first-order perturbation expression for the CD of electric dipole allowed transitions is readily verified using, for example, a simple vibronic coupling model. The procedure is analogous to that discussed elsewhere for vibronic contributions to DICD (dispersion-induced CD).[12] As the induced moment is itself first order in vibronic coupling, the CD strength is effectively third order overall (substitution of the square of the induced moment into a first-order CD term), and will thus be roughly about three orders of magnitude smaller than the chelate CD.

This contribution will also arise for magnetic dipole allowed d-d transitions, but it is then swamped by an effectively second-order term involving the magnetic transition moment.[3]

Applications

It follows from the previous theory that the signs of the CD of the chelate bands, and hence that of the CT bands, are direct indicators of the absolute configuration of the complex, provided that the polarizations of the bands are determined by some independent technique. The lower intensity and energy of the CT transitions make polarization data as determined from oriented crystal spectra more accessible than direct studies of the chelate bands. In addition, with the increasing development of linear dichroism (LD) techniques,[11] CT spectra may be assigned for certain tris bidentates (e.g., using flow orienting techniques for the complex bound to DNA). Such an approach in which all the quantities appearing in the CD expression may be determined by independent techniques should give considerably more reliable stereochemical correlations than alternative approaches involving, for example, energy splitting calculations of chelate bands[4] for systems exhibiting clear CT spectra. Conversely, of course, if the absolute configuration is known, the CT CD may be used to assign the CT transitions.

Similar applications may be envisaged for the CD of the magnetic dipole forbidden transitions, especially considering that polarization data for such transitions from solid-state studies are generally easily accessible. It is somewhat paradoxical that these transitions may ultimately give a more direct approach to assignment of absolute configuration than the intensively studied magnetic dipole allowed transitions. Of broader spectroscopic interest, however, is that the polarization and hence symmetry assignment of magnetic dipole forbidden transitions for complexes in which the absolute configuration is known follow readily from the sign of the CD.

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Vibrational Circular Dichroism in Amino Acids and Peptides.[1] 3. Solution- and Solid-Phase Spectra of Alanine and Serine

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Abstract: Vibrational circular dichroism (VCD) in solution and solid phases is reported for alanine and serine in vibrational modes involving hydrogen and deuterium stretching motions. The quality of the solution-phase VCD spectra was significantly enhanced by on-line computer averaging techniques. Solid-phase spectra were obtained as mulls in halocarbon oil and this method promises to be a useful new sampling technique in VCD spectroscopy. VCD signals in the solid phase are considerably larger than the solution spectra for the same molecules. The increased magnitude is attributed to such effects as conformational uniformity, local ordering, and crystal lattice interactions between molecules. A new vibrational assignment of solution-phase alanine in the carbon–hydrogen stretching region is presented as determined from the solid-phase Raman spectra of alanine-d_0 and alanine-C^*-d_1. The basic features of the observed VCD spectra of alanine and serine are discussed in terms of their constituent normal modes and the chiral perturbation of locally symmetric groups.

I. Introduction

Over the past few years, vibrational circular dichroism (VCD) and Raman optical activity (ROA) have been observed in a variety of optically active compounds,[3] and recent results of these studies suggest a unique sensitivity of these techniques to the elucidation of molecular stereochemistry.[4-6] In order to obtain results which can be interpreted in terms of empirical correlations, and in order to test existing models of the origin of VCD intensities, we have studied the vibrational circular dichroism of a number of amino acids and simple peptides. We have already reported results of our studies on alanine[6a] and some alanylpeptides;[6d] this series of molecules was of particular interest for the following reasons. First, alanine is one of the smallest molecules for which VCD has been observed and the small size allows a reasonably detailed vibrational analysis which in turn facilitates the interpretation of the observed spectral features. Second, this series allows comparison of the spectra of a parent molecule with the spectra of a number of derivatives,[6d] leading to the conclusion that VCD is, indeed, very sensitive to small changes in molecular structure and stereochemistry.

We have since obtained VCD spectra of a number of amino acids, among them valine, proline, arginine, and serine. We

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have found that in all of these compounds the observed VCD signals are considerably smaller than in alanine. For some amino acids, low solubility has prevented us from obtaining reliable VCD of aqueous solutions; for others, the possibility for a detailed interpretation of the VCD was reduced by the size of the molecules under investigation. In this publication, we report results of our studies on serine, which is very soluble in H_2O (or D_2O) and which exhibits an only slightly more complicated molecular structure than alanine. To further increase spectral quality where needed, experiments were controlled by a dedicated minicomputer which provided VCD signal averaging of multiple spectral scans.

However, even for amino acids soluble in D_2O the range of VCD measurements is restricted to a narrow region (2800–3100 cm^{-1}) between the HOD and D_2O absorptions (cf. Figure 1 of ref 6d). Thus, we attempted to obtain VCD spectra of polycrystalline, solid samples of amino acids. We have found that mulls yielded considerably better VCD results than KBr pellets, and as a result we also report the VCD of alanine and serine as mulls in halocarbon oil. The absence of solvent interference permits VCD spectra to be obtained over a wide frequency range (3600–1900 cm^{-1}) limited at high frequency by atmospheric water absorptions and at low frequency by the source envelope and detector cutoffs. Thus, we were able to study frequency regions corresponding to OH, NH, CH, OD, and ND stretching vibrations of amino acids and certain deuterated analogues.

We believe mull sampling techniques to be an important new approach in VCD spectroscopy. This is due to the simplicity of sample preparation, an increased magnitude of VCD intensities, and the absence of solvent interference, even though the interpretation of these VCD intensities generally will be complicated by solid-state effects. For some solid-phase samples, however, we may expect that VCD spectra can be interpreted more easily if crystal effects are small (less polar molecules) and the molecular conformation is known in the solid. In the case of most amino acids one finds large crystal effects owing to the zwitterionic character of the solids and thus, solid- and solution-phase spectra are expected to be rather different. This expectation is borne out, at least in the hydrogen stretching region, by the mull samples which we have observed thus far.

An additional benefit derived from studying solid-phase spectra is a more highly resolved view of the individual vibrational transitions. This not only leads to a more straightforward interpretation of the solid-phase VCD but also permits a more reliable interpretation of the solution-phase absorption and VCD spectra.

II. Experimental Section

The VCD and Raman spectrometers used for this study were described previously.[6d] The VCD unit now incorporates a dedicated minicomputer (Data General Noval 1210 with 8K memory) which allows multiscanning, data averaging, storage, and smoothing. The interface electronic system and the overall performance improvement have been described.[7] The instrument still may be operated in an analog output configuration as discussed earlier[6d] if quick survey spectra are desired or if the VCD features are very large (vide infra).

