complicates the pattern originally present for alanine-d_0.

In Table V we present the potential energy distribution (PED) among the force constants for each of the vibrational modes of alanine-d_0. Only force constants participating by greater than 10% in the PED of a given mode are shown. From this we see hardly any mixing beyond the functional group vibration for all modes above ν^a_{CN}. Furthermore, the methyl rocking modes, the CO_2^- modes, and the torsion modes are clearly identified, leaving only the skeletal stretching and bending modes to remain extensively mixed. These results agree with our previously deduced assignments based on the isotopic Raman spectra. The mode assigned to ν^a_{CCN} is primarily C-N stretch but there is also a 5% PED contribution due to the $C-C_M$ stretch; the internal coordinate composition of this mode shows a $C-C_M$ stretching motion out of phase with, and 58% of the magnitude of, the C-N stretch. This mode also naturally couples with NH_3^+ bending motion. The $\nu_{C(CO_2)}$ mode has very little $C-C_M$ or C-N character but mixes with both methyl and amine bending modes. Finally the ν^s_{CCN} mode is predominantly $C-C_M$ stretch and methyl bend, but the C-N and $C-C(O_2)$ stretches contribute in phase with amplitudes of 58 and 29% of the $C-C_M$ motion. The ν^s_{CCN} mode can be regarded as a skeletal breathing mode which is consistent with its large effect on the polarizability and hence is striking Raman intensity. This simple pattern for the stretching modes is disrupted by methyl and amine deuteration when both their deformation and rocking modes mix extensively with the skeletal stretches and with each other. Deuteration of the α position also disrupts the alanine-d_0 pattern, but to a lesser extent, and leaves the strong breathing mode essentially intact.

As a final point we wish to stress that although the force field for alanine was initially assembled from force-field descriptions of local symmetric regions as described above, this process should be regarded only as an efficient *means* of obtaining a zeroth-order field sufficiently accurate that FPERT could be applied effectively. The final force field has the same degree of intramolecular interaction that would be obtained if a whole molecule approach to the zeroth-order force field were used, provided that the same set of nonzero force constants was employed in both approaches.

Conclusions

The vibrational assignments and the Urey-Bradley force field presented here meet our objective of providing a good description of the vibrational modes of deuterated alanine isotopomers in solution. This force field provides a good foundation within the harmonic approximation for the calculation of vibrational circular dichroism intensities. With the Raman spectra of deuterated isotopic species representing selective deuteration of the three hydrogen-bearing substituents of alanine, CH_3, C*H, and NH_3^+, we have been able to make unambiguous assignments for all vibrational regions, excepting the obviously mixed region from 800 to 1100 cm^{-1}. We can now proceed to VCD calculations in CH stretching region, where spectral data are available, without concern that our lower frequency motions are improperly described, thereby distorting our results. This is not an idle concern, since to a certain degree all internal coordinate motions contribute to each normal mode in an asymmetric molecule and VCD is more sensitive to distant motions within a molecule than ordinary infrared absorption. In addition, this new force field should also provide a good basis for the calculation of mid-infrared VCD for alanine and related molecules when experimental data in this region become available.

Acknowledgment. Support from grants from the National Institutes of Health (GM-23567) and the National Science Foundation is acknowledge by one of us (L.A.N.), and support from a Cottrell grant from the Research Corporation as well as a CUNY PSCBHE faculty research award is acknowledged by another of us (M.D). We also express appreciation to Mr. Joel M. Kupfer for the preparation of our initial samples of alanine-C^*-d_1.

Registry No. Ala-d_0, 56-41-7; Ala-C^*-d_1, 21386-65-2; Ala-C-d_3, 63546-27-0; Ala-C-d_4, 18806-29-6; Ala-N-d_3, 19470-97-4.

Vibrational Circular Dichroism in Amino Acids and Peptides. 5. Carbon-Hydrogen Stretching Vibrational Circular Dichroism and Fixed Partial Charge Calculations for Deuterated Isotopomers of Alanine

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Abstract: Vibrational circular dichroism (VCD) in the CH stretching region for alanine-N-d_3, alanine-C^*-d_1-N-d_3, and alanine-C-d_3-N-d_3 are presented and empirically analyzed. It is shown that selective deuteration in alanine reveals direct insights into the origin of VCD intensity and vibrational coupling between the methyl and methyne substituents. By employing spectral curve analysis to the alanine CH absorption spectra, the frequencies, bandwidths, and intensities of overlapping vibrational bands were determined revealing the presence of several combination and overtone bands in addition to the expected four fundamentals. Analysis of two of the overtones with Fermi resonance methods yielded a revised set of observed frequencies for the fundamentals which in turn led to a refined Urey-Bradley description of these modes. Finally VCD intensities were calculated with the fixed partial charge model and compared with experiment. Conclusion regarding the limitations of the FPC model for alanine VCD calculations are discussed.

Vibrational circular dichroism[2-6] (VCD) has emerged over the past half-decade as a new spectroscopic probe of structure, conformation, and configuration of small chiral molecules and helical polymers in solution. Parallel developments in the related field,

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Raman optical activity (ROA), have also taken place over this same period.[2,6,7] Together, these two techniques constitute the new field of vibrational optical activity which provides stereochemical sensitivity to the study of vibrations in chiral, low-symmetry molecules.

