The magnitude of the IR and VCD intensities for these vibrations is about that calculated earlier via the fixed partial charge (FPC) formalism, whereas the signals in the methine deformations are much more intense than those calculated via the FPC approach.

For the above-mentioned $-NH_3^+$ rocking modes, $\Delta A/A$ of about 5×10^{-5}, and opposite sign, as expected from qualitative group theoretical considerations, was observed. The sharp peak at 1117 cm^{-1}, which exhibits $\Delta A/A$ of -4.7×10^{-5}, was previously[12] assigned to the "antisymmetric" C-C-N stretching mode (the response of the C-C and C-N stretching modes to deuteriation of the amine and the methyl function has suggested that these stretches are more adequately described in terms of a "symmetric" and an "antisymmetric" C-C-N stretching motion).[12]

On the basis of solid-phase Raman work, the peak at 1001 cm^{-1} actually contains two accidentally degenerate vibrations, the C^*-C_C stretching and a methyl rocking vibration.[10] Here, C_C denotes the carbon atom of the carboxylate group. This vibration does not exhibit any VCD intensity.

The low-frequency (900-1150 cm^{-1}) region of L-Ala-*N*-d_3 contains, according to the vibrational assignment published earlier, two methyl rocking modes (1060 and 918 cm^{-1}), the C^*-C_C (1103 cm^{-1}) and the C-C-N "antisymmetric" stretching modes. All these vibrations are strongly perturbed, as compared to the parent molecule. Among these vibrations, the C^*-C_C stretching vibration exhibits a clearly observable VCD signal, whereas the low-frequency $-CH_3$ rocking band at 920 cm^{-1} exhibits barely observable VCD ($S/N \approx 2$). This is, partially, due to the transmission cutoff of some optical elements[8] in the Hunter College VCD instrument, which can be eliminated relatively easily, opening the possibility of extending the range accessible to VCD measurements in aqueous solutions to about 800 cm^{-1}. The cuttoff in the spectra of L-Ala in H_2O, on the other hand, is due to water itself, and therefore, spectra probably cannot be extended further toward lower wavenumber.

The limitations of the solvents may explain why there is, at present, no apparent correlation between the L-Ala and the L-Ala-*N*-d_3 VCD spectra in the low-frequency region. This is particularly obvious when one tries to follow the vibrations upon isotopic substitution. The $-NH_3^+$ rocking modes, which show distinct VCD in L-Ala, cannot be observed in L-Ala-*N*-d_3, since they occur below 850 cm^{-1}. Similarily, the CCN "antisymmetric" stretching mode, which is observable in L-Ala, shifts to higher frequency in L-Ala-*N*-d_3 (1148 cm^{-1}) and is partially obscured by the D_2O deformation. Finally, the two modes that show VCD in L-Ala-*N*-d_3 have their corresponding vibrational frequencies in L-Ala just at the water cutoff at 1000 cm^{-1}.

Nevertheless, the ability to observe $-CH_3$ and $-NH_3^+$ rocking VCD from these aqueous solutions, as well as some of the skeletal stretching VCD, will open the possibilities to utilize these vibrations for purposes of vibrational assignments and conformational analyses.

Conclusions

The feasibility of observing VCD in aqueous solutions in the midinfrared region has been demonstrated. Aside from very intense methine deformation vibrations, VCD signal can be observed in rocking modes as well as skeletal stretching vibrations.

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Registry No. L-Ala, 56-41-7; L-Ala-*N*-d_3, 19470-97-4.

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Vibrational Circular Dichroism in the Methine Bending Modes of Amino Acids and Dipeptides

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Abstract: The vibrational circular dichroism (VCD) spectra of several amino acids and dipeptides in aqueous solution in the methine bending region (1200-1400 cm^{-1}) are reported. In both H_2O and D_2O solution, a characteristic (-,+) VCD pattern (negative at higher frequency) is observed for the two orthogonal methine bending modes in L-amino acids and L-amino acid residues only for a methine bond adjacent to a CO_2^- group. At low pH or for the N-terminus of a dipeptide, no VCD intensity is observed for these modes. The "amide III" NH bending motion does not contribute to the VCD in the dipeptides since deuteration of the nitrogen leaves the overall VCD pattern unchanged. An interpretation of the signs of the VCD features using the ring current mechanism is provided.