Samples of alanine (both D and L enantiomers) were obtained from Aldrich Chemical Co.; samples of D- and L-serine were purchased from Chemical Dynamics, Inc.; all samples were used without further purification. For solution spectra, the amine functions of the amino acids were deuterated as discussed previously.[6d] For serine, however, deuteration of the $-CH_2OH$ function to $-CH_2OD$ proceeds slowly to completion; thus, serine samples were dissolved in excess D_2O and kept in solution for ca. 4 days before evacuation to dryness.

Solid-phase samples of the amino acids and their deuterated analogues were prepared from ca. 20 mg of sample and ca. 10 drops of halocarbon oil (Halocarbon Products Corp., Hackensack, N.J.). The mulls were spread between CaF_2 windows and exhibited a typical transmission of 80% at 3300 cm^{-1}. Mulls of deuterated amino acids were prepared in an inert atmosphere to prevent deuterium exchange with the surrounding humidity. To ascertain that the observed spectral features were not due to birefringence of the samples, the windows containing the mull were rotated about the propagation direction of the infrared radiation. With carefully prepared samples, no dependence of the spectral features on window alignment was observed.

The solution spectra of alanine and serine were obtained using the computer-controlled instrument configuration, and represent the average of a large number of individual scans (cf. figure captions) The mull spectra represent results obtained from a single run of the spectrometer in the analog output configuration. The signal averaging was unnecessary owing to the large VCD and high transmission levels.

To facilitate vibrational assignments of alanine, the α-deuterated analogue, $NH_3^+-CD(CH_3)-CO_2^-$, was synthesized according to a previously published procedure.[8] The compound was characterized by its ^1H NMR and its vibrational spectra. The ^1H NMR of undeuterated alanine shows signals at 1.48 (doublet) and 3.70 (quartet) ppm, due to the methyl and methyne protons, respectively. For alanine-C^*-d_1 only a single peak is observed at 1.46 ppm in accordance with the expected spectral features.

III. Results

We first present the results of solution-phase VCD for alanine and serine. The quality of these spectra has been enhanced by on-line computer-controlled multiple scanning and signal averaging. In Figure 1 the VCD spectrum of L- and D-alanine in D_2O is shown for the carbon–hydrogen stretching region and can be compared to our previously reported single-scan results.[6a,d] The spectrum of each enantiomer was recorded as a 256-point array from 50 successive scans using a time constant of 3 s. A delay of 1 s was used between the arrival at a new wavelength position and the computer acquisition of the VCD value for that position. The time constant and the delay time (and hence the number of scans for a given total spectral acquisition time) were varied to determine the most efficient means of reducing spectral noise. We found that averaging a large number of fast scans at a low time constant was preferable to averaging a few slow scans with a high time constant since low-frequency drifting between successive scans remained in the averaged spectrum if only a few scans were recorded.

The computer-averaged VCD spectrum of L- and D-serine in D_2O in the C–H stretching region is given in Figure 2. The VCD signals for serine are smaller than those for alanine, making quality spectra more difficult to obtain. The serine data were obtained as 256 point arrays from 70 scans using a 10-s time constant and a 3-s delay. Although the serine spectra contain more noise than the alanine spectra in Figure 1, substantial improvement has been achieved over the single-scan VCD spectrum of serine, where only a rough outline of the spectrum was discernible.

The VCD results for the solid-phase spectra of alanine and serine and their deuterated analogues are given in Figures 3–6. The major VCD features of all these spectra are larger than those encountered in the solution phase. As a demonstration of the expanded wavelength accessibility of the mull sampling technique we note that all the hydrogen and deuterium stretching modes of the four molecular species investigated are represented in Figures 3–6. Since the VCD signals are large, signal averaging with multiple scans was not carried out.

As an aid to the interpretation of the VCD spectra of alanine we also report the Raman spectra of alanine in aqueous solution, polycrystalline alanine-d_0, and polycrystalline alanine-C^*-d_1, for the C–H stretching region. The increased resolution provided by these spectra facilitates the assignment of the individual vibrational modes of alanine as discussed below.

IV. Discussion

The results of computer-averaging techniques in VCD show

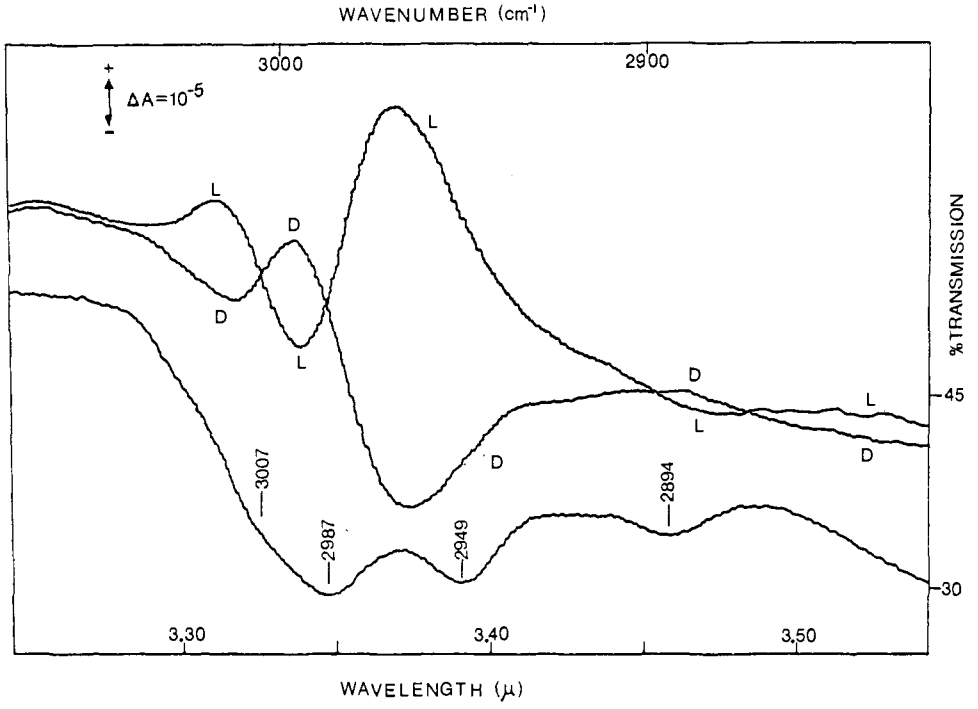

Figure 1. Solution transmission and VCD spectra of the L and D enantiomers of alanine-*N*-d_3 in D_2O (saturated solution). Experimental conditions: absorption path length ca. 150 μ, 50 scans computer coadded, scan time ca. 10 min/scan.