In this paper we apply the methods of isotopic substitution to further investigate VCD in alanine,[8] the simplest optically active amino acid. In particular, we will present and analyze the VCD of aqueous (D_2O) solutions of alanine-*N*-d_3, alanine-*C**-d_1-*N*-d_3, and alanine-*C*-d_3-*N*-d_3 in the CH stretching region. These spectra provide immediate empirical information regarding the origin of VCD in these molecules, the sensitivity of VCD to isotopic substitution, the vibrational coupling between the methyl and methyne substituents, and the positions of overlapping vibrational bands. Further analysis of the spectra using the methods of band contour analysis and taking into account Fermi resonance interactions of nearby overtone bands provide the basis for refinements of the Urey–Bradley force field in the CH stretching region. Using these refinements, VCD intensities were calculated within the fixed partial charge (FPC) model[9] and compared to experiment. Although the observed sign pattern for the VCD is closely predicted, the calculated intensities in most cases fall below experimental values, particularly for the CH deuterated isotopomers. This fundamental drawback to which the FPC model is susceptible is discussed together with those aspects of the FPC calculations that provide information regarding the structure, vibrational interactions, and local symmetry of alanine.

The basic aims of this paper are to demonstrate the effectiveness of selective isotopic substitution in vibrational optical activity, to provide an in-depth analysis of the vibrational spectroscopy of alanine in the CH stretching region, and to probe the utility and limitations of the FPC model of VCD intensities starting from the basis of a refined vibrational analysis as developed in both this paper and the previous one is this series.[10] In the following paper[11] we describe localized molecular orbital (LMO) model[12,13] calculations of these same spectra using the vibrational analysis established here.

Experimental Section

Both L and D enantiomers of alanine were obtained from Chemical Dynamics Corp.; L-alanine-*C*-d_3 was purchased from Merck Sharp and Dohme, and these samples were used without further purification. The α-deuterated analogue L-alanine-*C**-d_1 was synthesized according to the previously described procedure.[10] All samples used in the VCD measurements were repeatedly exchanged with D_2O to deuterate the amine function before sampling as a D_2O solution as described in earlier papers in this series.[8,10]

The VCD spectrometer used in this investigation has been discussed earlier;[8] however, some modifications have been made. These include the replacement of a ZnSe photoelastic modulator with a CaF_2 unit from Hinds-International, Inc., which operates at 57 kHz and the use of a 300-W xenon illuminator from the Eimac Division of Varian. These improvements have resulted in nearly an order of magnitude improvement in the signal-to-ratio of VCD spectra obtained in the CH stretching region.

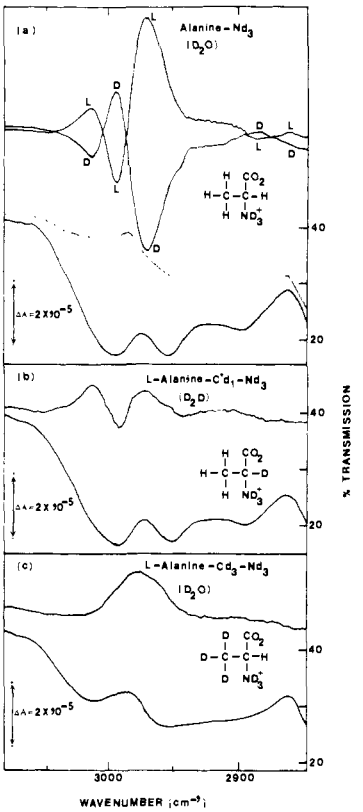

Figure 1. (a) VCD spectra of L- and D-alanine-*N*-d_3 in D_2O solution; below the VCD are two single-beam transmission curves, the upper representing the D_2O background (using the xenon lamp) and the lower representing the sample in D_2O solution. (b) The VCD of L-alanine-*C**-d_1-*N*-d_3 in D_2O above its single-beam transmission. (c) Similar spectra for L-alanine-*C*-d_3-*N*-d_3. Each VCD curve represents a 2-h scan using a 30-s lock-in time constant at a resolution of 12 cm^{-1}.

Calculations of VCD intensity using the FPC model and refinements of the Urey-Bradley force field were carried out at Syracuse University using a DEC-10 computer system as described in the previous paper.[10]

Absorbance spectra and their spectral contour analyses were obtained using a Nicolet 7199 Fourier transform infrared (FTIR) spectrometer system. Spectra were first obtained at 4-cm^{-1} resolution, with D_2O background absorption removed by spectral subtraction and spectral ratio, giving equivalent results. The resulting amino acid spectra were simulated using the Nicolet software program CAP (curve analysis program). The functional dependence of the component spectral bands could be varied continuously from pure Gaussian to pure Lorentzian form, and we used a value of 35% Gaussian–65% Lorentzian based on optimum fitting to isolated bands. The graphical comparison of the results of the FPC calculation of alanine-*N*-d_3 to experiment were also carried out using CAP with 100% Gaussian band character.

The optical purity of the L-alanine-*C**-d_1 which was synthesized for this work as described in the previous paper,[10] was checked by measuring its rotation, yielding $[\alpha]^{20}_D$ +15° (*c* 10, 1 N HCl) compared to the value of $[\alpha]^{20}_D$ +14° (*c* 6, 1 N HCl) for L-alanine-d_0, quoted by Aldrich Chemical Co. for our samples of L-alanine. Samples of L-alanine-*C*-d_3 and L-alanine-*C*-d_4 obtained from Merck Sharpe and Dohme, Ltd., Canada, were quoted as 98% pure in deuterium. From Raman studies in the previous paper, we estimate that our samples of alanine-*C**-d_1 contain approximately 10% contamination with alanine-d_0. All solutions of alanine isotopomers in D_2O were run at room temperature near saturation at a concentration of 1.50 M.

