similar to that for normal liquids the minimum can be enhanced. Because ΔV and the choices of α are also open to variation there seems to be no difficulty in choosing mutually reasonable values which will fit all of the data considered here. In particular a choice of $\Delta V^\circ = 4.0 \text{ cm}^3$, $V_A{}^\circ = 20.0 \text{ cm}^3$, and $V_B{}^\circ = 16.0 \text{ cm}^3$ with slightly larger values for α_A and smaller values for α_B will give much improved fits in the supercooled region.

Discussion

The structure of water and models of multicomponent systems to represent the properties of water have been proposed by many authors.[9,11] In most of these models relatively little attention has been given to the heat capacities and consequently most of these models have not reproduced C_p very well, this despite the fact that they have generally used more complex structures than that suggested here. All of the ones that have fitted either the thermodynamic properties or the molar volumes have arrived at ΔH_1 and ΔV_1 values similar to those used here.[12] The present results are of interest in that they suggest that the heat capacity data alone are sufficient to require a minimum two-component system to represent this property of water. The detailed molecular structure that satisfies these requirements is of course outside the scope of the present approach.[13] It is of interest to note, however, that the differences in energy between an ice-like, tetrahedral structure of specific hydrogen bonds and a structure of dipole-linked chains with coordination of about 6 is comparable to the ΔH_1 values we have considered here.[14]

References and Notes

(1) S. W. Benson, *J. Chem. Phys.*, **15**, 866 (1947).
(2) (a) J. H. Hildebrand, J. M. Prausnitz, and R. L. Scott, "Regular and Related Solutions", Van Nostrand-Reinhold, Princeton, N.J., 1970. (b) See also discussion in E. A. Moelwyn-Hughes, "Physical Chemistry", 2nd ed, Pergamon Press. Oxford, 1961, pp 692–696.
(3) R. Shaw, *J. Chem. Eng. Data*, **14**, 461 (1969).
(4) S. W. Benson, "Thermochemical Kinetics", 2nd ed, Wiley, New York, N.Y., 1976. D. W. Scott, *J. Chem. Phys.*, **60**, 3144 (1974), has made a very sophisticated correlation of the thermodynamic properties of hydrocarbons from which C_p for the gases can also be estimated to good accuracy (±0.1 eu).
(5) M. Luria and S. W. Benson, *J. Chem. Eng. Data*, **22**, 90 (1977).
(6) R. C. Reid, T. K. Sherwood, and J. M. Prausnitz, "The Properties of Gases and Liquids", 3rd ed, McGraw-Hill, New York, N.Y., 1976.
(7) If the asymmetry of the molecule is great and the barrier to rotation of the order of (2–5)RT then the individual rotational motions may resemble hindered rotations in molecules with an extreme contribution to C_p of 2.3 eu each.
(8) K. S. Pitzer et al.. *Ind. Eng. Chem.*, **50**, 265 (1958), and earlier work.
(9) D. Eisenberg and W. Kauzmann, "The Structure and Properties of Water", Oxford University Press, London, 1969, pp 171–175.
(10) G. S. Kell, *J. Chem. Eng. Data*, **12**, 67 (1967).
(11) A recent bibliography with 2000 references is given by D. T. Hawkins, *J. Solution Chem.*, **4**, 625 (1975).
(12) See summary of models in ref 9, p 258.
(13) A recent example of such efforts is given by J. C. Owicki and H. A. Scheraga, *J. Am. Chem. Soc.*, **99**, 7403 (1977).
(14) See discussion in ref 9, p 39. In a hypothetical chain-like structure, a near neighbor distance of about 2.8 Å between H_2O molecules in the chains and a mean interchain distance of about 3.1 Å would have the molar volume $V_B{}^\circ$ of water species B if the chains had an average of four nearest-neighbor chains. Six neighboring chains could relax the interchain distance to 3.3 Å.
(15) W. Kauzmann, *Colloq. Int. CNRS*, **246**, 63 (1976). I am much indebted to Professor Kauzmann for bringing this paper to my attention in the course of reviewing my work. He has made an analysis of the physical properties of water very similar to the one presented here.

Vibrational Circular Dichroism in Amino Acids and Peptides. 2. Simple Alanyl Peptides

Max Diem,* Paul J. Gotkin, Joel M. Kupfer, and Laurence A. Nafie*

Contribution from the Department of Chemistry, Syracuse University, Syracuse, New York 13210. Received December 27, 1977

Abstract: Vibrational circular dichroism (VCD) as well as Raman and infrared absorption spectra of alanine, alanylalanine, alanylalanylalanine, glycylalanine, and alanylglycine are reported in the 2800–3100·cm^{-1} region. Vibrational assignment of the carbon-hydrogen stretching modes of alanine is presented and an interpretation of the observed VCD spectrum is proposed. The arguments employed for the interpretation of alanine are subsequently utilized, along with considerations concerning subtle changes in chemical environment, to propose an interpretation of the VCD features of the alanyl peptides.

I. Introduction

The structure and conformation of amino acids, oligopeptides, polypeptides, and proteins have been studied extensively by spectroscopic methods owing to their direct relevance to the understanding of biochemical processes on a molecular level. Of these methods, X-ray diffraction of single crystals has provided the most accurate and detailed information. However, X-ray crystallographic methods are not applicable to solution phase samples where biological molecules may adopt altered conformations or undergo critical conformational changes while executing biological activity. Currently the most sensitive spectroscopic probes of solution phase conformation in proteins and related molecules are electronic optical activity, nuclear magnetic resonance, infrared absorption, and Raman scattering. Very often the results of several of these techniques must be combined to gain desired conformational inferences, since no individual method is significantly superior to the others, or is so conclusive that supporting results are not helpful.