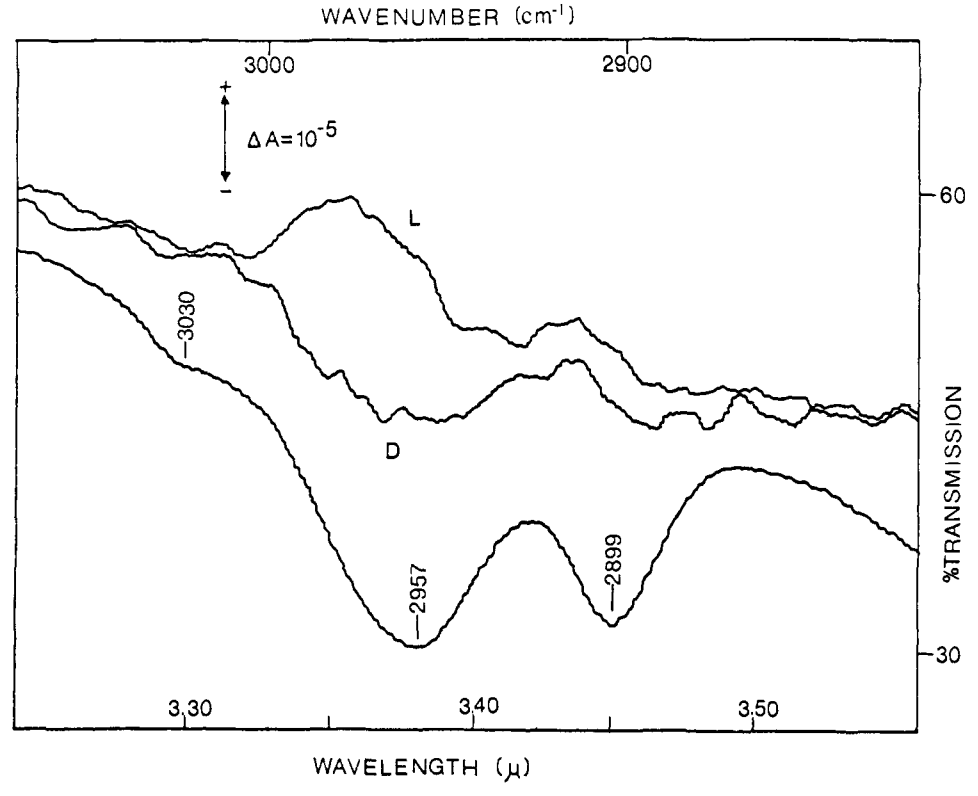

Figure 2. Solution transmission and VCD spectra of serine-d_4 in D_2O (saturated solution). Experimental conditions: absorption path length ca. 150 μ, 70 scans coadded, scan time ca. 18 min/scan.

that dramatic improvement in signal-to-noise ratio can be achieved. We have demonstrated this by improving our previously reported single scans of alanine[6a,d] and by obtaining the spectrum of serine, which would otherwise be only very weakly defined. Such improvement, although not unexpected, considerably enhances our ability to record VCD spectra. In general, we find that signal quality continues to improve as additional time is devoted to signal averaging and that slow instrumental drift is best overcome by averaging a large number of relatively fast scans, as opposed to a few slow scans (vide supra).

In considering the solid-phase mull spectra, we note that the VCD spectra are not only larger than the corresponding solution-phase spectra but they may also differ from the solution

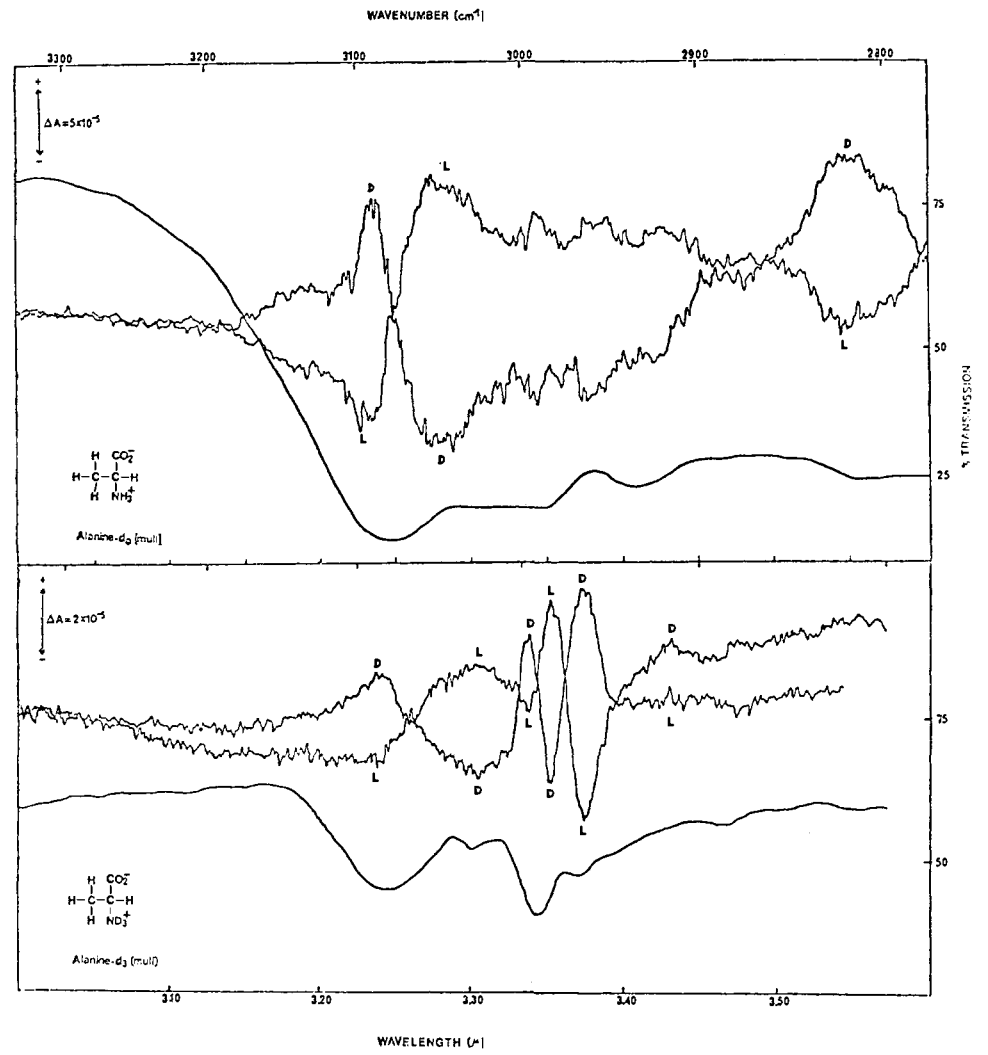

Figure 3. Solid-phase transmission and VCD spectra of alanine-d_0 (top) and alanine-N-d_3 (bottom), polycrystalline samples as mulls in halocarbon oil, 1 scan, VCD time constant 10 s.