Spectral Results

In Figure 1a we present the VCD of L- and D-alanine-*N*-d_3 in D_2O solution from 3100 to 2850 cm^{-1}. In agreement with our earlier interpretation[8c] the positive–negative VCD couplet of L-alanine centered near 3000 cm^{-1} is assigned to the nearly degenerate antisymmetric methyl stretching vibrations. The large positive VCD feature near 2970 cm^{-1} is due to the lone methyne stretch, but contrary to earlier interpretation, negative rather than positive VCD is assigned to the symmetric methyl band at 2950 cm^{-1}. This negative contribution is seen as diminishing the low-

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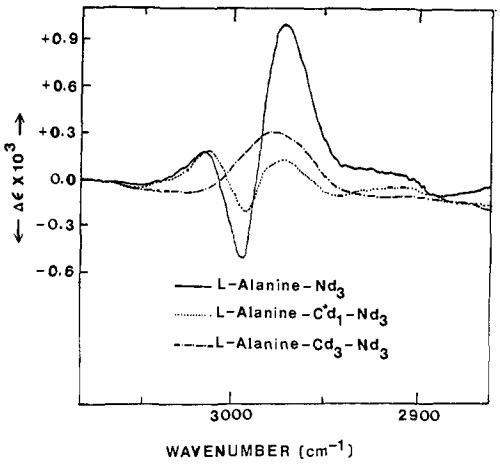

Figure 2. VCD spectra in D_2O of L-alanine-*N*-d_3 (—), L-alanine-*C**-d_1-*N*-d_3 (···), and L-alanine-Cd_3 (-·-·) plotted in units of $\Delta\epsilon$ (L cm^{-1} mol^{-1}) and overlayed for direct comparison.

Table I. Experimental VCD and Infrared Absorption Results[a] for Alanine and Deuterated Isotopomers

molecule	absorption peak frequency (cm^{-1})	peak intensity ϵ (L cm^{-1} mol^{-1})	VCD peak frequency (cm^{-1})	peak intensity $\Delta\epsilon \times 10^4$
Ala-*N*d_3	3006	7.0	3015	2.5
	2989	8.5	2995	−3.9
	2950	6.0	2970	11.1
	2893	2.0	2945	2.0
Ala-*C**d_1*N*d_3	3004	6.0	3013	2.5
	2987	8.0	2993	−1.7
	2948	4.5	2970	1.2
	2890	1.5	2945	−1.0
Ala-Cd_3*N*d_3	3012	2.5		
	2972	2.0	2974	3.5
	2949	1.5		
	2898	0.5		

[a] Apparent frequencies and Intensities are presented. These data represent only the net result of the overlapping contributions of Individual bands.

frequency side of the broad (vide infra) positive methyne VCD. A small negative VCD feature is noted at 2890 cm^{-1} and is attributed to the Fermi-enhanced overtone vibration at that same frequency.

The VCD and single beam transmission spectra of the isotopomers L-Ala-*C**-d_1-*N*-d_3 and L-Ala-*C*-d_3-*N*-d_3 are presented in Figures 1b and 1c, respectively. The contribution of the antisymmetric methyl stretching vibrations in the methyne-deuterated species is nearly unchanged from the parent spectrum, although the lower frequency negative component at 2987 cm^{-1} appears with diminished intensity. We attributed the positive feature at 2970 cm^{-1} to an approximately 10% impurity of non-methyne-deuterated compound (Ala-*N*-d_3) which is also apparent in the Ala-*C**-d_1 Raman spectrum presented in the previous paper.[10] Thus, the only remaining VCD features in this spectrum are the weak negative band at 2950 cm^{-1} associated with the symmetric methyl vibration and the slight negative dip near 2890 cm^{-1} for the overtone band. In Figure 1c the broad positive VCD feature extending from 3010 to 2940 cm^{-1} and centered at 2972 cm^{-1} is due to the lone C*–H stretching mode in the deuterated methyl isotopomer.

In Figure 2 we replot the VCD spectra as $\Delta\epsilon$ in units of (cm^{-1} L mol^{-1}) to provide a more direct comparison of these spectral results, and in Figure 3 we plot the corresponding FTIR absorption spectra as ϵ in the same units as the VCD results in Figure 2. In Table I we provide numerical estimates for the frequencies, intensities, and assignments of the observed VCD and absorption band maxima for the eight fundamental vibrations plus major overtone and combination bands.

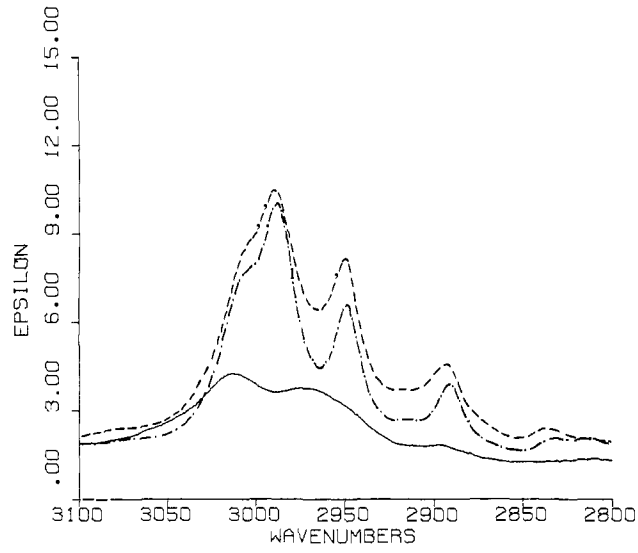

Figure 3. FTIR spectra in units of molar absorptivity, ϵ (L cm^{-1} mol^{-1}) of alanine-*N*-d_3 (- - -), alanine-*C**-d_1-*N*-d_3 (-·-), and alanine-*C*-d_3-*N*-d_3 (—) with D_2O background spectra digitally subtracted. The spectral resolution is 4 cm^{-1}.

The spectral results lead to a number of direct empirical conclusions. First, the signs and approximate intensities of the VCD bands do not change when the neighboring CH bearing substituent is deuterated. Thus, the VCD bands of the methyl and methyne moieties are reasonably independent. Nevertheless, considerable VCD enhancement (approximate ×3 near 2970 cm^{-1}) occurs in Ala-*N*-d_3 when these two groups couple vibrationally. Similar enhancements are not present in the absorption spectra which appear to be more additive in character and are not as sensitive to vibrational coupling.

