shifts with surface Lewis acidity must await the results of additional thermal desorption studies.

Conclusions

We have demonstrated that trimethylphosphine is a useful NMR probe molecule for acid site characterization of zeolites and, most likely, of catalytic surfaces in general. When trimethylphosphine is adsorbed on a 400 °C calcined H-Y zeolite, both $[(CH_3)_3P-H]^+$ species and, depending on desorption conditions, physisorbed $(CH_3)_3P$ are detected. Clear evidence for a phosphonium ion is found from well-resolved J_{P-H} coupling. Calcination of H-Y at 500 °C and above gives rise to a number of additional ^{31}P resonances that can be assigned to Lewis sites. These results support the concept of a heterogeneous Lewis site distribution in calcined H-Y zeolites. A resonance that can be ascribed to a $(CH_3)_3P/Al_2O_3$ complex is noted at 500 °C and above, providing independent evidence to support the previous findings of Freude et al.[22b] Poorly resolved coupling for another species in the 500 °C calcined sample provides evidence for a site that coordinates $(CH_3)_3P$ both to a proton and a Lewis acid. This type of site may be responsible for the strongly acidic properties of zeolites in heterogeneous catalysis.

Thermal desorption studies of the 400 °C calcined H-Y suggest that there are at least two $[(CH_3)_3PH]^+$ complexes: (1) a rather immobilized phosphonium complex with a chemical shift of −2.3 ppm that dominates the spectrum from the sample degassed at 80 °C for 0.5 h and (2) a mobile phosphonium ion with a chemical shift of −4.4 ppm which is observed for a sample degassed at 300 °C. The former complex appears to arise from interaction with 3550-cm^{-1} hydroxyl groups and the latter with 3650-cm^{-1} hydroxyl groups.

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Vibrational Circular Dichroism in Amino Acids and Peptides. 9. Carbon–Hydrogen Stretching Spectra of the Amino Acids and Selected Transition-Metal Complexes

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Abstract: Vibrational circular dichroism (VCD) spectra have been obtained in the carbon–hydrogen stretching region for lactic acid, 21 α-amino acids, 5 bis(amino acid)copper(II) complexes, and a tris(alaninato)cobalt(III) complex. The spectra of the amino acids, which include most of those that occur naturally, were obtained in D_2O without pD adjustment. A common feature of nearly all of these spectra is a positive VCD bias for the L-amino acids resulting from a broad band associated with the methine C_α*H stretching mode located near 2970 cm^{-1} in the free amino acids. Evidence is presented that associates the strong positive methine VCD intensity with the presence of an intramolecular hydrogen bond between the ND_3^+ and CO_2^- groups in the amino acids. Further intensification of this VCD band in the bis-copper (II) complexes is attributed to increased bonding strength between the amine and carboxylic acid groups via the transition-metal bonds. The mechanism of positive VCD intensity enhancement is proposed to arise from a current in a ring that is closed by the intramolecular hydrogen bond or the transition-metal bonds. The remaining VCD features are associated with the CH stretching modes of the side chains of the amino acids.

I. Introduction

The solution-state conformation of amino acids is of considerable interest owing to the role that these molecules play in the determination of the structure and function of small peptides and proteins. The conformation of an amino acid involves the geometry of the amine and carboxylic acid groups on the one hand and that of the side-chain group on the other. The problem of specifying the various torsional angles describing the conformational degrees of freedom for amino acids in solution is formidable, although significant progress has been achieved in recent years by using NMR spectroscopy.[1] Vibrational spectroscopy has contributed qualitatively to the problem; however, neither infrared nor Raman spectroscopy has yet been used for quantitative determination of amino acid conformations.[2]

A new approach to the determination of conformational details in chiral molecules known as vibrational circular dichroism (VCD) has been developed recently.[3] In previous publications we have reported VCD spectra in the CH stretching region for alanine, simple alanyl peptides, and serine.[4] The combination of the infrared absorption spectrum and the VCD spectrum, where the

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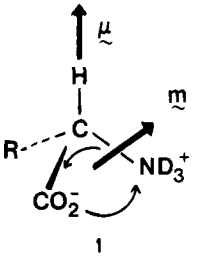
latter provides a new set of vibrational intensities possessing enhanced stereochemical sensitivity, serves to increase the information content of the vibrational spectra. Although a wide range of conformations may be consistent with the infrared absorption spectrum, a much narrower range of conformations will be consistent with both the VCD and the absorption spectrum. We have recently analyzed the CH stretching VCD spectra of alanine-N-d_3, alanine-C_α-d_1-N-d_3, and alanine-C_β-d_3-N-d_3 in D_2O solution and have found close agreement between the observed relative VCD intensities and calculated intensities using a localized molecular orbital model and CNDO wave functions.[5] This work confirmed our earlier qualitative assignments[4b] of the VCD spectrum of alanine.