Table I. Solution- and Solid-Phase VCD Frequencies and Intensities for Alanine

solution[a]			solid phase						
L-Ala-N-d_3/D_2O			L-Ala-d_0			L-Ala-N-d_3			
IR freq, cm^{-1}	VCD freq, cm^{-1}	ΔA^c $\times 10^5$	IR freq,[b] cm^{-1}	VCD freq, cm^{-1}	ΔA^c $\times 10^5$	IR freq,[b] cm^{-1}	VCD freq, cm^{-1}	ΔA^c $\times 10^5$	assignment
			3080	3090	−9.3				NH_3^+ antisym
				3053	+11.0				
3007	3015	+0.8				2991	2996	−1.1	CH_3 antisym
2987	2995	−1.5					2985	+2.8	
	2970	+4.5				2964	2962	−3.5	CH_3 sym + C*H
2949									
			2820	2820	+6.5				overtones (CO_2^-)
						2350	2250		overtones and combinations
							2226	−9.4	ND_3^+ antisym
							2161	+12.6	
							~2100	−4.5	ND_3^+ sym

[a] Cf. also values reported in ref 6d. [b] Cf. also Table III. [c] The uncertainty in the ΔA measurement is ca. $1/10$ of the ΔA value.

VCD in relative signal magnitude and sign for the individual vibrational modes. To consider this in more detail we will next discuss the basic features of the mull spectra.

A tabulation of the spectral results for Ala-d_0 and Ala-N-d_3 taken from Figures 3 and 4 is given in Table I, where for comparison the corresponding data for Ala-N-d_3 in D_2O solution is also given. The VCD spectrum of Ala-d_0, as shown in Figure 3, is dominated by a huge couplet centered under the NH_3^+ antisymmetric stretching peak at ca. 3080 cm^{-1} and a large, monosignate single at ca. 2820 cm^{-1}. The observed VCD features are almost entirely due to N–H vibrations, with C–H vibrations contributing only minor VCD intensity. The reasons for this assertion are as follows. The Raman and IR spectra of Ala-N-d_3 show that the N–D vibrations occur over

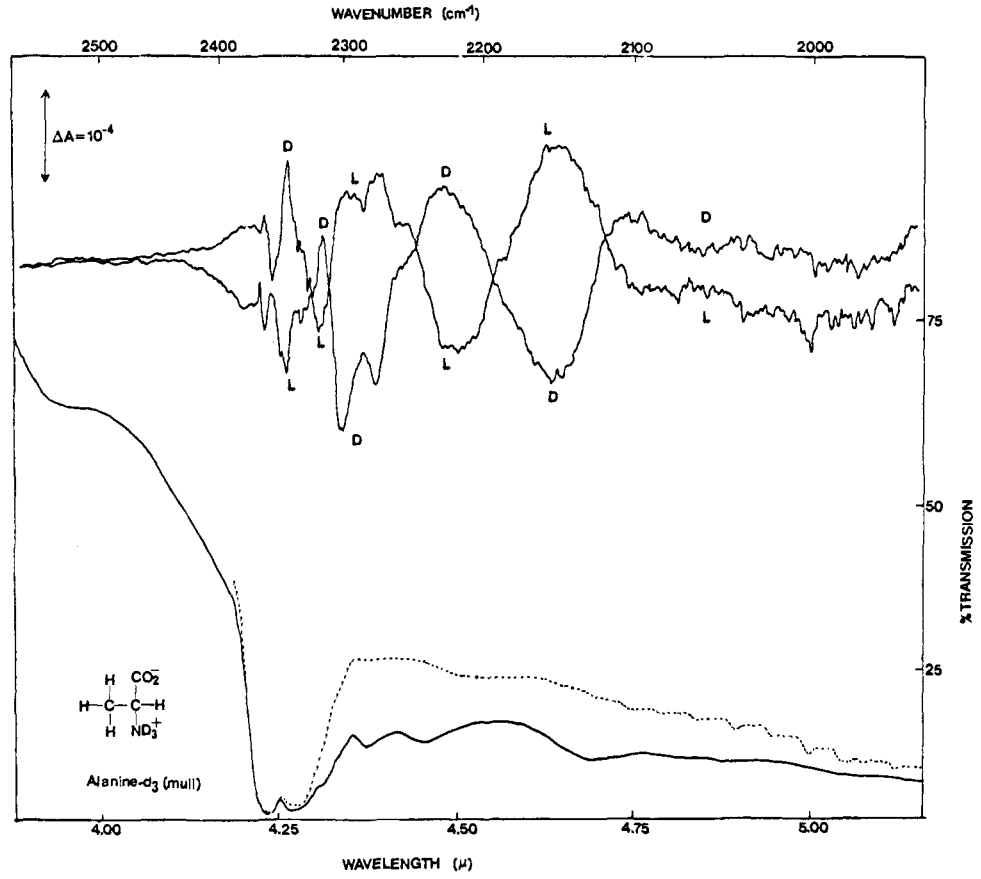

Figure 4. Solid-phase transmission and VCD spectra of alanine-*N*-d_3, polycrystalline samples as mulls in halocarbon oil, 1 scan, VCD time constant 30 s. Instrument transmission with sample cell and fluorocarbon oil is represented by a dotted line. The strong absorption at ca. 4.25 μ is due to atmospheric CO_2.

a wide frequency range between 2000 and 2300 cm^{-1}. Similarly, the Raman spectrum and Ala-d_0 shows peaks on both the high- and low-frequency side of the C–H bands; these peaks are not observed in the Ala-*N*-d_3 spectra and, thus, are undoubtedly due to N–H vibrations (N–H stretching modes above 3000 cm^{-1} and N–H deformation overtones between 2750 and 3000 cm^{-1}). Assuming that the N–H vibrations occur over as wide a frequency range as do the N–D vibrations, we can assume that the C–H spectral region is completely overlapped by N–H vibrations. Thus, the large couplet centered about 3070 cm^{-1} is due to the two NH_3^+ antisymmetric stretching vibrations. The broad, undefined VCD "background" between 3030 and 2900 cm^{-1} is due to the symmetric NH_3^+ stretching mode and a number of undefined NH_3^+ deformation overtones and combinations. The strong feature at ca. 2820 cm^{-1} may be due to the CO_2^- symmetric stretching overtone, since VCD intensity in this region does not disappear upon deuteration.