Vibrational circular dichroism (VCD)[1-4] studies of amino acids and simple peptides in aqueous solution provide information on absolute configuration, solution conformation, and intramolecular association. Previous investigations have focused primarily on the CH stretching region.[5-8] The large positive VCD intensity bias observed in this region for the L-enantiomers at neutral or high pH has been attributed to the stretching mode of a methine bond adjacent to a CO_2^- group.[7,8] The positive VCD intensity is not observed for amino acids at low pH[1,9] for the N-terminus of a

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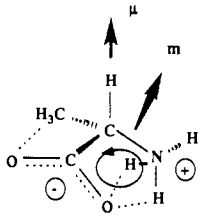
dipeptide[1,9] or for (*S*)-glycine-*C*-d_1.[8] A consistent explanation for these observations can be found by applying "rule 1" of the ring current mechanism for VCD.[1,10,11] According to this mechanism, the contraction of the methine bond generates electron flow, directed preferentially toward the nitrogen, in the ring closed by hydrogen bonding between the carboxylate and amino or peptide NH groups. As depicted in **1** for L-alanine, the resulting



1

positive current (opposite in direction to electron flow) at constant electron density in the closed ring produces a magnetic dipole transition moment **m** with a component parallel to the direction of the electric dipole transition moment μ for the methine contraction. For the elongation phase of the vibration, the current flow and both moments are in the opposite directions. VCD intensity, proportional to the rotational strength $R = \mathrm{Im}(\mu \cdot \mathbf{m})$, is predicted to be positive, as observed. A secondary intramolecular interaction between a carboxylate oxygen and a hydrogen on the β-carbon also appears to play a role in enhancing the CH-stretching VCD signal.[9] Current in this closed path can serve both to increase the magnitude of the magnetic dipole transition moment (which is proportional to the area of the ring) and to increase the positive overlap in the directions of μ and **m**. This secondary interaction is not possible in (*S*)-glycine-*C*-d_1, for the N-terminus amino acid of a dipeptide, or for amino acids at low pH, and no methine stretching VCD intensity enhancement is observed in these situations.

Although CH bending motion often contributes to a number of normal modes in the midinfrared region, in simple amino acids and dipeptides the two orthogonal $C_\alpha H$ bending motions are largely localized in two distinct bands between 1350 and 1250 cm^{-1}.[6a] A (−,+) VCD pattern has been observed for this pair of modes in L-alanine[9,12] and L-alanyl-L-alanine.[13] In this report we examine the methine bending VCD as a function of solvent, pH, and molecular environment for several amino acids and dipeptides, and provide an interpretation of the mechanism for generating the VCD intensity of the methine bending modes.

Experimental Section

The amino acids and dipeptides were obtained from standard commercial sources. An enzymatic method[14] was used to prepare (*S*)-glycine-C_α-d_1 and (*R*)-glycine-C_α-d_1 from glycine-d_0 and glycine-d_2, respectively. The neutral samples were either dissolved directly in distilled H_2O or exchanged at least twice with D_2O before preparing D_2O solutions. For the pH studies, the pH was adjusted with HCl or NaOH; for the highest and lowest pH samples, acid or base concentration was twice that of amino acid. Spectra were recorded by using a variable path length cell with BaF_2 windows. With the exception of the spectra of L-alanine in H_2O and D_2O, the path lengths have not been calibrated, and the reported path lengths may be larger than actually employed.

Absorption and VCD spectra were collected on a modified Nicolet 7199 Fourier transform infrared (FTIR) spectrometer as previously described,[15] at 4 cm^{-1} resolution. Absorption spectra (64 scans) are displayed with H_2O or D_2O background subtracted. The VCD spectra represent collections of 12 288 ac and 800 dc scans per enantiomer. VCD base lines were obtained by comparing raw VCD spectra for L- and D-enantiomers.