We have now extended our VCD studies in the CH stretching region to include the majority of the naturally occurring amino acids and a number of amino acid transition-metal complexes.[6] The most striking feature that appears in the VCD spectra is a strong bias toward positive VCD intensity for the L-amino acids. As a result of this bias and the observation that all of the VCD spectra exhibit a positive VCD maximum at the observed or expected location of the C_αH methine stretching mode, we recently proposed a VCD chirality rule for the CH stretching region in the amino acids and related molecules.[7]

Furthermore, we have postulated that this chirality rule arises from a new mechanism for the generation of VCD intensity in which the methine stretching motion induces an oscillating current in an intramolecular ring to which it is attached. The oscillating current then produces a magnetic moment that varies at the methine stretching frequency. In the amino acids the ring is formed by an intramolecular hydrogen bond between the amine and carboxylic acid groups, whereas the two amino acid ligand bonds close the ring in the transition-metal complexes. Elementary considerations supported by molecular orbital calculations indicate that very strong, positive VCD intensity can result from the positive vector dot product of a magnetic transition moment, $\mathbf{m}$, due to current flow in a loop including the CO_2^- and ND_3^+ functional groups, with the electric transition moment, $\boldsymbol{\mu}$, of the stretching of the C_αH methine bond as illustrated in **1**.



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The principal aim of this paper is to describe more fully the spectroscopic evidence that has led us to propose the methine chirality rule.[7] In addition, the proposed mechanism of the rule will be further examined in the light of recent LMO–VCD calculations for glycine-C_α-d_1. The correlation of intramolecular hydrogen bonding with bias in the observed VCD spectra of amino acids has significance for VCD as a probe of molecular self-association and conformation. For example, the presence of a ND_3^+–CO_2^- hydrogen bond places constraints on the equilibrium torsional angle of the CO_2^- group, which in turn has an influence on the conformational behavior of the amino acid side chain.

A secondary aim of this paper is to formulate a basis for the interpretation of VCD in the CH stretching region of amino acids and their transition-metal complexes. This formulation involves two contributions to the VCD: a broad positive methine stretching contribution and a perfect following contribution arising from the VCD of the side-chain and methine C_αH stretching vibrations. The latter involves consideration of the rotameric distribution of the side chain about the C_α–C_β bond as ascertained from NMR studies.

II. Experimental Section

VCD spectra were obtained by using a dispersive grating instrument that has been described previously.[4-6] Spectral resolution in the CH stretching region was 12–14 cm^{-1}. Absorption spectra were collected by using a Nicolet 7199 FT-IR spectrometer at 4-cm^{-1} resolution and are displayed as double-beam absorbance spectra with the D_2O background subtracted from the primary result. VCD base lines were established by comparing the VCD traces of the L enantiomer to that of the corresponding D enantiomer. The base line for alanine-C_β-d_3 has been described previously.[5b]

The amino acids were obtained from standard commercial sources. In the case of glycine, the enantiomers (*S*)-(−)-glycine-C_α-d_1 and (*R*)-(+)-glycine-C_α-d_1 were prepared enzymatically,[8] starting from glycine-d_0 and glycine-d_2, respectively. The Cu(II) complexes were prepared and purified according to published procedures.[9] The Co(III) complex was also prepared[6] according to previous work,[10] the $(\Delta)\alpha'$ isomer being separated from other spatial isomers by column chromatography. The free amino acids and the Cu(II) complexes all contain labile protons, and samples were first exchanged twice with D_2O before sampling in a variable path length cell.[4-6]

III. Results

In Table I we list the frequency and intensity of the spectral maxima for the VCD and absorption of the free amino acids and lactic acid. We note that all VCD maxima are positive, except for glycine-C_α-d_1, and that they occur in a range between 3×10^{-4} and 16×10^{-4} L cm^{-1} mol^{-1}. The absorbance maxima vary over a relatively wide range between 2 and 100 L cm^{-1} mol^{-1}. Also listed are estimates of the net VCD area in the CH stretching region, referred to as the VCD bias, the solubilities of the amino acids,[11] and percentages of rotamer populations about the C_α–C_β bond where this is relevant or known from NMR studies.[12-23]