In Ala-*N*-d_3, the signal centered at 3070 cm^{-1} drops drastically and the remaining signal (somewhat shifted) is attributed to residual Ala-d_0 (the deuterated mulls were sensitive to atmospheric moisture). Distinct peaks due to the C–H stretching vibrations can be observed in Ala-*N*-d_3. These peaks exhibit lower VCD intensities than the previously discussed N–H vibrations of Ala-d_0 and are masked in the VCD spectra of that molecule. As in the case of solution VCD, three distinct C–H peaks in Ala-*N*-d_3 are observed. The high-frequency couplet has its zero crossing exactly at the absorption maximum of the IR peak (2991 cm^{-1}), which could not be resolved, but exhibits two bands in the Raman spectrum (cf. Table III). The strong VCD feature at ca. 2960 cm^{-1} occurs a few wavenumbers below the corresponding absorption peak. We believe that the 2960-cm^{-1} band is due to a superposition of the C*–H stretching and the CH_3 symmetric stretching mode which also occurs in solution according to our revised VCD interpretation of alanine in D_2O, given below. Note, however, that the solid- and solution-phase VCD signals have opposite signs; this finding can only be explained by strong solid-state effects changing the absolute stereochemistry which the methyl groups experience. In addition to the C–H spectra, we have recorded the VCD of the N–D stretching region of Ala-*N*-d_3 between roughly 2350 and 2000 cm^{-1} as shown in Figure 4. The most remarkable aspect of these spectra is the extremely high VCD intensity, with $\Delta A/A$ values approaching 4×10^{-4}. Another notable feature is the lack of direct resemblance to the N–H stretching region VCD of Ala-d_0. This is most likely due to Fermi resonance and VCD enhancement of overlapping combination bands and overtones. Among these would be modes comprised of the N–D deformation fundamentals. One correlation does, however, appear likely. If the crossing of the N–H couplet at 3080 cm^{-1} in Ala-d_0 shifts by $1\sqrt{2}$ upon deuteration of the amine function, the crossing should appear at approximately 2180 cm^{-1}. Such a crossing does appear with bands of the corresponding sign of each side. Thus, the antisymmetric N–D stretching mode appears to be identified; however, the rest of the features are complex and beyond interpretation for the present.

A summary of the spectral data for serine is given in Table II along with the assignments of the observed vibrational features. The solid-state VCD spectrum of Ser-d_0 is dominated by signals from the O–H and N–H stretching modes (cf. Figure 5). A broad monosignate VCD band coincides with the O–H absorption band. This is followed by first a strong and then a weak VCD band which are most likely a distorted couplet from the degenerate antisymmetric N–H stretching modes. The zero crossing of these VCD bands occurs at the