In view of this situation, we have undertaken the application of the newly developed technique of vibrational circular dichroism[1a,b] (VCD) to the study of protein-related molecules. The overall aim of this work is to provide a new spectroscopic method of investigation which will combine the conformational and configurational sensitivity of electronic optical activity with the rich structural content of vibrational spectroscopy. In the present study of alanine and some of its simple oligopeptides we are investigating the VCD of a small group of closely related molecules in order to amplify some of the basic features of this new technique and to demonstrate the feasibility of further studies.

In a recent communication,[2] we reported the first observation of VCD of an amino acid, alanine, in the carbon–hydrogen

stretching region. In this publication, we present additional results of our continuing work on the vibrational optical activity of amino acids and small peptides. In particular, we will focus our attention on three principal areas. First, experimental conditions and methods will be described that were used to obtain VCD spectra with good signal to noise ratio from aqueous solutions of amino acids and small alanyl oligopeptides. Second, the observed VCD signals will be described and interpreted utilizing supporting vibrational data obtained in this work. Third, the unique sensitivity of VCD to probe subtle changes in molecular structure will be discussed.

We report vibrational circular dichroism and detailed vibrational data in the C–H stretching region of aqueous solutions of alanine (D-Ala and L-Ala), alanylalanine (D-Ala-D-Ala and L-Ala-L-Ala), and alanylalanylalanine (D-Ala-D-Ala-D-Ala and L-Ala-L-Ala-L-Ala) as well as the two noncyclic copeptides of alanine and glycine, glycylalanine (Gly-D-Ala and Gly-L-Ala) and alanylglycine (D-Ala-Gly and L-Ala-Gly). In a subsequent publication,[3] VCD of cyclic alanylalanine and alanylglycine (diketopiperazines) will be reported. The molecules listed above were selected because they allow the study of the interactions of near-identical subunits in larger molecular systems, without the complicating problems of additional vibrational assignment. Furthermore, the slight structural differences encountered between the subunits give valuable information about the origin of the observed VCD effects.

Interpretation of the observed vibrational circular dichroism requires detailed knowledge of the vibrational motions of the molecules. However, no reliable spectroscopic vibrational data in the carbon–hydrogen stretching region were available prior to this study. The reason for this lack of data is twofold. First, solution data are unavailable because water is the only practical solvent for most of the amino acids and small peptides; the water spectrum, however, masks the 2800–3100-cm^{-1} spectral region completely. Second, solid-state vibrational spectra (mostly in KBr pellets) do not exhibit a great deal of detail in the carbon–hydrogen stretching region because the hydrogen bonding in the solid state produces one broad, poorly resolved peak between 2600 and 3300 cm^{-1}.

Therefore, we decided to obtain Raman frequencies and depolarization ratios in the C–H stretching region for the molecules listed above as solutions in D$_2$O. Along with the infrared absorption spectra obtained simultaneously with the VCD spectra, the Raman data allow a reliable vibrational assignment. The infrared and Raman spectra of alanine, alanylalanine, and alanylalanylalanine proved to be nearly identical, whereas the VCD spectra show marked changes between the monomer and the dimer. This observation will be interpreted in terms of the sensitivity of VCD to probe small changes in the stereochemical environment which conventional vibrational techniques are too insensitive to probe. In particular, it is thought that the chemically different nitrogen atoms in the amine group and the peptide linkage may strongly influence the optical activity of the vicinal CH$_3$ vibrations. Other possible factors contributing to the observed vibrational optical activity, such as couplets due to near-degenerate methyl stretches and long-range methyl–methylene interactions, will also be discussed.

II. Experimental Section

The VCD spectrometer used in this investigation was briefly discussed earlier.[2] Here, we want to present a more detailed description of the unit. The basic design of our VCD spectrometer is similar to one reported earlier[1a] utilizing a double modulation technique. Light from a 1000-W tungsten halogen lamp (Sylvania 1000 Q/CL), operated a ca. 3000 °C, is mechanically chopped at ω_C (ca. 150 Hz) via a light chopper (Laser Precision Model CTX-534) and subsequently focused into a ¾-m monochromator (Spex Model 1702), incorporating

a 300 lines/mm grating blazed at 3 μ. The light emerging from the monochromator is passed through a 2.2-μ long wavelength pass filter (Optical Coating Laboratory, Inc.) to remove radiation diffracted by the grating at higher than first order. Next, the infrared radiation is polarized by a LiIO$_3$ polarizer (Interactive Radiation, Inc.), oriented at 45° with respect to the axes of the modulator. A ZnSe photoelastic modulator, constructed in our laboratory,[4] operates at ω_M (ca. 50.1 kHz) to produce alternately left and right circularly polarized light. The ZnSe modulator is followed by the sample contained in a variable path length cell (Beckman Instruments) equipped with CaF$_2$ windows. The infrared radiation is detected by a 12 × 12 mm, liquid nitrogen cooled InSb detector (Spectronix, Inc.) having a D^* (λ peak) = 1.0 × 10^{11} cm Hz$^{1/2}$/W. In order to obtain ΔA, the difference in absorbance of left and right circularly polarized radiation, it is necessary[1a] to ratio the signals observed at ω_M and ω_C. This normalization is performed by a variable gain amplifier[1a] in conjunction with a lock-in amplifier (PAR Model 128A) tuned to ω_C. The AC signal modulated at ω_M is filtered (to remove the ω_C component) and amplified by a second lock-in amplifier (PAR Model 124A) tuned to ω_M. High stray signal rejection is obtained by operating a third amplifier (PAR Model 186A), tuned to ω_C, in tandem with the ω_M lock-in amplifier. A fourth lock-in (PAR Model 121) permits recording of the transmission spectrum simultaneously with the VCD spectrum. Both signals are displayed on a dual channel strip chart recorder (Soltec Model B-281). Since our first communication,[2] we were able to increase the light level through the spectrometer significantly, resulting in an improved signal to noise ratio. Furthermore, the VCD baseline now is a straight line (within experimental noise) exhibiting a slope of +1 × 10^{-5} ΔA unit in the range of 2800–3100 cm^{-1}, independent of sample absorption. The magnitude of the VCD signal is calibrated following a previously reported procedure[1a] employing a retardation plate and a second polarizer. The sign of the VCD signal was calibrated against published spectra of camphor,[1a] and also against camphor spectra recorded by Moskowitz and Overend.[5] Absorption baselines[6] (obtained from an optically active sample but representing the signal of a racemic modification) were obtained for a number of samples. These spectra were straight lines (within the limits mentioned above) but are not reproduced in the figures for clarity of the displays.