The VCD bias is significant because it is an indicator of vibrational motion of electronic charge that does not perfectly follow the nuclear motion. Perfect nuclear following by electronic charge is embodied in the fixed partial charge (FPC) model,[24] and this model gives nearly zero VCD bias for a set of vibrational modes, like the hydrogen stretching vibrations, that do not couple significantly with other vibrational modes.[5b,c] The FPC model thus predicts a local sum rule of nearly zero for VCD intensity in the CH stretching region. Such a sum rule is clearly not obeyed for the amino acids and is not required by VCD models[25-29] that allow

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Table I. VCD and Infrared Absorption Maxima, VCD Bias, Solubility, and C_α–C_β Rotomeric Conformational Distribution for the Amino Acids and Lactic Acid

L-amino acid	$\Delta\epsilon_{max} \times 10^4$, L cm^{-1} mol^{-1}	$\bar{\nu}_{max}$, cm^{-1} ±2	VCD bias[a] $\times 10^4$, L cm^{-2} mol^{-1}	ϵ_{max}, L cm^{-1} mol^{-1}	$\bar{\nu}_{max}$, cm^{-1} ±2	solubility[b] (H_2O, 25 °C), g/kg	I (I′)	II (II′)	III[c] (III′)
glycine-C_α-d_1	−2	3020	−85	5	3020	250			
alanine-C_β-d_3	+5	2970	+200	2	3020	167			
alanine	+11	2970	+285	14	2995	167			
lactic acid	+5.2	2920	+380	9	2995	v sol			
serine	+3	2970	+120	15	2960	422	28[d]	10	62
cysteine	+9	2975	+360	8	2955	100	45[e]	17	38
cysteine deuteriochloride	+1	3000	+60	10	2950				
asparagine	+6	2970	+300	8	2955	28	50[d]	12	38
glutamine	+6	2970	+325	16	2945	26			
phenylalanine	+5	2970	+90	12	2940	28	52[f]	20	28
histidine	+5	2985	+275	8	2935	42	48[g]	23	29
valine	+7	2975	+175	70	2980	58	(59)[f]	(24)	(17)
leucine	+16	2965	+200	100	2970	22	67[h]	21	12
isoleucine	+15	2970	+105	100	2980	34.5	(50)[i]	(12)	(50)
threonine	+12	2975	+50	32	2985	v sol	(72)[f]	(21)	(7)
allo-threonine	+5	2970	+250	31	2990	v sol	(77)[f]	(15)	(8)
penicillamine	+9	2960	+120	38	2990	100			
methionine	+6	2980	+300	23	2930	56	51[j]	40	9
lysine deuteriochloride	+5	2980	+325	37	2950	737			
arginine deuteriochloride	+5	2970	+275	25	2950	856			
proline	+7	2990	+455	18	3000	1620	$C_\gamma$exo[k]	$C_\gamma$endo	
4-hydroxyproline	+9	2990	+320	14	2950	361	$C_\gamma$exo[l]		
allo-4-hydroxyproline	+7.5	2970	+340	15	2955		$C_\gamma$exo[l]		

[a] Net integrated VCD area $\int \Delta\epsilon \, d\bar{\nu}$. [b] Reference 11. [c] See text for definitions of I, II, III, I′, II′, and III′. Primed rotameric forms I′, II′, and III′ are listed under the corresponding unprimed heading in parentheses. [d] Reference 13. [e] Reference 15; data were obtained at pH ≈14. [f] Reference 17. [g] Reference 18. [h] Reference 19; populations I′ and III′ were deteremined by ^{13}C chemical shifts and population II′ from coupling constants. [i] Reference 20. [j] Reference 21; data were obtained at pD ≈2. [k] Reference 22. [l] Reference 23.

Table II. VCD and Infrared Absorption Maxima and VCD Bias for the Cu(II) Complexes and Co(III) Complexes of Amino Acids

complex	$\Delta\epsilon_{max} \times 10^4$, L cm^{-1} mol^{-1}	$\bar{\nu}_{max}$, cm^{-1}	$\Delta\epsilon$ bias, L cm^{-2} mol^{-1}	$\Delta\epsilon_{max}$, L cm^{-1} mol^{-1}	$\bar{\nu}_{max}$, cm^{-1}	solvent
bis(L-alaninato)copper(II)	+35	2950	+1400	32	2985	D_2O
(Δ)α′-tris(L-alaninato)cobalt(III)	+25	2955	+1625	33	2950	D_2O
bis(L-serinato)copper(II)	+15	2950	+900	35	2960	D_2O
bis(L-valinato)copper(II)	+25	2970	+1625	170	2975	D_2O
bis(L-threoninato)copper(II)	+30	2980	+1200	75	2980	D_2O
bis(L-prolinato)copper(II)	+30	2950	+1650	75	2990	D_2O

charge flow or currents to arise that do not perfectly follow the nuclear trajectories.