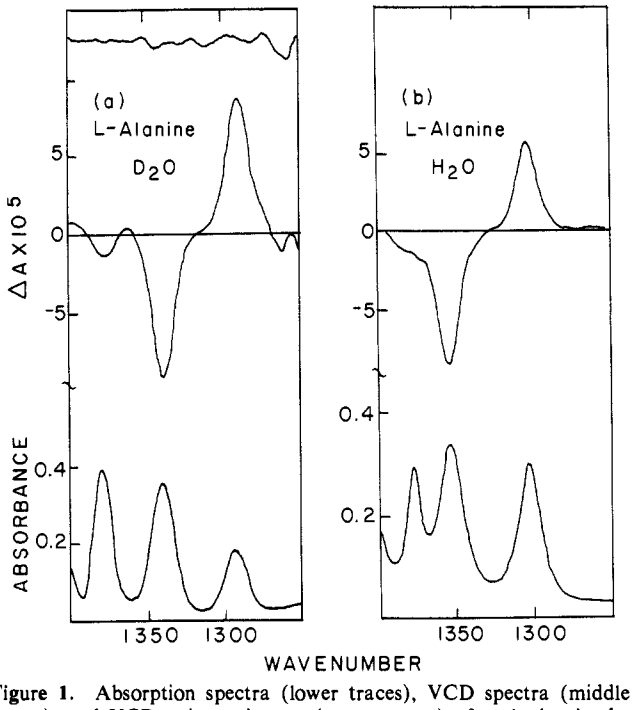


Figure 1. Absorption spectra (lower traces), VCD spectra (middle traces), and VCD noise estimates (upper traces) of L-alanine in the methine bending region: (a) 1.2 M in D_2O solution, ~35-μm path length; (b) 1.7 M in H_2O solution, ~15-μm path length.

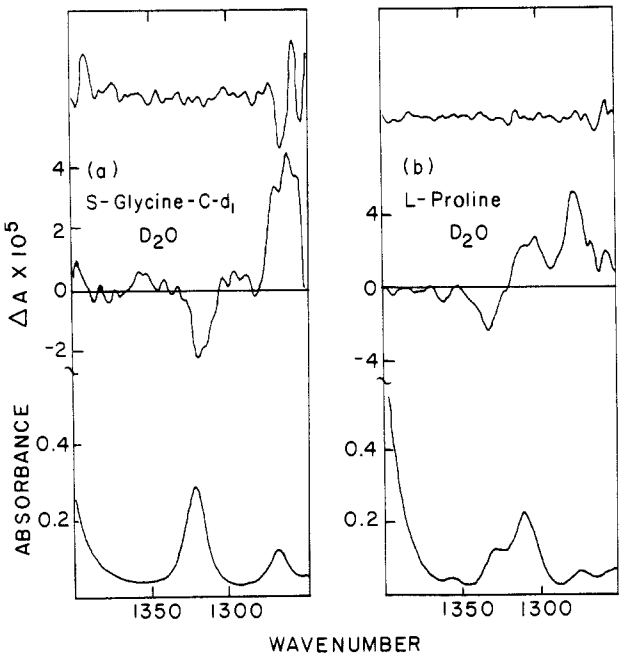


Figure 2. Methine bending absorption and VCD spectra for D_2O solutions of (*S*)-glycine-*C*-d_1 (0.6 M, ~35-μm path length) and L-proline (1.2 M, ~35-μm path length). Upper traces are VCD noise estimates.

Results

The VCD spectra in the 1400–1250-cm^{-1} region of L-alanine in H_2O and D_2O are compared in Figure 1. Normal coordinate analyses based on several deuterated isotopomers have assigned the bands at 1351 and 1301 cm^{-1} in L-alanine, and 1337 and 1291 cm^{-1} in L-alanine-*N*-d_3 to the two approximately orthogonal bending motions of the $C_\alpha H$ bond.[6a] In both H_2O and D_2O, the higher frequency methine bend exhibits negative VCD intensity and the lower frequency bend exhibits positive VCD intensity.