The absorption spectra reported with the VCD spectra are single beam transmission spectra of solvent and solute; they are not compensated for instrument response.

Raman spectra were obtained using the 514.5-mm line of an argon ion laser (50–400 mW) for excitation. Raman scattered light is analyzed by a double monochromator (Spex Model 1401) and direct current amplification. Spectral resolution was about 3 cm^{-1} at 2900 cm^{-1}. Depolarization measurements were carried out using an analyzer and a polarization scrambler placed between sample and monochromator. Samples were dissolved in D$_2$O and placed in melting point capillaries for measurement. Some dipeptides, notably Gly-Ala and Ala-Gly, were slightly yellow when purchased and exhibited a strong background scattering. Purification over activated charcoal was necessary to obtain quality Raman spectra; however, the polarization measurement was still somewhat unreliable for these two peptides.

Samples of the various di- and tripeptides were purchased from either Aldrich Chemical Co. or Chemical Dynamics Corp. For the VCD measurements, the samples were used without further purification. VCD samples were prepared by dissolving ca. 250 mg of the peptides in D$_2$O and boiling to dryness in vacuo three times to deuterate the amine function. Samples then were redissolved in a minimum amount of D$_2$O (ca. 0.5 mL) to obtain sample concentration as high as possible. Generally, the solutions were near saturation. VCD spectra of both enantiomeric forms were obtained for all compounds reported, not only to check instrumental performance, but also to ensure chemical identity and optical purity of the materials under investigation.

III. Results

In Table I are listed the compounds utilized in this study along with the commonly used abbreviations. A typical single beam transmission spectrum of alanine (which represents well the observed infrared spectra of the molecules studied) in the 2000–4000-cm^{-1} range is shown in Figure 1, the arrows indicating the range over which VCD measurements were un-
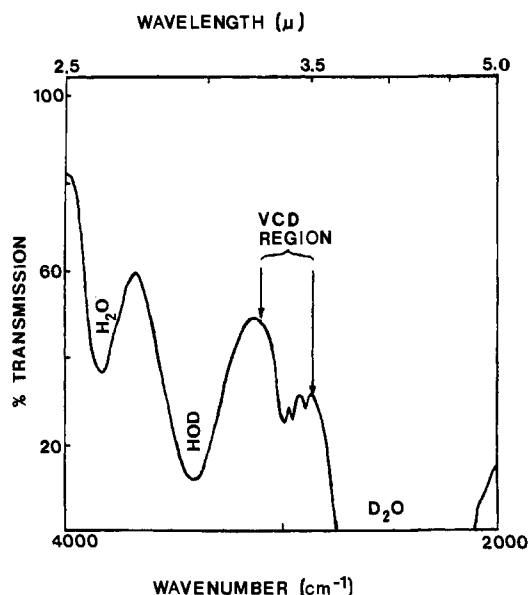
Figure 1. Single beam transmission spectrum of a saturated solution of D-alanine-d_3 in D_2O at 150-μ path length. VCD measurements are obtained in the spectral region marked.

Table I. List of Compounds Studied

symbol	compd[a]
Ala	ND_3^+-C*H(CH_3)-CO_2^-
Ala-Ala	ND_3^+-C*H(CH_3)-CONH-C*H(CH_3)-CO_2^-
Ala-Ala-Ala	ND_3^+-C*H(CH_3)-CONH-C*H(CH_3)-CONH-C*H(CH_3)-CO_2^-
Gly-Ala	ND_3^+-CH_2-CONH-C*H(CH_3)-CO_2^-
Ala-Gly	ND_3^+-C*H(CH_3)-CONH-CH_2-CO_2^-

[a] Asterisks denote asymmetrically substituted C atoms.

dertaken. This figure also demonstrates the high solvent absorption in the spectral range of interest. Failure to deuterate the amine function prior to dissolving the samples leads to increased absorption at 3400 cm^{-1} and thus to considerably less detailed solute spectra between 2800 and 3100 cm^{-1}.