Solubilities are included in the table because they are relevant to the ease of recording VCD spectra. The option of increasing the path length for low-solubility samples is not available due to a strong absorption of the solvent, D_2O, even though we are working in a window of relatively low background absorption. In fact, we are unable to obtain the VCD spectra of tyrosine, tryptophan, glutamic acid, and aspartic acid at neutral pD due to their very low solubilities.

In Table II we provide a similar compilation of results for the metal complexes. Solubilities were not a problem with the complexes, and NMR data concerning C_α–C_β rotamer populations of the Cu(II) complexes are not available due to paramagnetic line broadening.

In the remainder of this section we will describe three groups of spectra in a progression toward increasing complexity. By establishing empirical results and discussing detailed assignments for the more simple molecules, we will form a basis for drawing conclusions and identifying trends for subsequent spectra.

Glycine-C_α-d_1 and Alanine-C_β-d_3. From the perspective of CH stretching vibrations, these two molecules each have only a lone α-methine stretching mode and represent the simplest of the amino acid spectra. The alanine spectrum has been discussed previously[5] and consists of the broad C_αH stretching fundamental at 2968 cm^{-1} and three bands at 3015, 2944, and 2894 cm^{-1}, which are combination bands of the strong antisymmetric CO_2^- stretching fundamental at 1607 cm^{-1} with the three next lower fundamental transitions, the symmetric CO_2^- stretch at 1410 cm^{-1}, and two methine bending modes at 1341 cm^{-1} and 1291 cm^{-1}. As seen from Figure 1 the methine C_αH stretch fundamental is the only transition to exhibit a large VCD intensity. Its anisotropy ratio is quite strong, $g = \Delta\epsilon/\epsilon = 2.5 \times 10^{-4}$. A weaker broad negative VCD band at 3030 cm^{-1} originates in the $\nu_{CO_2^-}{}^{as} + \nu_{CH_2}{}^{s}$ combination band with $\Delta\epsilon/\epsilon \sim -0.4 \times 10^{-4}$.

In the same figure, we display the corresponding absorption and VCD spectra for glycine-C_α-d_1-N-d_3. Again there are four bands. The C_αH stretch fundamental has shifted to higher frequency and appears as a shoulder at 2990 cm^{-1}. The combination bands occur at 3018, 2940, and 2888 cm^{-1} and arise from the same four mid-infrared fundamentals as in alanine-C_β-d_3-N-d_3, namely $\nu_{CO_2^-}{}^{as}$ at 1616 cm^{-1}, $\nu_{CO_2^-}{}^{s}$ at 1413 cm^{-1}, and $\delta_{C_\alpha H}$ modes at 1324 and 1270 cm^{-1}. In glycine-C-d_1-N-d_3, however, the methine C_αH stretching mode exhibits little if any VCD. On the other hand, the CO_2^- combination band at 3018 cm^{-1} has nearly doubled in strength in both absorption and VCD spectra while maintaining its anisotropy ratio at -0.4×10^{-4}. In both alanine and glycine the intensity of the C_αH absorption band is $\epsilon = 2$ L cm^{-1} mol^{-1}.

From Table I we see that these two molecules have widely differing values for the VCD bias, +200 L cm^{-2} mol^{-1} for alanine and −85 L cm^{-2} mol^{-1} for glycine. It follows that the nature of the electronic charge flow, as distinct from the nuclear motion, is quite different in these two molecules. Two possible structural reasons for differences in electronic motion are evident. In glycine, no charge can flow through the β-position since it terminates with a deuterium; however, in alanine the β-carbon is a branch point for three additional bonds. A second difference involves the

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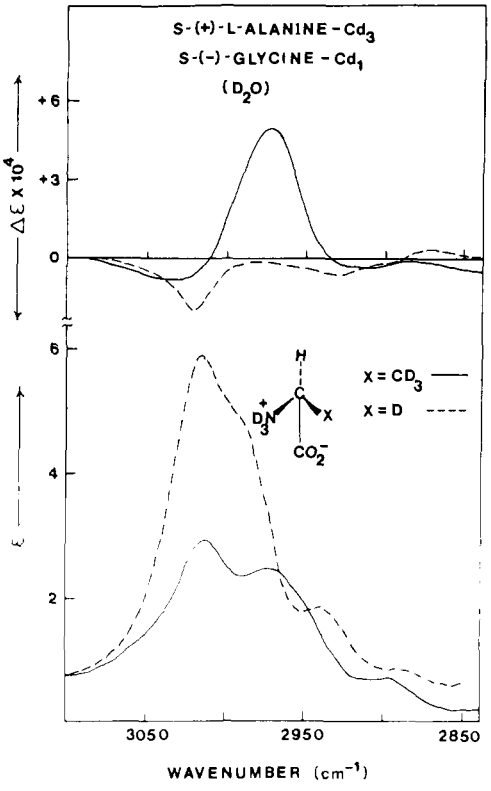