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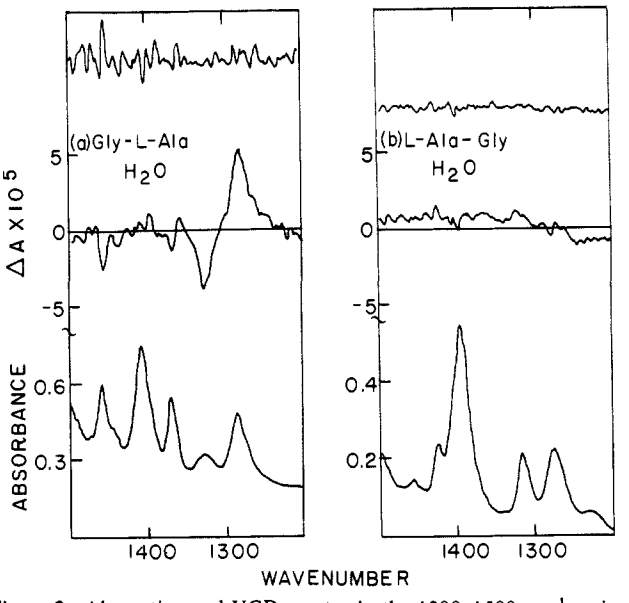

Figure 3. Absorption and VCD spectra in the 1200–1500-cm^{-1} region of glycyl-L-alanine and L-alanylglycine, 1.7 M in H_2O, ~35-μm path length.

The methyl umbrella mode at 1375 cm^{-1} consistently gives rise to a weak negative VCD feature. A similar methine bending VCD pattern is observed in (*S*)-glycine-*C*-d_1 (Figure 2a) corresponding to the absorption bands at 1323 and 1268 cm^{-1}. The increased noise observed for the lower frequency VCD band is due to its proximity to an intense D_2O solvent absorption. In L-proline (Figure 2b), the overall (–,+) VCD pattern is also present; in this molecule, however, methylene bending modes also occur in this region, and the pattern is less distinct. Comparison of the alanine and proline spectra indicates that the major methine bending contributions for proline are at 1331 and 1275 cm^{-1}.

We have also investigated the VCD spectrum of L-alanine in H_2O in the 1550–950-cm^{-1} region as a function of pH.[9] In the absorption spectra, distinct features grow in owing to generation of the species with COOH or NH_2 moieties as the pH is lowered or raised, respectively. Between pH 3 and 12, only the (–,+) features corresponding to the methine bends in the zwitterion are observed in the VCD spectrum. These two features decrease in intensity as the pH is raised or lowered from neutrality, and at pH 1.5 or 13.5, no VCD features are observed. Because of overlapping carboxyl or other modes, distinct features in the absorption spectrum attributable to the methine modes are not readily identified at the two extreme pH values.

Three dipeptides have also been investigated, glycyl-L-alanine and L-alanylglycine in H_2O solution, and L-alanyl-L-alanine in H_2O and D_2O solution, shown in Figures 3 and 4. For both Gly-L-Ala and L-Ala-Gly, distinct absorption features corresponding to the methine bends of the alanyl residue are observed at 1330, 1286 cm^{-1} and 1315, 1271 cm^{-1}, respectively. The peptide NH "amide III" bend also contributes in this region.[16,17] The characteristic (–,+) methine bending VCD pattern is only observed for Gly-L-Ala; no VCD signal is observed in this region for L-Ala-Gly.