The VCD spectra of alanine, alanylalanine, and alanyl-alanylalanine are reproduced in Figure 2. Although the spectrum of alanine was reported previously, we include the spectrum which we obtained while recording the VCD spectra of the oligopeptide for completeness and direct comparison. VCD and single beam transmission spectra were obtained at a resolution of ca. 14 cm^{-1} at 2950 cm^{-1}. The spectra are direct strip chart reproductions without data smoothing or background subtraction. Figure 3 displays Raman, infrared, and VCD spectra after normalization and background subtraction. Since the sample concentrations were not known exactly (vide supra) and since the sample path length in the VCD measurement was varied to optimize the signal to noise ratio, the infrared transmission spectra were normalized (after proper conversion to wavenumber and solute absorbance units) such that the total C-H peak areas of all compounds were identical. The scale factor found for each compound was used to replot the corresponding VCD spectrum. Thus, in Figure 3 all VCD spectra are normalized for equal sample absorption. This method allows comparison of the observed VCD signals without reference to the exact experimental path lengths and concentrations. Similarly, the Raman spectra were normalized to allow comparison of relative intensities of the bands in the various Raman spectra. Table II lists observed Raman and IR frequencies, Raman depolarization ratio, and absorbance values for all five compounds studied.
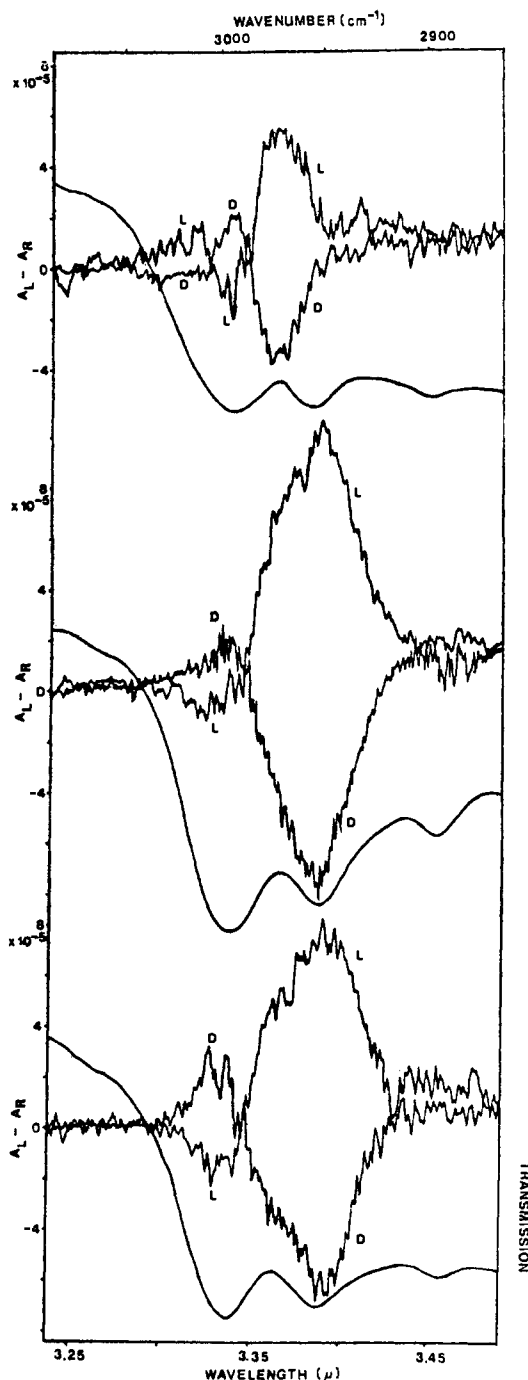

Figure 2. VCD and transmission spectra of D-alanine-d_3 and L-alanine-d_3 (top), D-alanyl-D-alanine-d_3 and L-alanyl-L-alanine-d_3 (middle), and D-alanyl-D-alanyl-D-alanine-d_3 and L-alanyl-L-alanyl-L-alanine-d_3 (bottom) in D_2O.

Figure 4 shows the VCD and transmission spectra of gly-cylalanine and alanylglycine. The normalized Raman, infrared, and VCD spectra are displayed in Figure 5.

IV. Discussion

In the following discussion, we shall attempt to present a simple, straightforward interpretation of the basic features of all observed data. In light of the lack of previously available vibrational information as well as the lack of suitable models for the interpretation of VCD (the coupled oscillator model[7] does not seem to be applicable), our interpretation of the observed data should by no means be considered unique. However, the obtained vibrational data along with some very distinct VCD signals suggest an acceptable interpretation. It

Table II. Observed Frequencies and Intensities[e]

	Raman		infrared		VCD		
	freq, cm⁻¹	depol ratio[a]	freq, cm⁻¹	peak absorbance	freq, cm⁻¹	$\Delta A \times 10^5$ L enantiomer	assignment
Ala	2895[b]	0.04	2894[b]	0.14			overtones and combinations
	2951	0.06	2949	0.29			CH₃ sym
					2970[c]	+4.5 ± 0.07	
	2994	0.38	2987	0.37			CH₃ antisym
					2995	−1.5 ± 0.5	
	3010 sh	0.47	3008 sh	0.24			C*-H
					3015	+0.8 ± 0.5	
Ala-Ala	2888	0.02	2889	0.19			overtones and combinations
	2946	0.04	2947	0.52	2950	+9.0 ± 0.7	CH₃ sym
					2970 sh	+6.0 ± 0.7	
	2998 br	0.48	2991	0.74			C*-H, CH₃ antisym
					3000	−1.0 ± 0.5	
Ala-Ala-Ala	2885	0.03	2887	0.18			overtones and combinations
	2946	0.04	2946	0.46	2950	+7.0 ± 0.7	CH₃ sym
					2970 sh	+4.7 ± 0.7	
	2998 br	0.36	2994	0.58			C*-H, CH₃ antisym
					3000	−2.2 ± 0.6	
Gly-Ala	2890	d	2988	0.14			overtones and combinations
	2945		2944	0.43			CH₃ sym, CH₂ sym
					2950	+6.0 ± 0.7	
	2981 br		2987	0.54			CH₃/CH₂ antisym
					2990	−1.5 ± 0.5	
			3008 sh	0.37			C*-H
Ala-Gly	2885	d	2882	0.07			overtones and combinations
					2935	+0.5 ± 0.3	
	2946		2946	0.33			CH₃ sym, CH₂ sym
					2975	−1.2 ± 0.5	
	2996 br		2988	0.25			CH₃/CH₂ antisym C*-H
					3010	+0.7 ± 0.4	

[a] Reported depolarization ratios are for linearly polarized exciting light (ρ_l). [b] Frequencies are rounded to the nearest wavenumber. They are considered accurate to +1 cm⁻¹. [c] Frequencies of VCD peaks are given to the nearest 5 cm⁻¹. [d] Reliable depolarization ratios could not be obtained because of sample fluorescence. [e] Abbreviations: sh, shoulder; br, broad.

should also be kept in mind, however, that the arguments presented, which are applicable to all five molecules, represent the first interpretation of VCD of a set of related molecules. These arguments shall serve as a working hypothesis until more experimental and theoretical results are available.