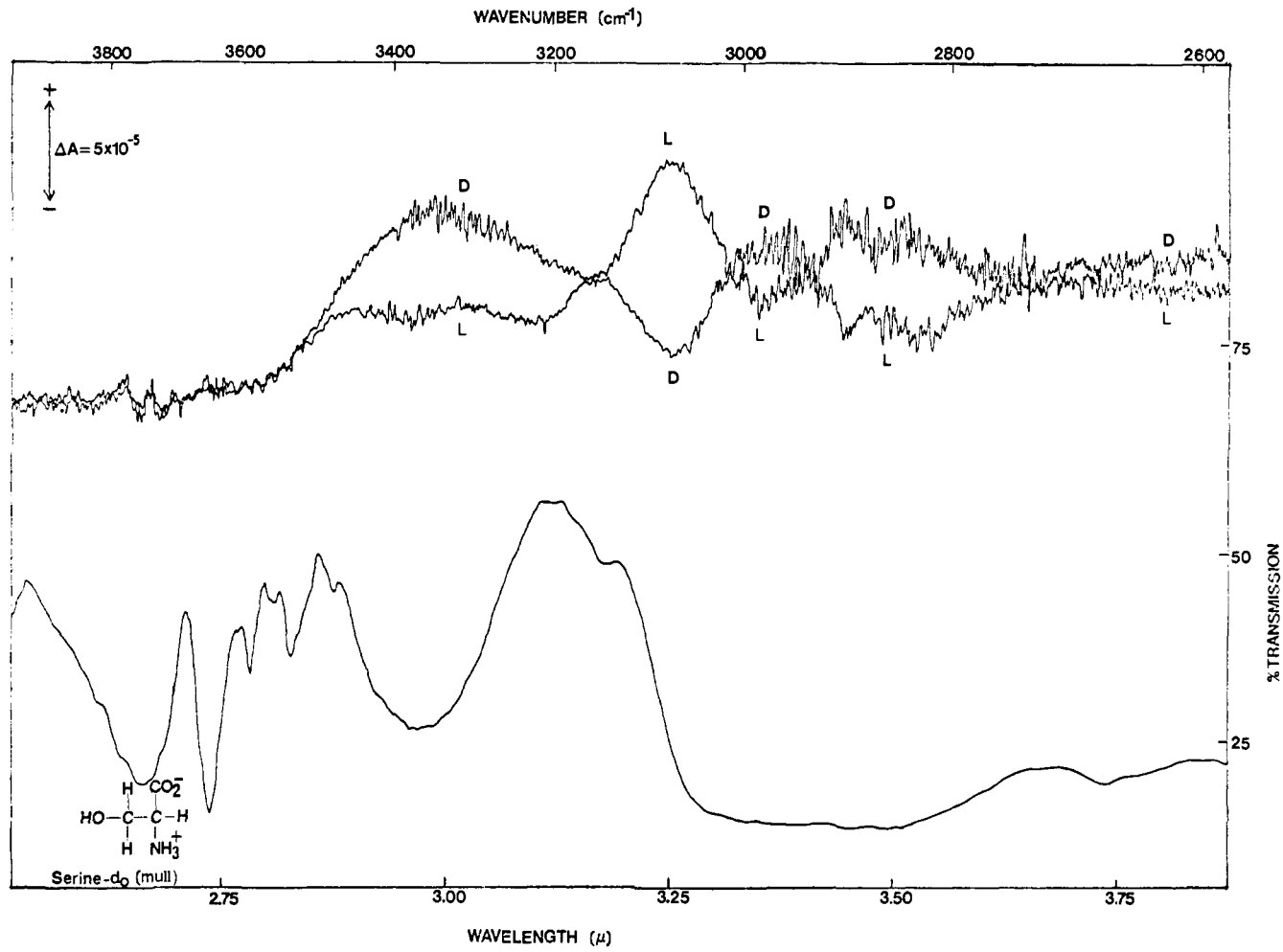

Figure 5. Solid-phase transmission and VCD spectra of serine-d_0, polycrystalline sample as mulls in halocarbon oil, 1 scan, VCD time constant 10 s. Transmission features below 2.9 μ are due to atmospheric absorption.

Table II. Solution- and Solid-Phase Frequencies and VCD Intensities of Serine

solution L-serine-d_4/D_2O				solid phase L-serine-d_0				L-serine-d_4				
IR freq, cm^{-1}	Raman, cm^{-1}	VCD freq, cm^{-1}	ΔA^a $\times 10^{-5}$	IR freq, cm^{-1}	Raman, cm^{-1}	VCD freq, cm^{-1}	ΔA^a $\times 10^5$	IR freq, cm^{-1}	Raman, cm^{-1}	VCD freq, cm^{-1}	ΔA^a $\times 10^5$	assignment
				3380		3370	−3.4					OH
				3040		3095	+5.1					NH_3^+ antisym
3030												
		2970	+1.2					3000	3001	3000	+2.1	CH_2 antisym
2957	2960							2960	2965			CH_2 sym + C*H
2899	2900			2910			−2.2	2910	2908			overtone
										2580	−2.3	OD
								2550		2504	+5.6	OD

[a] For accuracy of VCD intensity, cf. footnote *c*, Table I.

maximum of the broad N-H absorption band. Following the usual pattern the remainder of absorption and VCD spectra corresponds to the N-H symmetric stretching. The small variations in the VCD spectrum below 3000 cm^{-1} correspond to contributions from the C-H stretching modes which are masked by the N-H absorptions.

The C-H stretching modes are revealed clearly in the Ser-d_4 VCD and absorption spectra in Figure 6. Small residual signals due to remaining undeuterated species are evident. In the O-D stretching band a large bisignate VCD signal appears, although it should be noted that Figure 6 has a 2.5 times more sensitive VCD scale than Figure 5. The change in the form of the O-H VCD upon deuteration is unexpected and requires an explanation involving the coupling of the O-D mode with another mode which does not couple with the corresponding O-H mode in the undeuterated species.

We next discuss the solution-phase vibrational assignments and VCD spectra for alanine and serine. For alanine a number of normal coordinate calculations have been carried out;[9-13] however, these treatments suffer from a lack of detail in the C-H stretching region. The only C-H stretching frequencies reported in the literature are for Raman spectra of L-alanine single crystals[14] and in solution by authors of this publication.[6a,d] In Table III we list our Raman data for polycrystalline Ala-d_0 and Ala-C^*-d_1 from Figure 7 together with the available Raman single crystal data.[14] Experimental data for

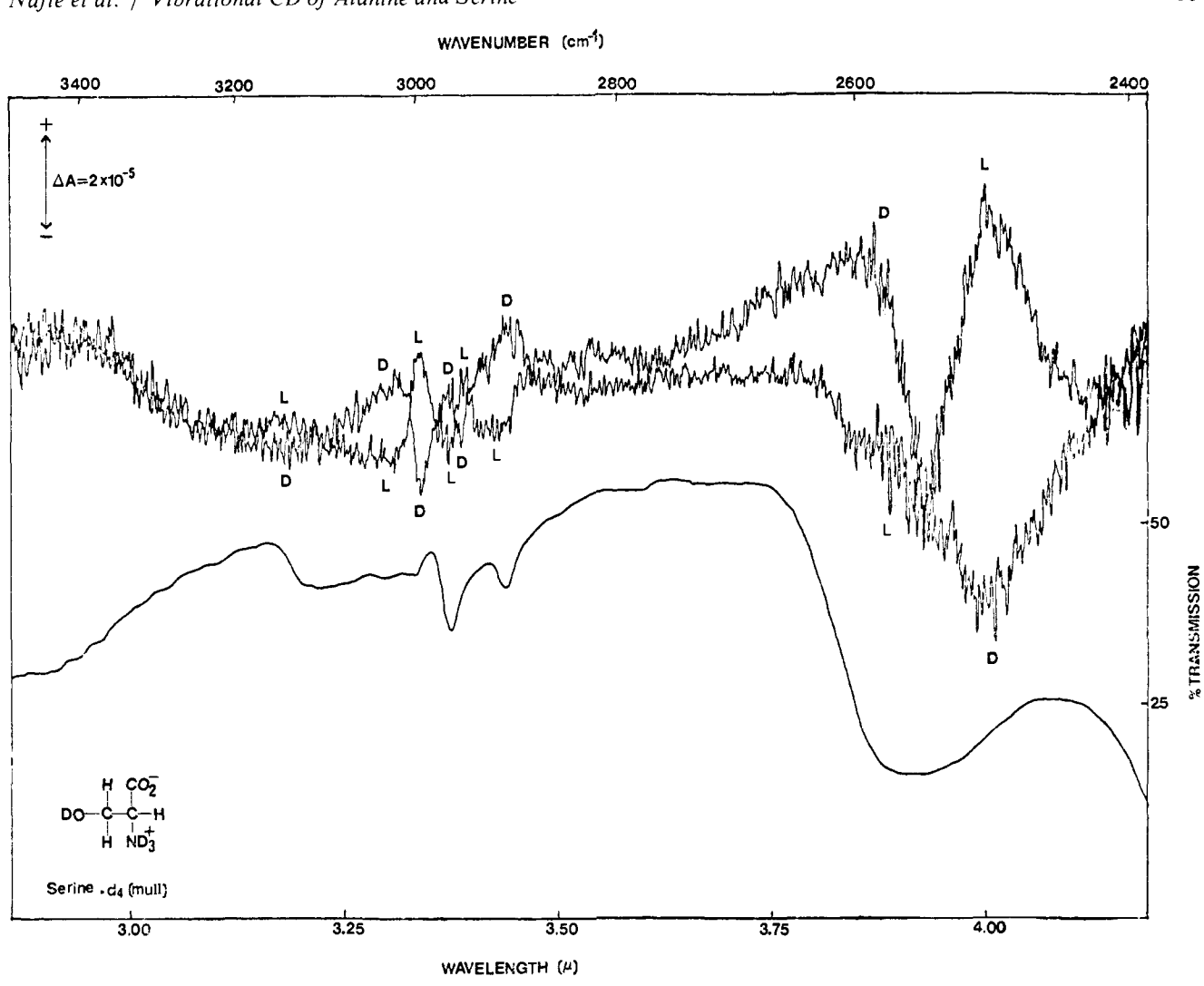

Figure 6. Solid-phase transmission spectra of serine-d_4, polycrystalline mull in halocarbon oil, 1 scan, VCD time constant 30 s.