The empirical analysis can be carried still further by first considering the correlation of the VCD of alanine-*C**-d_1-*N*-d_3 and alanine-*C*-d_3-*N*-d_3 to previous VCD observations and calculations. In L-alanine-*C**-d_1-*N*-d_3 the observed sigmoidal couplet in the antisymmetric methyl stretching modes can be interpreted in terms of the perturbed degenerate mode (PDM) approach advanced originally by Barron[14] for ROA and extended recently to VCD in our laboratory[15] through a series of model calculations. In L-alanine-*C*-d_3-*N*-d_3 the spectrum of the methyne CH stretch provides unequivocal evidence for a positive VCD effect from this isolated vibration. Empirically this feature can be correlated to the positive VCD observed in *l*-tartaric-*O*-d_2 acid-*O*-d_2 (DOOC-CH(OD)–CH(OD)COOD)[16] having the same absolute configuration as alanine, as well as to L-lactic[17] acid and a number of other L-amino acids, such as serine,[7c] threonine,[17] and valine,[17] which all show positive VCD in the region associated with the methyne stretching frequency (2970 cm^{-1} in the amino acids and ~2930 cm^{-1} in lactic acid and tartaric acid). While exceptions to this correlation may yet appear, there is a growing body of experimetnal evidence suggesting that the α-methyne CH stretching vibration in amino acids and hydroxy acids exhibits positive circular dichroism.

Spectral Contour Analysis

In order to obtain accurate vibrational frequencies, bandwidths, and intensities for the absorption bands in the CH stretching region of alanine-*N*-d_3 and its deuterated isotopomers, we carried out detailed spectral contour analyses of their FTIR spectra. The results of these spectral decompositions are given in Figure 4 where the total simulated curves (dashed) are also compared with the

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Table II. Results of Spectral Contour Analysis of the Absorbance Spectra of Alanine and Deuterated Isotopomers

molecule	frequency (cm^{-1})	bandwidth FWHH (cm^{-1})	integrated intensity (%)	mode assignment
Ala-Nd_3	3009.0	32.9	24.9	$\nu^a CH_3$
	2988.7	25.3	25.3	$\nu^a CH_3$
	2970.2	50.6	19.3	νC^*H
	2949.4	19.2	14.9	$\nu^s CH_3$
	2915.3	31.4	4.4	$2 \times \delta^a CH_3$
	2892.4	22.8	8.8	$2 \times \delta^a CH_3$
Ala-$C^*d_1Nd_3$	3007.9	30.3	30.4	$\nu^a CH_3$
	2986.6	23.3	37.4	$\nu^a CH_3$
	2948.1	20.0	20.0	$\nu^s CH_3$
	2912.2	19.4	1.7	$2 \times \delta^a CH_3$
	2890.6	18.5	8.9	$2 \times \delta^a CH_3$
Ala-Cd_3Nd_3	3056.1	48.4	5.1	
	3014.6	46.9	45.0	$\nu^s CO_2^- + \nu^a CO_2^-$ or $\zeta CD_3 + \nu^a CD_3$
	2967.6	53.6	41.0	νC^*H
	2943.7	40.8	4.3	$\delta C^*H + \nu^a CO_2$
	2894.1	27.6	3.7	$\delta C^*H + \nu^a CO_2$
Ala-Cd_4Nd_3	3003.0	49.4	100.0	$\nu^s CO_2^- - \nu^a CO_2^-$ or $\zeta CD_3 + \nu^a CD_3$

experimental curves. The quantitative data pertaining to the frequencies, full bandwidths at half-height, and percent of total integrated intensity are given in Table II. The total simulated curves have root-mean-square errors of less than 1.5% for alanine-*N*-d_3 and alanine-*C*-d_1-*N*-d_3 and less than 2.0% for alanine-*C*-d_3-*N*-d_3. Owing to internal laser calibration of the FTIR spectrometer, the observed frequencies are accurate to much less than a wavenumber, limited only by our ability to judge the peak position of the band with a CRT marker. The intensity and bandwidth of the spectral decompositions are subject to somewhat larger error, but the results reported here should be accurate to within 5%.

Several important points regarding these absorption spectra were learned from the curve analyses. First, the absorption spectrum of alanine-*N*-d_3 can be obtained by starting with the results for alanine-*C**-d_1-*N*-d_3 and adding a broad band at 2970 cm^{-1} corresponding to the band observed at that location in alanine-*C*-d_3-*N*-d_3. Second, a weak band near 2915 cm^{-1} is required to produce the spectral plateau observed in this region for alanine-*N*-d_3 and one at 2912 cm^{-1} in alanine-*C**-d_1-*N*-d_3, where it is nearly resolved. Third, the bands in alanine-*C*-d_3-*N*-d_3 at 3015, 2944, and 2894 cm^{-1} are not due to the same vibrational modes as those which are prominent near the same frequencies in alanine-*N*-d_3 and alanine-*C**-d_1-*N*-d_3. The primary evidence for this is the large bandwidth of these bands in alanine-*C*-d_3-*N*-d_3, but in addition the frequencies are higher and the intensities are lower in the CD_3 species. This does not rule out the presence of these bands as weak underlying components in the CH_3-bearing isotopomers, particularly when it is recognized that the intensity scale of the alanine-*C*-d_3-*N*-d_3 spectrum is enhanced in Figure 4 relative to the two spectra above it. We have assigned the bands at 3015, 2944, and 2894 to combinations due to CO_2^- stretching and C*H bending modes based on frequencies reported in the previous paper.[10] The band at 3015 could also be a combination band involving the CD_3 rocking mode 758 cm^{-1} and the antisymmetric CD_3 stretch at 2551 cm^{-1}. We also note that the spectrum of alanine-*C*-d_4-*N*-d_3 in the CH-stretching region shows one band at 3003 cm^{-1}, which we tentatively assign to the $\nu^a CO_2^- + \nu^s CO_2^-$ combination band but which also may be a CD_3 rocking–stretching combination band; the spectral information for this band is listed in Table II. Finally, these spectral decompositions clearly establish a splitting between the components of the degenerate methyl stretching modes of approximately 20 cm^{-1}, in agreement with the Raman spectra presented in the previous paper[10] which showed partially resolved peaks having 10- to 15-cm^{-1} separations.