The most striking feature in the observed VCD spectra is the presence or absence of a very intense ($\Delta A/A > 10^{-4}$), VCD peak centered under the symmetric methyl stretching vibrations which occurs between 2946 and 2951 cm⁻¹ for the different compounds. In alanine and alanylglycine this feature is essentially absent with $\Delta A/A \sim 10^{-5}$. The large VCD signal observed in alanine, however, is not directly associated with the symmetric methyl stretches and appears at about 20 cm⁻¹ higher frequency. On the other hand, in alanylalanine, alanylalanylalanine, and glycylalanine intense symmetric methyl stretching signals with $\Delta A/A$ values of 1.7, 1.5, and 1.5 × 10⁻⁴, respectively, are observed. Inspection of Table I yields that in the two molecules exhibiting small signals a highly symmetric ND_3^+ group is attached to the asymmetric carbon to which also is attached the methyl group whose symmetric stretches are observed around 2950 cm⁻¹. However, whenever a methyl group is attached to the asymmetric carbon atom which bears a nitrogen involved in a peptide linkage, instead of an amine nitrogen, the symmetric stretches of the methyl group exhibit a large VCD signal. Based on this general interpretation of this dominant feature, a detailed discussion of each of the compounds will now be presented.
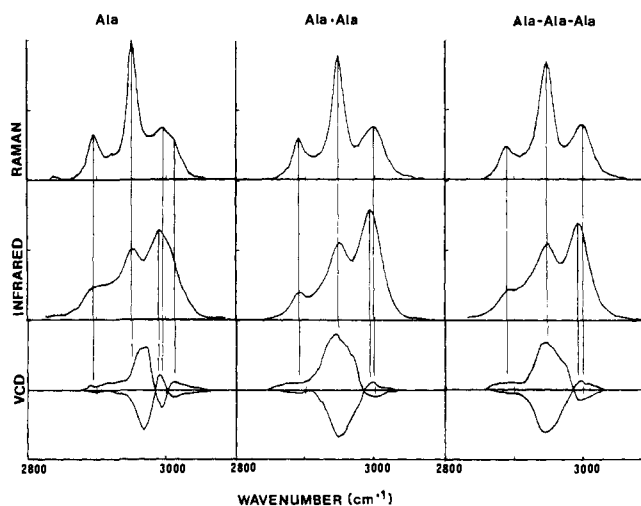

Figure 3. Normalized Raman, infrared, and VCD spectra of alanine, alanylalanine, and alanylalanylalanine. All spectra are presented in arbitrary intensity or absorbance units (cf. text).

Both Raman and infrared spectra of alanine show three major bands in the carbon–hydrogen stretching region, in addition to a weak, unresolved shoulder on the high-frequency side of the highest frequency band (cf. Figure 3). The low-
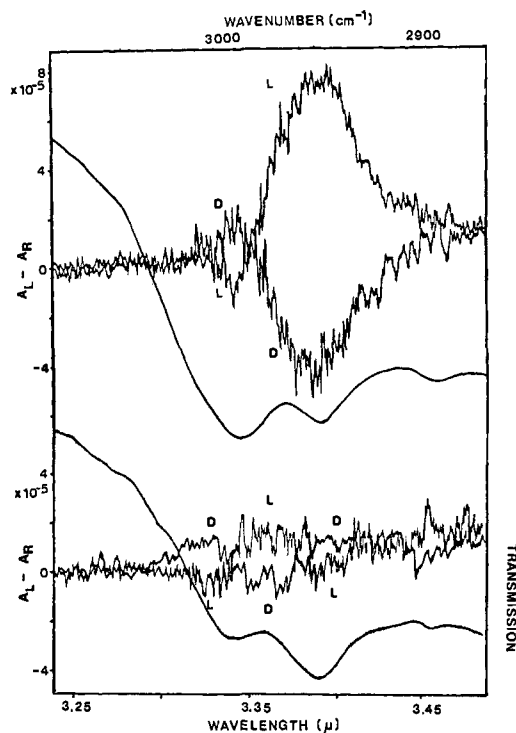
Figure 4. VCD and transmission spectra of glycyl-D-alanine and glycyl-L-alanine (top) and D-alanylglycine-d_3 and L-alanylglycine-d_3 (bottom) in D_2O.
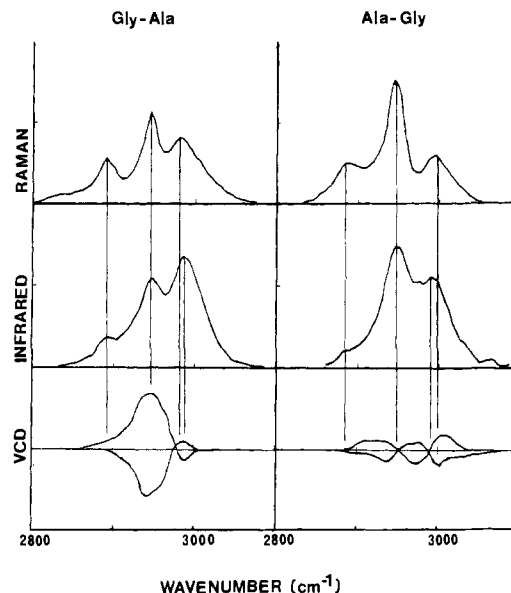