In this region in L-Ala-L-Ala (Figure 4), there are two methine bending modes from each residue, plus the peptide NH bend for the sample in H_2O solution. Diem and co-workers have assigned the bands at 1355, 1329, 1305, and 1276 cm^{-1} for L-Ala-L-Ala in D_2O solution to the four methine bends.[16,17] The 1355- and 1329-cm^{-1} modes exhibit negative VCD intensity and the 1305- and 1276-cm^{-1} modes, positive VCD intensity. The largest VCD intensity corresponds to the highest and lowest frequency modes. In H_2O solution only two distinct features are observed for the five modes, a band near 1325 cm^{-1} exhibiting negative VCD intensity and a band at 1280 cm^{-1} exhibiting positive VCD intensity.

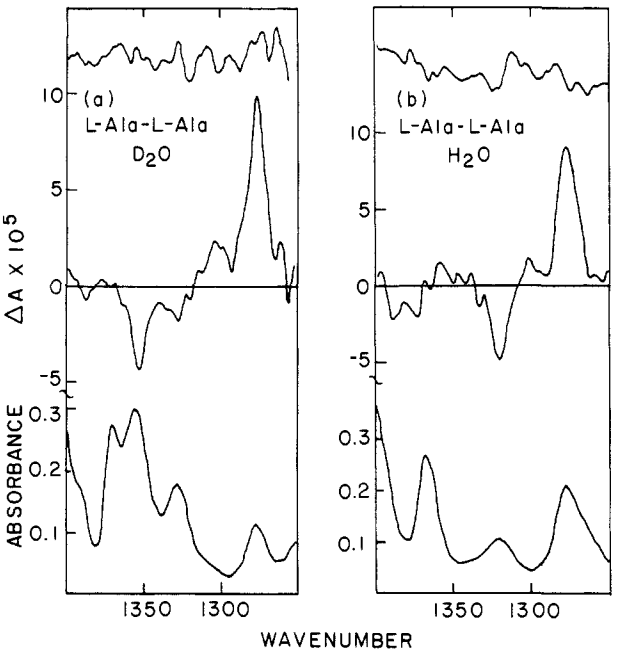

Figure 4. Absorption spectra, VCD spectra, and VCD noise estimates for the methine bending modes of L-alanyl-L-alanine: (a) 0.5 M in D_2O, ~60-μm path length; (b) 1.7 M in H_2O, ~30-μm path length.

Table I. Frequencies and Intensities of Absorption and VCD Spectra in the Methine Bending Region of Amino Acids and Dipeptides

molecule (solvent)	absorption		VCD	
	freq, cm^{-1}	absorbance	freq, cm^{-1}	$10^5\Delta A$
L-alanine	1338	0.33	1338	−9.2
(D_2O)	1294	0.15	1292	+8.8
L-alanine	1354	0.27	1354	−8.4
(H_2O)	1304	0.25	1304	+5.6
(*S*)-glycine-*C*-d_1	1323	0.26	1320	−2.5
(D_2O)	1268	0.10	1265	+4.2
L-proline	1331	0.10	1331	−2.4
(D_2O)	1310	0.19	1305	+2.4
	1275	0.05	1275	+5.2
glycyl-L-alanine	1330	0.08	1330	−3.8
(H_2O)	1286	0.26	1286	+5.0
L-alanylglycine	1315	0.16	no VCD features	
(H_2O)	1271	0.20	observed	
L-alanyl-L-alanine	1355	0.25	1355	−4.4
(D_2O)	1329	0.14	1330	−1.8
	1305	0.03	1305	+2.0
	1280	0.08	1278	+9.8
L-alanyl-L-alanine	1322	0.06	1320	−4.9
(H_2O)	1278	0.16	1278	+9.1

Both the absorption and VCD features for the methine modes in Gly-L-Ala are still observed at pH 13.[9] At pH 2, the absorption spectrum near 1300 cm^{-1} becomes considerably more complex, and no VCD signal is observed.