The fact that the methyne stretching vibration exhibits a full width of approximately 50 cm^{-1} in the VCD spectrum Ala-*C*-d_3-*N*-d_3 in Figures 1 and 2 and in its curve-resolved absorption spectra of Ala-*C*-d_3-*N*-d_3 and Ala-*N*-d_3 in Figure 3 (and Table II) has direct consequences on the interpretation of the VCD spectrum of Ala-*N*-d_3 described in the previous section. Namely, the width of this band together with its large positive magnitude forces one to conclude that the symmetric methyl VCD contribution must be negative to explain the rapid drop in VCD intensity between 2960 and 2940 cm^{-1}. If positive or zero VCD intensity were present in the symmetric methyl stretch the decrease in VCD intensity in this region would be much more gradual. This point can be further appreciated when simulations of VCD spectra are presented later in this paper and in the one to follow.[11]

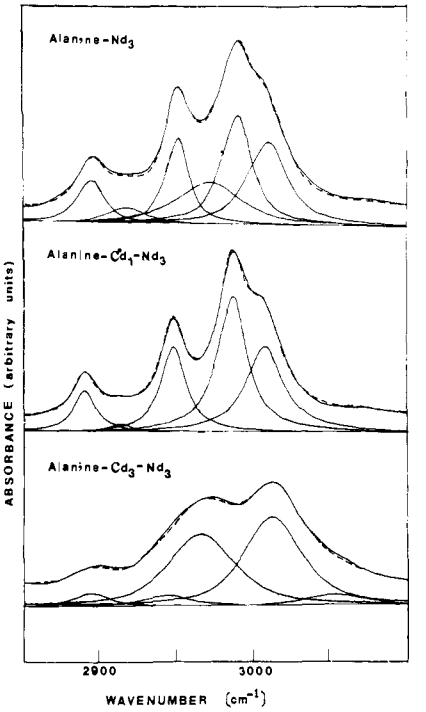

Figure 4. Spectral contour analysis of the FTIR absorbance spectra (—) for alanine-*N*-d_3, alanine-*C**-d_1-*N*-d_3, and alanine-*C*-d_3-*N*-d_3. The dashed curves (---) represent the spectral simulations obtained by adding the individual component bands shown just below in each case. The frequency, intensity, and bandwidth of each component were adjusted in obtaining the simulations.

Fermi Resonance Analysis

In this section we consider the origin of the bands near 2890 and 2915 cm^{-1} in alanine-*N*-d_3 and alanine-*C**-d_1-*N*-d_3. Methyl compounds are known to exhibit Fermi resonance between the symmetric methyl stretching fundamental and the overtones of the methyl deformation modes.[18] From the Raman studies in the previous paper[10] we assign a band in alanine-*N*-d_3 occurring at 1461 cm^{-1} to the antisymmetric methyl deformations. The FTIR spectra of alanine-*N*-d_3 and alanine-*C**-d_1-*N*-d_3 show vibrational bands at 1465 and 1461 cm^{-1}, respectively. The overtones of the antisymmetric methyl deformations would thus be expected to occur quite near the CH stretching fundamentals. The infrared intensity and negative VCD intensity near 2890 cm^{-1} can be explained on the basis of Fermi resonance which mixes these overtones with the symmetric methyl stretching fundamental.

The are several ways to estimate the extent of the resonance interaction and the frequencies of the unperturbed modes. If we consider the deformation modes, denoted Q_a and Q_b, to transform as x and y, respectively, in the E representation for local C_{3v} symmetry, there will be three degenerate overtones $Q_a^2 + Q_b^2$, $(Q_a^2 - Q_b^2, Q_aQ_b)$ transforming as A and E, respectively. Only the $Q_a^2 + Q_b^2$ species and the symmetric methyl stretching fundamentals, Q_s, have the same symmetry and can undergo Fermi resonance. However, the observed splitting of the antisymmetric

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metyl stretching vibration indicates that the local symmetry of the methyl group is, in fact, not C_{3v}. If we lower the local symmetry slightly to C_s, the species of the overtones $Q_a^2 + Q_b^2$, $Q_a^2 - Q_b^2$, Q_aQ_b become A′, A′, and A″, respectively. The Q_aQ_b mode, a combination band, cannot undergo resonance; however, we now have a Fermi resonance triad among the modes Q_s, $Q_a^2 + Q_b^2$, and $Q_a^2 - Q_b^2$. The relationship between the frequencies of the unperturbed levels, represented by wave functions ψ_s^0, $\psi^0_{a^2+b^2}$, and $\psi^0_{a^2-b^2}$, and the perturbed (observed) frequencies is given by the secular equation (eq 1), where $W_{a^2\pm b^2,s}$ are the Fermi

$$\begin{vmatrix} E^0_s - E & W_{a^2+b^2,s} & W_{a^2-b^2,s} \\ W_{a^2+b^2,s} & E^0_{a^2+b^2} - E & 0 \\ W_{a^2-b^2,s} & 0 & E^0_{a^2-b^2} - E \end{vmatrix} = 0 \qquad (1)$$

resonance interaction parameters,[18,19] and we assume no interaction between the two overtones.

Since no splitting is resolved for the fundamentals of antisymmetric deformations, we assume that the local methyl environment is not greatly distorted from C_{3v} symmetry and thus $W_{a^2+b^2,s} \gg W_{a^2-b^2,s} \approx 0$. In this case there is a strong interaction between Q_s and $Q_a + Q_b^2$ and a weaker, secondary interaction with $Q_a^2 - Q_b^2$. Therefore we have $E_{a^2-b^2} \approx E^0_{a^2-b^2} \approx E^0_{a^2+b^2}$.