Figure 5. Normalized Raman, infrared, and VCD spectra of glycylalanine and alanylglycine. All spectra are represented in arbitrary intensity or absorbance units (cf. text).

frequency band, observed at 2895 and 2894 cm^{-1} in the Raman and infrared spectra, respectively, was assigned to a Fermi resonance enhanced overtone of methyl deformation modes. The strong, highly polarized Raman band at 2951 cm^{-1} and the corresponding infrared peak at 2949 cm^{-1} were assigned to the symmetric methyl stretching motion. The agreement between Raman and infrared frequencies for these two bands is excellent. However, there is a difference of ca. 7 cm^{-1} between the Raman and infrared frequenices of the high-frequency band (Raman, 2994 cm^{-1}; infrared, 2987 cm^{-1}). We attribute this shift to the occurrence of two "antisymmetric" methyl stretching modes due to the chiral perturbation which removes the local C_{3v} symmetry of the methyl group and therewith lifts the degeneracy between the two antisymmetric stretching modes. Assuming that one of the two modes dominates the Raman intensity whereas the other dominates the infrared intensity, a resulting frequency shift of 7 cm^{-1} can easily be explained. The shoulder on the high-frequency side of the antisymmetric methyl peak was assigned to the C*–H stretching mode. This peak is well resolved in the depolarized Raman spectrum. Our vibrational assignment is confirmed by previously reported assignments on 1,1-disubstituted ethanes,[8] single crystal alanine Raman data,[9] and frequencies calculated from a Urey–Bradley force field.[10]

The observed VCD spectrum was previously[2] assigned using arguments transferred from the discussion of antisymmetric methyl deformation at about 1450 cm^{-1} in Raman optical activity (ROA).[11,12] The methyl deformations also lose their degeneracy upon introduction of a chiral perturbation. The two no longer degenerate antisymmetric deformations actually can be resolved into two components in the liquid-state Raman spectra.[11] The ROA spectrum of these deformation modes exhibits a conservative, bisignate couplet centered between the two components.[11]

Thus, we postulate that a similar couplet, centered at about 2990 cm^{-1}, interferes with VCD signals due to the methyl symmetric stretches and the C*–H stretch to produce the observed spectrum in the following manner. The high-frequency

component of the couplet interferes with a VCD band of opposite sign due to the C*–H stretching mode, thus reducing greatly the observed signal. The low-frequency component of the couplet overlaps a relatively close by, broad signal due to the symmetric methyl stretch, which produces a relatively large signal, the peak of which falls between the absorption maxima due to the symmetric and antisymmetric methyl stretching modes. It is this signal due to the symmetric methyl stretch which we claim is strongly dependent on the other groups attached to the asymmetrically substituted carbon atom.

Before discussing the magnitudes of the observed VCD signals a few comments about the above interpretation are in order. Intuitively one might expect that the zero crossing of the couplet should be in between the Raman and the infrared maxima of the antisymmetric methyl stretches, i.e., about 2990 cm^{-1}. However, it is observed at the low-frequency side of the infrared absorption maximum. This observation might be explained by different coupling of the two antisymmetric stretching modes with other molecular vibrations leading to an asymmetric couplet. Similar behavior is thought to occur in the methyl deformation modes in α-methylbenzylamine and its para-brominated analogue in Raman optical activity,[13] thus complicating the simple model. In addition, other mechanisms, including anharmonic effects, hydrogen bonding, and conformational splitting, may contribute to the observed VCD signals. However, the interpretation above is presently the only simple model which also seems to be able to explain the VCD spectra of the following compounds (vide infra).

The maximum $\Delta A/A$ value for alanine is hard to estimate because the VCD and the absorption maxima do not coincide. However, we estimate that $\Delta A/A$ at the VCD peak is about 1.5×10^{-4}, which is of the same order of magnitude as the values reported by Sugeta et al.[6] for the C–H stretches in tartaric acid-d_4. For most of the other molecules presented, more meaningful $\Delta A/A$ values can be obtained.

We now turn to the discussion of the spectra of the dimer and trimer of alanine. Figure 3 shows that the Raman and infrared spectra of alanine, alanylalanine, and alanylalanylalanine are very similar. The most notable differences are first, the less intense, but slightly broader symmetric methyl stretching bands, and second, the disappearance of the high-frequency shoulder on the antisymmetric methyl stretching band. The depolarized Raman spectrum, which exhibited two

distinct peaks in this region in alanine, only exhibits one broad peak for the dimer and the trimer.

Thus, one can argue that the vibrational spectra of the dimer and trimer are merely superpositions of the monomer spectrum. The slight peak broadening and the apparent disappearance of the C*-H shoulder can be explained in terms of the slightly different chemical surroundings that each of the methyl groups and the α hydrogens experience. Indeed, a shift of only a few wavenumbers toward lower energy will cause the C*-H modes to disappear under the peak due to the antisymmetric methyl stretches.