Table III. Solid-State C-H Vibrational Frequencies for Alanine and Alanine-C^*-d_1

Wang and Storms[a] Ala-d_0 single crystal		this work				Ala-N-d_3 polycrystalline (mull)
		Ala-d_0 polycrystalline		Ala-C^*-d_1 polycrystalline		
Raman freq, cm^{-1}	assignment	Raman freq, cm^{-1}	assignment	Raman freq, cm^{-1}	assignment	VCD peaks freq, cm^{-1}
3002	CH modes	2998	CH_3 antisym	3006	CH_3 antisym	2996
2998		2984	CH_3 antisym	2984	CH_3 antisym	2985
2964		2964	CH_3 sym + C*-H	2960	CH_3 sym	2962
2932		2928	2 × CH_3 antisym def (1480 cm^{-1})	2930	2 × CH_3 antisym def (1481 cm^{-1})	
2891		2885	2 × CH_3 antisym def (1458 cm^{-1})	2885	2 × CH_3 antisym def (1460 cm^{-1})	
				2212	C*-D	

[a] Reference 14.

polycrystalline Ala-N-d_3 are nearly identical with that of Ala-d_0 in the C-H stretching region and are not separately displayed.

Our previous vibrational assignment of Ala-N-d_3 in D_2O was based on a published alanine vibrational calculation and on the vibrational assignment of the alanine-mass-analogue molecule CH_3CHFCl,[15] where in both cases the highest frequency C-H stretching mode was assigned to the methyne C-H stretch. We then assigned the 3010-cm^{-1} band to the methyne stretch and the near-degenerate antisymmetric modes to the 2994-cm^{-1} Raman band (2987 cm^{-1} in the IR). However, the solid-state Raman spectrum of Ala-d_0 indicates a new assignment which is further strengthened by the Raman spectrum of Ala-C^*-d_1. As seen in Figure 7b there are two distinct, well-resolved bands in Ala-d_0 observed at 2984 and 2988 cm^{-1} and, in addition, a previously unobserved band at 2928 cm^{-1}. Upon deuteration of the α hydrogen, the strongest band at 2964 cm^{-1} loses intensity and shifts to 2960 cm^{-1}; also

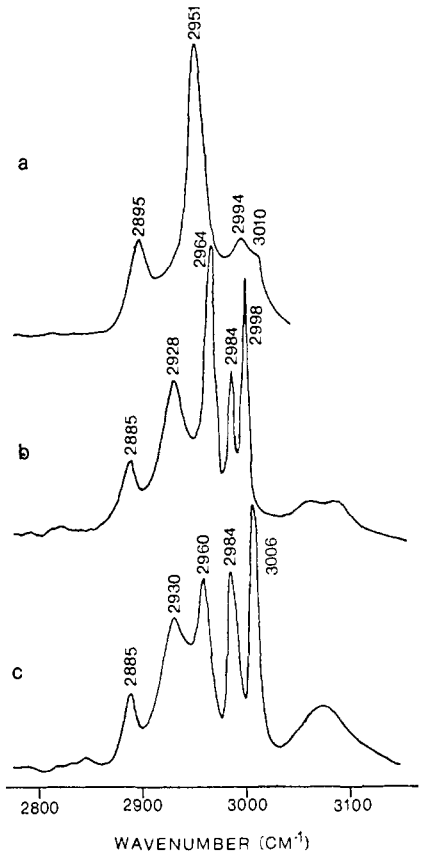

Figure 7. Raman spectra of alanine: (a) alanine-d_0, 1 M in H_2O (H_2O background subtracted); (b) alanine-d_0, polycrystalline solid; (c) alanine-C*-d_1, polycrystalline solid. Experimental conditions: laser power ca. 400 mW at 514.5 nm (laser plasma lines removed by grating filter), resolution ca. 4 cm^{-1}. All spectra were obtained using melting point capillaries for sample cells.

a strong peak corresponding to the C*-D stretching mode appears at 2212 cm^{-1} and the highest frequency band shifts from 2998 to 3006 cm^{-1}. Based on this evidence, the following vibrational assignment is proposed. The two broad, medium intense bands at 2885 and 2928 cm^{-1} are assigned to Fermi resonance enhanced overtones of the methyl antisymmetric deformations, observed at 1458 and 1480 cm^{-1}. The strong band at 2964 cm^{-1} in Ala-d_0 then consists of the methyl symmetric stretching vibration along with the C*-H stretching, and the two high-frequency bands at 2984 and 2998 cm^{-1} correspond to the antisymmetric methyl stretching modes. Upon deuteration, the C*-H mode shifts to 2212 cm^{-1}, leaving the symmetric methyl stretch at 2960 cm^{-1}. The 8-cm^{-1} shift of the highest frequency band upon deuteration indicates considerable coupling between one of the methyl antisymmetric stretchings and the C*-H stretching mode. Recently a vibrational analysis of alanine and its methyl-d_3 analogues was reported[16] which includes excellent detail in the C-H stretching region. The results of this new work are in complete agreement with our present revised interpretation of the alanine C-H stretching region.