Assinging the weak bands at 2915 cm^{-1} (alanine-N-d_3) and 2912 cm^{-1} (alanine-C^*-d_1-N-d_3) to the only slightly perturbed overtone, and the bands at 2949 and 2892 cm^{-1} in alanine-N-d_3 and at 2948 and 2891 cm^{-1} in alanine-C^*-d_1-N-d_3 to the strongly perturbed levels, we estimate the frequency of the unperturbed symmetric methyl stretching fundamental to lie near 2927 cm^{-1} in both isotopomers, giving a Fermi shift of ~22 cm^{-1}.

As a second method of approximating the extent of Fermi interactions, we consider the observed intensities of the perturbed modes. Ignoring the weaker Fermi interaction, we can express the perturbed wave functions as

$$\psi^+ = a\psi^0_s + b\psi^0_{a^2+b^2}$$

$$\psi^- = b\psi^0_s - a\psi^0_{a^2+b^2}$$

where the coefficients are normalized as $a^2 + b^2 = 1$. If it is assumed that the unperturbed intensity of the overtone is zero, we have for the ratio of observed intensities[18]

$$I^+/I^- = a^2/b^2$$

The ratio of the areas of the bands at 2948 and 2890 cm^{-1} in the spectral decomposition for alanine-C^*-d_1-N-d_3 is 2.25. We base the analysis on this isotopomer since there is less overlap of the two bands with the other fundamentals. Since

$$a^2 = (\Delta + \delta)/2\Delta$$

$$b^2 = (\Delta - \delta)/2\Delta$$

where Δ is the separation of the perturbed levels and δ the separation of unperturbed levels,[18,19] we can calculate the Fermi resonance shift, $(\Delta - \delta)/2 = 17.7$ cm^{-1}, and unperturbed frequenciese, 2930 and 2908 cm^{-1}. A similar analysis, based on the relative intensities of the Raman bands observed at 2947 and 2888 cm^{-1} in alanine-C^*-d,[10] yield a Fermi shift of 14.7 cm^{-1} and unperturbed frequencies 2932 and 2903 cm^{-1}.

These Fermi shifts are in the range calculated for other methyl compounds.[18] The range of unperturbed frequencies calculated for the deformation overtone are consistent with the anharmonicity parameters suggested for a series of methyl compounds with C_{3v} or C_s methyl site symmetry.[20] Based on this analysis, in our subsequent refinement of the harmonic vibrational force field and interpretation of the infrared absorption intensities and VCD intensities, we have chosen 2930 cm^{-1} as the approximate unperturbed vibrational frequency of the symmetric methyl stretching mode. Approximately 30% of the unperturbed infrared intensity and (negative) VCD intensity of this vibration lie near 2890 cm^{-1} in the observed (perturbed) spectra. Finally, our assignment of the weak band near 2915 cm^{-1} to the second component of the bending overtone is consistent with a local methyl environment slightly distorted from C_{3v} symmetry.

According to this analysis, results of spectral calculations are improved by including the effects of Fermi resonance interactions which provide improved (unperturbed) frequencies for the symmetric stretching fundamental and allows the strongly mixing overtone to borrow intensity from the fundamental according to the factors a^2 (fundamental) and b^2 (overtone). The fact that negative VCD is observed in the strongly mixing overtone further reinforces the assignment of negative VCD intensity to the symmetric methyl stretching fundamental.

Table III. Altered Urey-Bradley Force Constants[a] for Refined Vibrational Analysis of CH Stretching Vibrations of Alanine and Deuterated Isotopomers

force constant[b]	original values[a]	Fermi-adjusted values	split methyl and Fermi-adjusted values
C*-H	4.101	4.110	4.120
C_M-H (11)	4.553	4.565	4.520
C_M-H (12, 13)	4.553	4.565	4.596
H-C_M-H	0.487	0.499	0.499
H···C*···C_M	0.490	0.490	0.496
C*···C_M···H	0.432	0.435	0.415
H···C_M···H	0.100	0.073	0.074

[a] See preceding paper[10] for remaining force constants which were not adjusted for the present vibrational refinements.

Fixed Partial Charge Calculations

Having determined more accurate experimental frequencies for a harmonic force-field description of the alanine CH stretching region, we proceed first to refine the general force field described in the previous paper[10] and then to the calculation of VCD and infrared absorption intensities using the fixed partial charge model. As a *first step* in the force-field refinement we adjusted the force constants in the methyl and methyne moieties to bring the methyne stretching mode close to 2970 cm^{-1}, to move the Fermi-shifted symmetric methyl stretch to its unshifted position near 2930 cm^{-1}, and to maintain the antisymmetric methyl stretches near 3000 cm^{-1}, midway between the location of the two components resolved by the curve analysis. A listing of the adjusted values of the force constants affected by this refinement is given in Table III together with their original values as determined in the previous paper.[10]

Using this force field we next calculated the dipole strengths and rotational strengths of the eight fundamental stretching modes in L-alanine-N-d_3, -C^*-d_1-N-d_3, and -C-d_3-N-d_3. The results of these calculations are given in Table IV under FPC calculation I. For comparison we also list experimental estimates for the rotational and dipole strengths for the transitions in this region. We employed the results of the curve analysis to obtain ϵ-peak values of the overlapping absorption bands and combined them with the bandwidths (see Figure 4 and Table II) to arrive at relative areas and dipole strengths. The experimental VCD curves were analyzed in the same manner (without changing the bandwidths from the dipole strength determination) to obtain estimates for the experimental rotational strength.