However, the drastic difference observed in the VCD spectra can only be explained in terms of different chemical environments that each of the subunits experiences. These differences in the surroundings are apparently too subtle for conventional vibrational spectroscopy to distinguish; however, vibrational optical activity is quite sensitive to them. We believe that the observed features can be derived from the alanine VCD spectrum as follows. The VCD of the C*-H stretching modes, shifted toward lower wavenumbers, merges with the previously discussed high-frequency portion of a couplet due to the antisymmetric methyl, thus producing only one, relatively weak signal centered at about 3000 cm^{-1}. The low-frequency component of the couplet is superimposed on a very intense peak due to the two symmetric methyl stretches. We believe that the methyl group located in the molecular region, ND_3^+-C*H(CH$_3$)C-, exhibits a small signal similar to the one observed in alanine; however, the methyl group in the region, -HNC*H(CH$_3$)CO$_2^-$, contributes a much larger signal under the symmetric methyl stretching band to produce the very broad (half-width ca. 50 cm^{-1}), very intense signals observed in alanylalanine and alanylalanylalanine. The $\Delta A/A$ values in the symmetric methyl stretching bands are in the order of 1.7×10^{-4} and 1.5×10^{-4} for the dimer and trimer, respectively, and 1.4×10^{-5} and 3.8×10^{-5} for the high-frequency VCD signals located under the antisymmetric methyl stretching modes.

The arguments presented above can be applied virtually unchanged to the interpretation of the VCD spectra of alanylglycine and glycylalanine. However, it is in order to first discuss in detail the vibrational spectra of these two dipeptides, since quite distinctly different Raman and infrared spectra were observed.

One might expect the vibrational spectra of alanylglycine and glycylalanine to be very similar in the carbon–hydrogen stretching region, since both molecules incorporate a methyl and a methylene group and one α hydrogen. As a matter of fact, however, the two compounds have quite different infrared spectra. The absorption spectrum of glycylalanine closely resembles the alanylalanine spectrum except for a weak shoulder on the high-frequency side (ca. 3008 cm^{-1}) of the antisymmetric methyl stretches. The absorption spectrum of alanylglycine, on the other hand, is distinctly different, with the band at ca. 2880, cm^{-1} appearing very weak and with the intensities of the symmetric and antisymmetric, methyl and methylene modes reversed, The Raman spectra contribute little additional information; no resolved bands due to CH$_2$ and CH$_3$ group vibrations could be detected. The assignment of the vibrational modes[14] is summarized in Table II.

The most striking result in this study was the difference in the VCD spectra of glycylalanine and alanylglycine. The basic interpretation of this result utilizes and emphasizes the arguments presented earlier. In glycylalanine, the methyl group is in close proximity to an amide nitrogen, similar to one of the methyl groups in alanylalanine. Thus, the symmetric stretching mode contributes a VCD signal similar in magnitude to the corresponding methyl group in alanylalanine. In alanylglycine, however, the methyl group is in close proximity to an ND$_3^+$ group and thus experiences a chemical surrounding similar to

the surrounding it would experience in alanine; thus both alanylglycine and alanine do not exhibit the large VCD feature due to the symmetric methyl stretching modes.

In the following section a rationale for the very low VCD intensity of alanylalanine at 2975 cm^{-1} ($\Delta A = 1.2 \times 10^{-5}$) as compared to alanine ($\Delta A = 4.5 \times 10^{-5}$ at 2970 cm^{-1}) will be presented. Again, it should be kept in mind that the arguments only present a tentative interpretation. The infrared band observed at about 2988 cm^{-1} in alanylglycine is due to both antisymmetric methylene as well as the two antisymmetric methyl stretching modes, calculated[14] to occur at 2970, 2988, and 2989 cm^{-1}, respectively, for alanylglycine. The small, negative peak observed in alanylglycine at about 2975 cm^{-1} may thus be due to the contribution of the antisymmetric methylene stretching motion, which is thought to contribute with opposite sign to the spectrum of glycylalanine. These opposite contributions can be explained using arguments commonly employed in the interpretation of electronic optical activity. Assuming for simplicity trans peptide conformations and a fairly rigid peptide linkage (PL) between the two amino acid units, one may represent the molecules as follows:

Ala-Gly

Gly-Ala
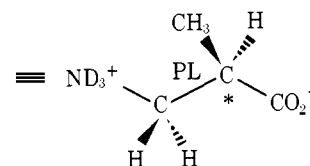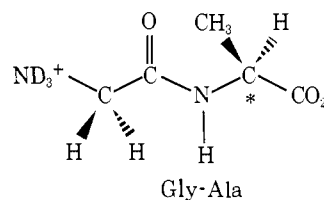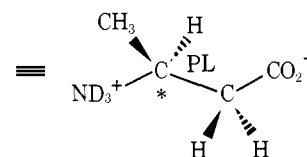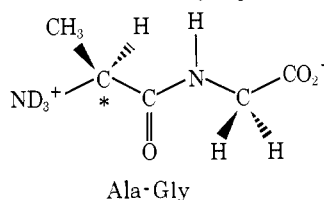
It can now be seen that the two CH$_2$ groups in alanylglycine and glycylalanine probe opposite chiral environments at the asymmetric carbon atom. Thus, in alanylglycine, the antisymmetric CH$_2$ contributions might subtract from, and in glycylalanine add to, the VCD signals of the other carbon–hydrogen stretching motions.

The arguments presented above are, as mentioned previously, the first attempt to interpret VCD signals of a number of closely related molecules. Efforts are underway to investigate further the above interpretation by studying other binary combinations of amino acids to check for a similar behavior to that found in alanylglycine and glycylalanine. Furthermore, preliminary calculations[15] of VCD intensities of asymmetrically perturbed methyl vibrations have been completed. Unfortunately, the results are still inconclusive owing to difficulties in the force fields utilized. Finally, a great deal of information about the validity of the interpretation presented can be obtained by using amino acids and peptides where methyl, methylene, or methyne protons are substituted by deuterium atoms, shifting certain vibrational modes and thus reducing interference and facilitating interpretation.