We have only reported the VCD spectra for a narrow region in the midinfrared for which consistent, reproducible patterns have been obtained. Our VCD spectra for L-alanine and L-Ala-L-Ala in H_2O are quite similar to those recently obtained by Diem and co-workers with a dispersive VCD instrument.[12,13] Although we have some evidence for weak positive VCD corresponding to the symmetric CO_2^- stretch for L-alanine in H_2O and weak negative VCD for the antisymmetric CO_2^- stretch in L-Ala-L-Ala in D_2O (in agreement with the observations reported by Diem et al.[12,13]), these data are less certain. No significant, reproducible VCD intensity is observed for other modes in the 1200–1700-cm^{-1} region. Relevant frequencies and intensities are presented in Table I.

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Discussion

The VCD spectra presented here identify a characteristic (−,+) VCD pattern for the two orthogonal methine bends in an L-amino acid or L-amino acid peptide residue, when the $C_\alpha H$ bond is adjacent to a CO_2^- group. These modes thus provide a probe of the configuration at the α-carbon. Further examination of our previous normal coordinate analyses[6a] for L-alanine indicates that the higher frequency bending motion is directed toward the carboxylate group and the lower frequency motion toward the amino group. The contributions from the motions of the other nuclei to these two modes are much smaller. For example, the methine bend directed toward the carboxylate group couples slightly with the CO_2^- symmetric stretch, and the methine mode directed toward the amino group has a small contribution from the antisymmetric CO_2^- stretch. The methyl symmetric deformation couples weakly with both modes.

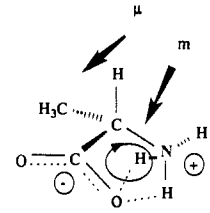
Since the overall (−,+) pattern is observed for L-Ala-L-Ala in both H_2O and D_2O solution, the VCD intensity in both solutions must arise from the methine bending motions alone, with little contribution from the peptide NH-bending "amide III" motion that has been proposed to contribute to the absorption features between 1250 and 1350 cm^{-1} in the H_2O solution.[16,17] The amide III′ in-plane ND peptide deformation occurs near 1000 cm^{-1} and does not mix with the methine bends in D_2O solution.[16,17] Furthermore, since the L-alanine residue exhibits methine bending VCD in Gly-L-Ala, but not in L-Ala-Gly, the observation of some VCD intensity for each of the four methine bending modes for N-deuterated L-Ala-L-Ala (in D_2O) is indicative of coupling among the modes. From the signs and intensities of the VCD bands, we conclude that the bend of the methine bond on the C-terminus residue that is directed toward the carboxylate has a large contribution to the mode at 1355 cm^{-1} and a smaller contribution at 1329 cm^{-1}. This assignment is the reverse of the earlier one[16,17] that assigned the methine bend at the N-terminus at 1355 cm^{-1}. The bend of the C-terminus methine that is directed toward the peptide ND group has a large contribution at 1276 cm^{-1} and a smaller contribution at 1305 cm^{-1}.

There are a number of similarities between the behavior of the VCD of the methine stretch[7-9] and bends in different environments. In both regions the anisotropy ratio, $\Delta A/A$, is larger than 2×10^{-4}. For both types of motion, VCD intensity is observed for the C-terminus, but not the N-terminus amino acid in a dipeptide, and for both, the VCD intensity in L-alanine is absent at low pH. However, in contrast to the $C_\alpha H$-stretching mode, the methine bends exhibit intense VCD in (*S*)-glycine-*C*-d_1, and at high pH no methine bending VCD is observed in L-alanine. The $C_\alpha H$ stretch in Gly-L-Ala exhibits strong VCD at both high and low pH, whereas the $C_\alpha H$ bending VCD is not observed at low pH.