The values of the fixed partial charges used in these calculations are listed in Table V, with the atomic numbering given in the stereoprojection of L-alanine shown in Figure 5. The FPC values originated from a GAMESS ab initio calculation of net atomic charge population for alanine.[21] The values were then averaged for equivalent nuclei to arrive at the charge set listed in the table. However, these charges are also very close to an earlier charge

(19) Herzberg, G. "Infrared and Raman Spectra of Polyatomic Molecules"; Van Nostrand: New York, 1945; pp 215–217.

(20) Lavalley, J. C.; Sheppard, N. *Spectrochim. Acta, Part A* **1972**, *28*, 2091.

(21) These calculations were carried out by one of use (P.L.P.) at the NRCC/QCPE Summer School in Bloomington, Ind. in Aug 1979. GAMESS is an acronym for Generalized Atomic and Molecular Structure System and is based on Gaussian-type basis functions.

Table IV. Comparison of Experimental to Calculated Rotational and Dipole Strengths for the CH Vibrations in L-Alanine and Deuterated Isotopomers

	experiment[a]			FPC calculation I[b]			FPC calculation II[c]		
molecule	frequency (cm^{-1})	$R \times 10^{44}$ ($esu^2\ cm^2$)	$D \times 10^{39}$ ($esu^2\ cm^2$)	frequency (cm^{-1})	$R \times 10^{44}$ ($esu^2\ cm^2$)	$D \times 10^{39}$ ($esu^2\ cm^2$)	frequency (cm^{-1})	$R \times 10^{44}$ ($esu^2\ cm^2$)	$D \times 10^{39}$ ($esu^2\ cm^2$)
Ala-*N*-d_3	3009	0.54	0.57	3000	0.472	1.52	3007	0.545	1.42
	2989	−2.43	0.58	2999	−0.705	1.28	2993	−1.709	1.34
	2970	5.58	0.45	2972	0.318	0.46	2970	1.588	0.45
	2949	−0.75	0.34	2950	−0.018[c]	0.25[c]	2950	−0.258[d]	0.29[d]
	2915		0.10						
	2892	−0.40	0.20	2892	−0.008	0.10	2892	−0.107	0.12
Ala-*C**-d_1-*N*-d_3	3008	0.70	0.53	3000	0.0067	1.25	3007	−0.0653	1.25
	2987	−0.53	0.67	2999	−0.0011	1.25	2993	0.0727	1.22
	2948	−0.31	0.36	2951	0.0002[c]	0.29[c]	2951	0.0011[d]	0.31[d]
	2912		0.03						
	2891	−0.08	0.016	2893	0.0001	0.12	2893	0.0005	0.13
Ala-*C*-d_3-*N*-d_3	3056		0.05						
	3015		0.39						
	2967	2.11	0.37	2972	0.0351	0.73	2970	0.0345	0.73
	2943		0.04						
	2894		0.02						

[a] Estimates of integrated band intensities based on experimentally determined band frequencies and half-widths. [b] Using the refined Fermi-adjusted force constants listed in Table III. [c] Using the refined split methyl and Fermi-adjusted force constants listed in Table III. [d] 29.3% of the intensity calculated for this vibration has been transferred to the overtone band at 2892 cm^{-1} in Ala-*N*d_3 and 2893 in Ala-*C**d_1*N*d_3.

Table V. Fixed Partial Charges for Alanine and Deuterated Isotomers

no.	atom	partial charge
1	O	−0.468
2	C	0.228
3	C*	0.025
4	N	−0.339
5	H	0.301
6	O	−0.468
7	H	0.076
8	C	−0.186
9	H	0.300
10	H	0.300
11	H	0.074
12	H	0.074
13	H	0.074

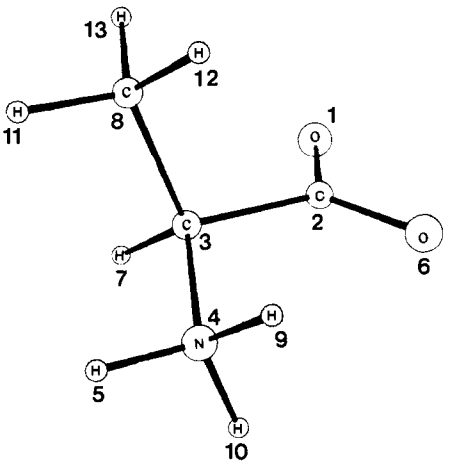

Figure 5. Stereoprojection of L-alanine showing the atomic numbering system used in Table V for the assignment of fixed partial charges and the identification of the unique methyl hydrogen (11) in the split methyl force field.

set employed for alanine in our laboratory[22] obtained from considerations of bond dipole moments.

In order to describe the observed frequency splitting of the antisymmetric methyl stretching modes, we *further* refined the methyl and methyne force constants as given in Table III. This was achieved by allowing the C_M–H stretching force constant for the methyl hydrogen *trans* to the carboxylic group (H_{11}, see Figure 5) to assume a value different from the other two C_M–H stretching force constants (H_{12}, H_{13}). While this choice of hydrogen (H_{11}) is not unique, the results of VCD calculations, particularly the LMO calculations described in the following paper, yielded much more favorable sign patterns for the eight vibrational modes when compared with experiment than did the other two choices. This choice of unique hydrogen also produces the same nuclear displacement pattern for the two antisymmetric modes that naturally arises in alanine-*N*-d_3 using three equal force constants. So in effect we are further encouraging alanine-*N*-d_3 to vibrate along the same trajectories as it did before this refinement. For alanine-*C**-d_1-*N*-d_3, the methyl motions are heavily mixed from the classical degenerate patterns of (2, -1, -1) and (0, 1, −1) when equal force constants are employed. For the choice of H_{11} as unique, the methyl motions of alanine-*C**-d_1-*N*-d_3 fall in line with those of alanine-*N*-d_3.