Finally we note that the peptides studied in this paper are not sufficiently long to adopt a stable helical structure. This contention is supported by the similarity of the VCD spectra of the dimer and trimer of alanine. Consequently our interpretation of the data has focused on the local environment of the individual chiral centers and not upon any long-range helical effects as considered in the earlier theoretical models of vibrational optical activity.[19]

V. Conclusions

We have demonstrated that VCD can combine the structural sensitivity of vibrational spectroscopy with the stereochemical and configurational sensitivity of chiroptical techniques. The new spectra are more sensitive to structural details than ordinary vibrational spectra, but currently they are more subtle and difficult to interpret. In this study we have approached the problem of interpretation on a qualitative basis by investigating a group of closely related simple molecules. The major features of the VCD spectra appear to be compatible with some simple interpretive arguments, but verification of these ideas must await further experimentation or theoretical computation.

References and Notes

(1) (a) L. A. Nafie, T. A. Keiderling, and P. J. Stephens, *J. Am. Chem. Soc.*, **98**, 2715 (1976). (b) For earlier work on vibrational effects in optical rotation see H. F. Hameka, *J. Chem. Phys.*, **41**, 3612 (1964).
(2) M. Diem, P. J. Gotkin, J. M. Kupfer, A. G. Tindall, and L. A. Nafie, *J. Am. Chem. Soc.*, **99**, 8103 (1977).
(3) H. Khouri, P. J. Gotkin, M. Diem, and L. A. Nafie, to be published.
(4) J. C. Cheng, L. A. Nafie, S. D. Allen, and A. I. Braunstein, *Appl. Opt.*, **15**, 1960 (1976).
(5) A. Moscowitz and J. Overend, private communication.
(6) H. Sugeta, C. Marcott, T. R. Faulkner, J. Overend, and A. Moscowitz, *Chem. Phys. Lett.* **40**, 397 (1976).
(7) G. Holzwarth and I. Chabay, *J. Chem. Phys.*, **57**, 1632 (1972).
(8) J. R. Durig, C. J. Wurrey, W. E. Bucy, and A. E. Sloan, *Spectrochim. Acta, Part A*, **32**, 175 (1976).
(9) C. H. Wang and R. D. Storms, *J. Chem. Phys.*, **55**, 3291 (1971).
(10) R. B. Srivastava and V. D. Gupta, *Indian J. Pure Appl. Phys.*, **10**, 596 (1972).
(11) L. D. Barron, *Nature (London)*, **255**, 458 (1975).
(12) L. D. Barron, *Adv. Infrared Raman Spectrosc.*, in press.
(13) L. D. Barron, *J. Chem. Soc., Perkin Trans. 2*, 1790 (1977).
(14) A. M. Dwivedi, R. B. Srivastava, and V. D. Gupta, *Indian J. Pure Appl. Phys.*, **11**, 764 (1973).
(15) Calculations are being carried out using a harmonic force field and a fixed partial charge model for the molecules 1-bromo-1-chloro-1-fluoroethane, 1-chloro-1-fluoroethane, and alanine. For further details of this method, see ref 16–18.
(16) J. A. Schellman, *J. Chem. Phys.*, **58**, 2882 (1973).
(17) T. R. Faulkner, C. Marcott, A. Moscowitz, and J. Overend, *J. Am. Chem. Soc.*, **99**, 8160 (1977).
(18) C. Marcott, T. R. Faulkner, A. Moscowitz, and J. Overend, **99**, 8169 (1977).
(19) C. W. Deutsche and A. Moscowitz, *J. Chem. Phys.*, **49**, 3257 (1969); **53**, 2630 (1970).

Ground States of Molecules. 49.[1] MINDO/3 Study of the Retro-Diels–Alder Reaction of Cyclohexene

Michael J. S. Dewar,* Santiago Olivella,[2a] and Henry S. Rzepa[2b]

Contribution from the Department of Chemistry, The University of Texas, Austin, Texas 78712. Received October 11, 1977

Abstract: The retro-Diels–Alder reaction of cyclohexene to form ethylene and butadiene has been studied, using MINDO/3. The transition state is predicted to be very unsymmetric, corresponding to weakening of one of the two breaking CC bonds. The calculated entropy of activation agrees well with experiment and the calculated secondary isotope effects for 4,4-dideuteriocyclohexene and 4,4,5,5-tetradeuteriocyclohexene are similar to those measured for an analogous reaction by Taagepera and Thornton. Discrepancies between the conclusions reached here and those from recent ab-initio calculations are discussed.

Introduction

A major problem in studies of chemical reactions is to determine the timing of the bond breaking and bond forming processes that occur during them. The course of a reaction cannot be followed directly by any available experimental technique, so conclusions concerning mechanistic details of this kind can be drawn only very indirectly from experiment. This is an area where theoretical calculations would be especially useful, if they could be carried out by procedures able to give reliable results of sufficient accuracy.

One of the most striking examples is provided by the Diels–Alder reaction, whose detailed mechanism is still unknown after more than 40 years work by numerous investigations.[3] Opinion has vacillated concerning the timing of the formation of the two new bonds in reactions of this kind, from one extreme where the reaction is regarded as completely synchronous to the other extreme where it is supposed to occur in steps via an intermediate biradical or zwitterion.[3] In recent years the former view seems to have been generally favored, partly as a result of the views expressed by Woodward and Hoffmann and partly on the basis of experimental studies of secondary deuterium isotope effects[4] and rates of reaction of anthracene derivatives.[5] However, none of these arguments are unequivocal.

Various attempts have been made from time to time to study the Diels–Alder reaction theoretically, but until recently the methods available have been far too inaccurate to lead to useful conclusions. Thus early CNDO[6] studies of the prototype reaction, ethylene (**1**) + 1,3-butadiene (**2**) → cyclohexene (**3**), predicted it to occur without activation. The situation has now changed with the development of computers able to handle RH (Roothaan–Hall; "ab initio SCF")[7] calculations for systems of this size and of semiempirical procedures (MINDO/2;