for the rotatory strengths and conformational energies, if the averaging process is to be meaningful. Thus, the ability of the Boltzmann average to correctly predict the sign of the ^1L_b Cotton effect in only one of the solvent systems is not surprising.

These problems are alleviated to a certain extent in the averaging of the rotatory strengths arising from the ^1L_a and $n-\pi^*$ transitions. This is so because the signs of the rotatory strengths of these bands do not undergo so many fluctuations as a function of conformation, and, furthermore, the calculated rotatory strengths are clearly of one sign in the vicinity of the global minimum of the conformational energy, i.e., the nodal lines do not pass near the energy minimum.

The results which are presented in Table III indicate that the intense negative Cotton effect which occurs in neutral aqueous solution at 199 nm and the positive Cotton effect that occurs to the blue of it are probably caused by the aromatic ^1B transitions and the peptide $\pi-\pi^*$ transitions, respectively. Both of the aromatic ^1B transitions are predicted to give rise to negative Cotton effects, whereas the lower energy peptide $\pi-\pi^*$ band is predicted to give rise to a strong positive Cotton effect. The presence of this latter Cotton effect, to the blue of the negative aromatic Cotton effects, would have the effect of producing a sharp negative Cotton effect with an apparent extremum near 200 nm, followed by a sharp rise in ellipticity immediately to the blue. This is the pattern that is observed experimentally.

Our primary objectives in this work have been to correlate the optical activity which is associated with the lower energy phenolic transitions and the $n-\pi^*$ transitions of the diketopiperazine ring with the conformation of the L-AHPGDKP molecule in solution and to demonstrate that the theoretical formalism which has been used is capable of accounting for the optical properties of the molecule in a reasonable way. We are certainly gratified at the agreement which was achieved for the ^1L_a and $n-\pi^*$ transitions. The sign of the ^1L_b Cotton effect has not been correctly predicted insofar as polar solvent systems are concerned, but agreement is good for triethyl phosphate solutions. However, even the solvent dependence of the sign of this Cotton effect is clearly understandable in terms of the results of this study. In conclusion, it appears that the theoretical procedures which have been employed are capable of accounting for the chiroptical properties of yet another molecule which incorporates both peptide and phenolic chromophores. Furthermore, when the results of the optical calculations are considered in conjunction with the results of the conformational energy calculations, it appears likely that the L-AHPGDKP molecule assumes a variety of conformations in solution, but conformations which are characterized with negative values of the conformational angle β and χ_1 values from approximately 120 to 180° are probably dominant.

References and Notes

(1) T. M. Hooker, Jr., and J. A. Schellman, *Biopolymers*, **9**, 1319 (1970).
(2) J. W. Snow and T. M. Hooker, Jr., *J. Am. Chem. Soc.*, **96**, 7800 (1974).
(3) T. M. Hooker, Jr., P. M. Bayley, W. Radding, and J. A. Schellman, *Biopolymers*, **13**, 549 (1974).
(4) P. E. Grebow and T. M. Hooker, Jr., *Biopolymers*, in press.
(5) K. D. Kopple and H. G. Ghazarian, *J. Org. Chem.*, **33**, 862 (1968).
(6) R. Fletcher and M. J. D. Powell, *Comput. J.*, **6**, 163 (1963).
(7) J. R. Platt, *J. Chem. Phys.*, **17**, 484 (1949).
(8) T. M. Hooker, Jr., and C. Tanford, *J. Am. Chem. Soc.*, **86**, 4989 (1964).
(9) D. A. Brant, W. G. Miller, and P. J. Flory, *J. Mol. Biol.*, **23**, 47 (1967).
(10) R. A. Scott and H. A. Scheraga, *J. Chem. Phys.*, **45**, 2091 (1966).
(11) T. Ooi, R. A. Scott, G. Vanderooi, and H. A. Scheraga, *J. Chem. Phys.*, **46**, 4410 (1967).
(12) G. N. Ramachandran and V. Sasisekharan, *Adv. Protein Chem.*, **23**, 284 (1968).
(13) R. B. Corey, *J. Am. Chem. Soc.*, **60**, 1598 (1938); R. Degeilh and R. E. Marsh, *Acta Crystallogr.*, **12**, 1007 (1959).
(14) L. Pauling, "Nature of the Chemical Bond", 3rd ed, Cornell University Press, Ithaca, N.Y., 1960.
(15) R. Srinivasan, *Proc. Indian Acad. Sci.*, **50**, 19 (1959).
(16) IUPAC-IUB, *Biochemistry*, **9**, 3471 (1970).
(17) G. Del Re, *J. Chem. Soc.*, 4031 (1958); *Theor. Chim. Acta*, **1**, 188 (1963); G. Del Re, B. Pullman, and T. Yonezawa, *Biochim. Biophys. Acta*, **75**, 153 (1963).
(18) D. Poland and H. A. Scheraga, *Biochemistry*, **6**, 3791 (1967).
(19) S. Karplus and S. Lifson, *Biopolymers*, **10**, 1973 (1971).
(20) D. A. Brant and P. J. Flory, *J. Am. Chem. Soc.*, **87**, 663, 2791 (1965).
(21) P. M. Bayley, E. B. Nielsen, and J. A. Schellman, *J. Phys. Chem.*, **73**, 228 (1969).
(22) J. G. Kirkwood, *J. Chem. Phys.*, **5**, 479 (1937).
(23) W. Kuhn in "Stereochemie," K. Freudenberg, Ed., Deuticke, Leipzig, 1933, p 317.
(24) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956).
(25) L. L. Jones, Ph.D. Thesis, University of Utah, Salt Lake City, 1961.
(26) D. Caldwell and H. Eyring, *Rev. Mod. Phys.*, **35**, 577 (1963).
(27) R. W. Woody and I. Tinoco, *J. Chem. Phys.*, **46**, 4927 (1967).
(28) E. G. Hohn and O. E. Weigang, *J. Chem. Phys.*, **48**, 1127 (1968).
(29) E. V. Condon, W. Altar, and H. Eyring, *J. Chem. Phys.*, **5**, 753 (1937).