The results of the first FPC calculation in Table IV shows reasonably close agreement of calculated to observed dipole strength and close agreement in the sign of the calculated rotational strengths, the only exception being the symmetric methyl stretch in alanine-*C**-d_1-*N*-d_3. For alanine-*N*-d_3 the VCD intensities for the antisymmetric methyl modes are approximately half of the observed value; however, the methyne and symmetric methyl modes have calculated values that are an order of magnitude below experiment, particularly since the observed intensity in the 2892-cm^{-1} band has been borrowed from the symmetric methyl mode. In the deuterated species these same four vibrations are calculated to be much weaker than in the parent molecule, alanine-*N*-d_3. The reasons for this drastic loss in intensity in our calculations is due to the absence of significant vibrational coupling between the methyl and the methyne moieties in the CH deuterated molecules. Although some loss of intensity is experimentally observed, these vibrations maintain approximately 50% of their original VCD intensity. At this point we are not in a position to conclusively say whether the poor showing of the FPC calculation for the deuterated molecules (as well as for the methyne and symmetric methyl modes in alaine-*N*-d_3) is due to an inadequate force-field description or a deficiency in the FPC model itself, namely, its insensitivity to electronic motion at the vibrational frequency which differs from the nuclear motion. This point is considered in detail in the following paper.[11]

In the second FPC calculation, a distinct improvement in the rotational strengths for alanine-*N*-d_3 is achieved. The antisymmetric methyl vibrations agree closely and the methyne and

(22) Diem, M.; Nafie, L. A., unpublished results.

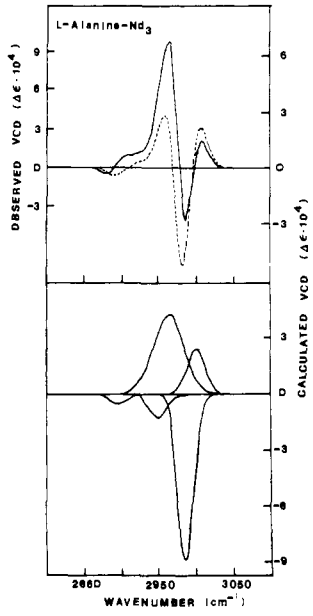

Figure 6. Spectral simulation (---) of the VCD spectrum of Ala-N-d_3 in the CH stretching region based on the intensities of the FPC calculation II in Table IV. The frequency positions and widths of the individual Gaussian bands for the contributing vibrational modes (shown below) are based on the spectral curve resolutions in Figure 4 and Table II. The experimental VCD spectrum(—) plotted on a different intensity scale is overlayed for comparison.

symmetric methyl vibrations are only a factor of 2 to 3 too small. The deuterated species also improve in intensity and are now only about an order of magnitude too small, except for the symmetric methyl mode in analine-C^*-d_1-N-d_3 which is more than two orders of magnitude below experiment. The problem of the sign reversal in the antisymmetric modes of this molecule is an additional point of disagreement with experiment. The overall improvement in rotational strength intensities indicates that a less symmetric description of the methyl group force field moves the calculations in the right direction compared to experiment. This change in the force field is also in agreement with conclusions reached in the Fermi resonance analysis section where the methyl group is best viewed in an environment which is slightly, but definitely, perturbed away from C_{3v} symmetry.

Throughout our studies of FPC calculations in these molecules were have found that the calculated VCD intensities depend more on the vibrational force field and, in particular, the internal coordinate composition of the normal modes than upon the variations in fixed partial charges. We have also found that shifting the frequency of a band without drastically altering its vibrational character usually has only a minor effect on its calculated rotational strength. For instance, the FPC calculations using the original force constants in Table III yield nearly the same results as those obtained for the Fermi resonance calculation listed under calculation I in Table II. Consequently, the results of the FPC calculations that we give here are actually representative of a wider range and variety of calculations which yield similar results.

In order to provide a more visual comparison of the FPC results to experiment, we present in Figure 6 a simulation of the VCD spectrum of Ala-N-d_3 using the FPC intensities determined in calculation II described above and the band positions and widths determined from the spectral contour analysis also described above. The simulated curve can be compared in shape to the experimental curve plotted on a different scale; the individual band components are displayed below on a separate baseline. These results show that the calculated negative antisymmetric methyl mode at 2989 cm^{-1} is too intense and that the methyne stretch at 2970 cm^{-1} is too weak. The calculated VCD spectra of the deuterated species Ala-$C^*d_1Nd_3$ and Ala-Cd_3Nd_3 are too small to be seen on the same scale (which is optimum for Ala-Nd_3) and are therefore not plotted. This should further emphasize the fact that although deuteration has no effect on fixed partial charge values (intensity scale), the FPC–VCD intensities for alanine decrease dramatically upon CH deuteration.

Conclusions

As the result of this study, we have now established definitive assignments of VCD and absorption features in alanine-N-d_3 and its deuterated isotopomers. In addition, through the methods of spectral curve analysis and considerations of Fermi resonance interactions, we have obtained a more accurate and detailed description of the vibrational force field and the normal mode displacements in the CH stretching region of these molecules. The results of FPC–VCD calculations are favorable for alanine-N-d_3 but not for the deuterated species, suggesting that the FPC model does well when vibrational coupling is important and is properly described. However, when vibrational coupling is greatly reduced by deuterium substitution, the FPC model is not capable of describing the electronic vibrational coupling throughout the molecule which is important in explaining the experimental intensity.

Acknowledgment. Support for this research is acknowledged from grants from the National Science Foundation (CHE-8005227) and the National Institutes of Health (GM-23567) awarded to L.A.N and support from a Cottrell grant from the Research Corporation and a CUNY PBSBHE faculty research award (M.D.).

Registry No. L-Alanine-C^*-d_1, 21386-65-2; L-alanine-d_0, 56-41-7; L-alanine-C-d_3, 63546-27-0; L-alanine-C-d_4, 18806-29-6.