We now consider mechanisms for generating the methine bending VCD intensity that can account for the above observations. Although a nearly balanced bisignate (+,−) or (−,+) VCD pattern is often indicative of VCD intensity arising from the coupled oscillator mechanism for two chirally oriented, nearly degenerate or degenerate oscillators,[18] these methine modes are each due to a single deformation only weakly mixed with displacements of other nuclei in the molecule. In numerous previous interpretations of VCD intensity that has large anisotropy ratio and is associated with localized vibrational motion in a molecule, the ring current mechanism has provided a consistent explanation for the intensity enhancement and the signs of the VCD signals.[1,7-11,19-24] Although we have primarily focused on stretching modes, we have also considered the VCD intensity generated by methylene scissors and wagging modes in the ethylenediamine rings.[21]

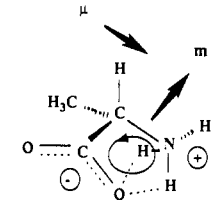
The major premise of the ring current mechanism is that nuclear oscillatory motion within or adjacent to a closed ring can generate oscillatory electron flow at constant electron density within the ring. Excitation of a vibrational mode involving such nuclear motion results in a large magnetic dipole transition moment due to the ring current.[10] Vibrationally generated ring currents require that nuclear momentum and electronic momentum cannot be independent, consistent with the non-Born–Oppenheimer nature of VCD.[25,26]

At neutral pH, the $C_\alpha H$ bond is adjacent to a ring closed by hydrogen bonding between the charged carboxylate and amino groups in an amino acid or between the carboxylate and peptide NH bond for the acid terminus residue in a dipeptide. As shown in **2** and **3** for L-alanine, the methine bend is proposed to generate



R < 0

2



R > 0

3

electron flow in the direction of the hydrogen motion, initiating positive ring current in the opposite direction.

For a methine bend, the positive direction of the electric dipole transition moment μ is in the direction of the hydrogen motion.[27] In both **2** and **3**, μ has a primary component out of the page. In **2**, methine bending motion directed toward the carboxylate carbon generates clockwise positive ring current that produces a magnetic dipole transition moment **m** with a primary component into the page. The resulting rotational strength *R* for this mode, the scalar product of μ and **m**, is negative. In **3**, the methine bend toward the amino group generates counterclockwise positive ring current, which results in **m** with a primary component out of the page and positive rotational strength. These predicted signs agree with the observed VCD spectra. We also proposed electron flow in the direction of the hydrogen motion in our earlier interpretation of methylene scissors and wagging VCD spectra.[21]

To account for the absence of enhanced $C_\alpha H$ stretching VCD in (*S*)-glycine-*C*-d_1, we proposed an equilibrium among carboxylate conformations with hydrogen bonding to the NH trans to $C_\alpha H$, the NH bond gauche to $C_\alpha H$, or to both in a bifurcated structure. In addition, as shown in **1**, a secondary $CO\cdots HC_\beta$ ring for the amion acids with β-CH bond can form when the carboxylate interacts with the gauche NH bond.[28] The magnetic dipole transition moment **m** due to current in the ring involving the trans NH bond has only a small positive overlap with the electric dipole transition moment for the methine stretch, but large overlaps with the electric dipole transition moments for both methine bends. In L-alanine and the other α-amino acids we have studied with the exception of (*S*)-glycine-*C*-d_1, the additional interaction between a $C_\beta H$ bond and the second carboxylate oxygen results in a current pathway generating a magnetic dipole transition moment having a large overlap with μ for the methine stretch and small overlaps with μ for either methine bend. The ring current mechanism thus accounts for the similarity in the VCD spectra of (*S*)-glycine-*C*-d_1 and the L-α-amino acids in the methine bending region, where conformations with trans NH to

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(28) Zuk, W. M.; Freedman, T. B.; Nafie, L. A., submitted for publication.

carboxylate hydrogen bonding make the largest contribution, and the contrasting VCD spectra in the methine stretching region, where conformations with gauche NH···CO and CO···HC_β rings are required for enhancement of the VCD intensity.