Based on the discussion above a new interpretation of the VCD spectrum of alanine in Figure 1 can be given. In solution only one band in the methyl deformation region is observed at 1465 cm^{-1}; consequently, the band at 2894 cm^{-1} is assigned to be the overtone of the unresolved methyl deformation fundamentals at 1465 cm^{-1}. The band at 2894 cm^{-1} shows only minimal VCD activity. The two high-frequency VCD signals of opposite sign, centered around 2995 and 3015 cm^{-1}, can then be attributed to the two antisymmetric methyl stretching modes in good agreement with the observed Raman frequencies (2994 and 3010 cm^{-1}). The large VCD signal observed at about 2970 cm^{-1} is attributed to the superposition of VCD bands due to the α hydrogen and the symmetric methyl stretching modes. Although this interpretation is somewhat different from the one previously published,[6d] it does not differ with regard to our earlier assumption of the perturbed degenerate methyl modes giving rise to a VCD positive-negative couplet. Actually, this interpretation is simpler since the observed antisymmetric methyl stretching couplet is now seen without significant interference from neighboring vibrations.

The Raman and IR solution-phase spectra of serine-d_4, (Ser-d_4, ND_3^+-$CH(CH_2OD)$-CO_2^-) exhibit two strong bands at 2900 and 2960 cm^{-1}. In addition, a weak shoulder is observed in the IR spectrum at about 3030 cm^{-1} (cf. Figure 2). The broad, strong feature observed in the solution Raman spectrum at 2960 cm^{-1} splits into two distinct, sharp bands at 2965 and 3001 cm^{-1} in the solid phase. These two bands are assigned as the symmetric and antisymmetric methylene stretching modes, respectively, in accordance with group frequencies reported for the $-CH_2OH$ function.[17] This assignment assumes that these two modes both contribute to the strong, broad peak observed in solution. However, the methyne hydrogen stretching motion cannot be comfortably assigned to either the low-frequency (2901 cm^{-1}) band or the high-frequency (3030 cm^{-1}) shoulder, and thus we assume that the methyne stretching mode overlaps the band due to the symmetric and antisymmetric methylene motions (cf. Table II).

The only sizable solution VCD signal in Figure 2 is centered at ca. 2970 cm^{-1}, i.e., at ca. 10 cm^{-1} higher than the corresponding absorption maximum. This signal may correspond to the strong narrow VCD feature observed in the solid state (ca. 3001 cm^{-1}) which was assigned to the antisymmetric methylene stretching mode (Table II). This interpretation requires the frequency of the antisymmetric methylene motion to shift by ca. 30 cm^{-1} toward lower frequency in the solution phase than in the solid phase. On the other hand, the solution VCD feature could be due to the α-hydrogen motion, if it indeed overlaps the two methylene modes, but 2970 cm^{-1} is above the typical range of α-hydrogen stretching mode.

The discussion in this paper has dealt in part with VCD features arising from locally symmetric groups which show vibrational optical activity as a result of perturbations from their chiral molecular environment. A particularly useful aspect of this effect is the appearance of a positive-negative couplet for each pair of near-degenerate antisymmetric stretching vibrations in XY_3 molecular groups (e.g., $-CH_3$, $-NH_3^+$, and ND_3^+). This correlation pervades both the solution- and the solid-phase VCD spectra of both alanine and serine and should prove to be a useful general tool in the evaluation of VCD data.

Another overall trend of the VCD spectra reported here is that for comparable spectral regions and experimental conditions alanine exhibits larger VCD features than serine. A simple explanation for this observation is the increased conformation freedom of serine. This freedom, due to the O-H or O-D moiety, has two aspects. The first is the structural capability of serine to assume three *distinct* rotameric forms of the CH_2OH group with respect to the remainder of the molecule which alanine does not possess. Second, the O-H group itself may assume different orientations with respect to the entire molecule. Since different conformational forms can be expected to contribute differently, and in some cases oppositely, to the observed VCD signal, diminished overall VCD intensity for serine is not an unexpected result.

Finally, we point out that both amino acids show well-resolved solid-phase VCD in the carbon-hydrogen stretching modes if the N-H intensity is reduced by deuteration. The frequencies of the C-H VCD signals coincide very well with

the Raman frequencies obtained for polycrystalline samples. The infrared peaks in the solid phase, however, generally cannot be resolved sufficiently to allow a direct correspondence of VCD and IR signals. This is apparently due to inherently broader bands in the infrared and consequently Raman spectra are required to obtain a complete and detailed picture of the vibrational frequencies. Thus, in the solid phase, there is a direct correspondence between ordinary vibrational features and VCD peaks. In solution, however, the dominant VCD peaks in alanine and serine occur at frequencies different from the peak frequencies of vibrational bands. The transitions responsible for VCD intensity often do not produce distinct peaks in the vibrational spectra, thus making the correlation between the vibrational and VCD features rather difficult. On the other hand, vibrational optical activity may prove very helpful from the standpoint of the vibrational spectroscopist by locating transitions which are unobserved in conventional vibrational techniques.

V. Conclusion

We have reported the first detailed VCD observations of molecules in a polycrystalline, solid phase. We have found that VCD can be obtained from transparent mulls, and that the spectra show very detailed features in both N-H and C-H stretching regions. Solid-phase spectra have aided in the assignment of solution VCD, although a direct correlation between solid- and solution-phase VCD is not generally observed. We have also found that computer control of the spectrometer can produce a significant increase in the sensitivity of the experiment, especially under the adverse conditions encountered in the solution spectra of serine.

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Contribution of Minor Species to Paramagnetic Line Broadening of ^{1}H Nuclear Magnetic Resonance Spectra in Peptide-Copper(II) System

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Abstract: pH dependence of the paramagnetic line broadening of ^{1}H NMR spectra has been observed for histidine (Gly·Gly·His) and glycine (Gly, Gly·Gly, Gly·Gly·Gly, and Gly·Gly·Gly·Gly) peptide-copper(II) systems. The effective line width at half-height, $(\pi T_{2P})^{-1}$, of the ^{1}H NMR signals showed a bell-shaped curve with a maximum for each system. The cause of the bell-shaped curve was examined in the Gly·Gly·His-copper(II) system as the most typical and dramatic case. It was found that the line broadening was caused by the small amount of incomplete complexes present in the solution and that an extreme decrease of the incomplete complexes and exclusive formation of a complete complex having a large τ_M result in narrowing of the line width. This finding indicates that the ^{1}H NMR line broadening does not always reflect the dominant complex species present in solution, so care is required in the use of line-broadening data in the study of metal binding sites.

Selective broadening of ^{1}H NMR spectra by a paramagnetic ion has often been used to investigate metal ion binding sites.[2] Among others, the amino acid and/or peptide-copper(II) systems are the ones most often studied. However, recently a question has been raised as to whether the broadening truly means or represents selective binding of the metal ion to the ligand.[3,4] This is because there are two successive restrictions when we combine the line broadening data with the

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