The pH dependence of the methine bending VCD spectra does not always parallel that of the methine stretches. These results can also be understood within the ring current framework. In L-alanine at low pH, protonation of the carboxylate group eliminates the interaction with the β-hydrogen, and the conformation with carboxyl to trans NH hydrogen bonding predominates, removing the source of the intensity enhancement of the methine stretch. The NH···OC interaction may also be weaker with a neutral carboxyl group. In the bending region at low pH, the symmetric carboxyl stretch (primarily single-bond stretch) occurs at 1262 cm^{-1}, and two distinct methine bending modes are no longer observed. Both the weakening of the hydrogen bond and the alteration of the nature of the normal modes serve to decrease the methine bending VCD intensity. Similarly, at high pH, isolated methine bending bands are not observed, possibly because of mixing with NH_2 motions, but a localized methine stretching mode and the β-hydrogen interaction are maintained. Thus enhanced methine stretching VCD, but not bending VCD, is observed at high pH.

In Gly-L-Ala, enhanced methine stretching VCD is observed at both low and high pH. To account for the low pH results, we postulated a conformation with a seven-membered ring closed by hydrogen bonding between the peptide C=O and carboxyl OH groups.[9] The methine stretch can generate current around this ring, enhancing the VCD intensity. However, compared to the conformation at neutral pH, the molecular environment for the methine bends is quite altered. Isolated methine bending modes are no longer observed, and no VCD intensity is generated. Since high pH alters the glycyl residue, but not the L-alanyl residue, enhanced VCD for both the methine stretch and bends is observed for Gly-L-Ala at high pH.

Finally, we note that Diem has recently carried out a study of the midinfrared VCD spectra of L-alanine in H_2O and D_2O, using a dispersive VCD instrument, that includes the 1600- and 1250–900-cm^{-1} regions.[29] In the frequency region (1250–1500 cm^{-1}) that our studies overlap, the VCD spectra are nearly identical in pattern.

Conclusions

The methine bending VCD spectra of L-amino acids and dipeptides can provide information on absolute configuration and local intramolecular association. The occurrence of the characteristic (−,+) VCD pattern depends on several factors: (1) the presence of two isolated, approximately orthogonal methine bending modes directed primarily toward the carboxylate and amino groups; (2) the presence of a CO_2^- group adjacent to the methine bond; (3) the presence of a ring closed by hydrogen bonding between a carboxylate oxygen and the peptide NH bond or the NH bond trans to the methine. Since the methine bends occur in the midinfrared region with numerous other types of deformation and stretching motion, in many environments the methine bends may mix extensively with other motions. In such a delocalized mode, the methine bends may not be directed toward the amino or carboxylate group, and thus may not generate any ring current. Alternatively, the net electric dipole transition moment for the mixed mode may not overlap favorably with any ring current magnetic moment generated by methine motion. For these reasons enhanced methine bending VCD may not be observed even in cases for which the methine stretch exhibits strong ring-current enhanced VCD intensity. However, since the methine bends, but not the methine stretches, can be observed in H_2O solution, VCD spectra in both vibrational regions provide valuable conformational and configurational probes for amino acids and dipeptides.

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Principal Component–Three Component Self-Modeling Analysis Applied to *trans*-1,2-Di(2-naphthyl)ethene Fluorescence[1]

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Abstract: Fluorescence spectra of *trans*-1,2-di(2-naphthyl)ethene, DNE, obtained under varying conditions of excitation wavelength and oxygen and carbon tetrachloride concentrations in methylcyclohexane were resolved into three distinct components by application of principal component analysis combined with a three-component self-modeling technique. Spectral matrices for O_2 and CCl_4 quenching, treated separately, yielded nearly identical pure component fluorescence spectra. The spectra were assigned to each of the three conformers of DNE by comparison with spectra of 3-methyl derivatives. Stern–Volmer quenching plots for the individual conformers were shown to be independent of excitation wavelength, indicating that each conformer has a single lifetime.

Upon excitation, flexible molecules containing conjugated double bonds undergo reversal of single/double bond order. Conformational changes which occur freely in the ground state are often too slow in singlet and triplet lowest excited states leading to noninterconverting excited molecules with different structures and properties. Since each ground-state equilibrium conformation has its own distinctive absorption spectrum, different excitation wavelengths, λ_{exc}, can lead to different compositions of excited

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