Figure 1. VCD and absorbance spectra of (*S*)-(+)-L-alanine-C_β-d_3 and (*S*)-(−)-glycine-C-d_1 in D_2O. Spectral conditions: alanine, concentration 1.7 M, path length 200 μm, Δε uncertainty ±0.1 × 10^{-4}; glycine, concentration 2.1 M, path length 300 μm, Δε uncertainty ±0.2 × 10^{-4}.

torsional angle of the CO_2^- group, which may vary over a wider range or have a different equilibrium position in glycine due to the absence of the β-carbon with its greater steric influence. These points will be addressed further in sections IV and V.

Alanine and Lactic Acid. Both L-alanine-*N*-d_3 and L-lactic acid-*O*-d_2 possess an α-hydrogen and three β-hydrogens. Their CH stretching absorption and VCD spectra bear a strong similarity as shown in Figure 2. Their major difference is the position of the $C_\alpha H$ stretching mode, which lies at 2970 cm^{-1} in alanine and 2917 cm^{-1} in lactic acid.[3f] In alanine, this mode is more strongly mixed with the two antisymmetric methyl modes near 3000 cm^{-1}, whereas in lactic acid the characteristically broad $C_\alpha H$ band is more isolated, lying below the symmetric methyl stretch which occurs near 2950 cm^{-1} in both molecules. The VCD associated with the symmetric methyl stretch and the Fermi-enhanced methyl bending overtone in alanine is negative[5] whereas the sign of these bands appears to be positive in lactic acid. The change in sign is attributed to the shift of the methine band from above the symmetric methyl band in alanine to below it in lactic acid as discussed in section IV.

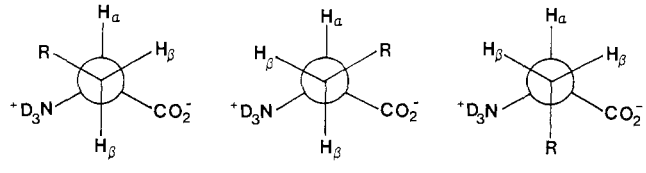
The amount of VCD bias is between +250 and +300 L cm^{-2} mol^{-1} in both molecules, which is also close to the value of +200 L cm^{-2} mol^{-1} for alanine-C_β-d_3-*N*-d_3. This is strong evidence that the $C_\alpha H$ stretching mode is responsible for the magnitude of VCD bias. The bias in lactic acid supports the idea of intramolecular hydrogen bonding between the OD and COOD groups in lactic acid in analogy to the proposed ND_3^+–CO_2^- bond in alanine. It is also noteworthy that the VCD spectrum of alanine-C_α-d_1-*N*-d_3 published earlier[5] shows methyl CH stretching VCD intensity but virtually no VCD bias, again implicating the $C_\alpha H$ mode as the source of bias in this region.

Turning to the transition-metal complexes bis(L-alaninato)-copper(II) and (Δ)α′-tris(L-alaninato)cobalt(III), we first note that the broad methine absorption and VCD bands have shifted from 2970 cm^{-1} in alanine to 2950 cm^{-1} in the Cu(II) complex and 2955 cm^{-1} in the Co(III) complex.[3f] There is only weak VCD, if any, arising from the narrow symmetric methyl stretching absorption band in this same region for both complexes. The spectra have an appearance that is midway between that of alanine and lactic acid. The metal complex spectra have absorption intensities that are stronger by a factor of ~3 compared to alanine. An enhancement factor of 2 is expected for the Cu complex due to a doubling in the number of alanine units per molecule, and a factor of 3 for the Co complex. The VCD spectra of the two complexes are similar in methine intensity, but the VCD couplet due to the two methyl antisymmetric modes is much larger in the case of Cu(II) due in part to the increased splitting of these two modes, as can be seen in their absorption spectra. Due to the general similarity in the appearance of these VCD spectra to those of alanine and lactic acid, there apparently is little coupling or splitting in this spectral region between vibrational transitions on different ligands. It is also likely that the conformation in alanine in the complexes is not drastically different from that of the monomer in solution. It is of interest to note that a shoulder at ~3030 cm^{-1} due to the CO_2^- combination band can be seen in the absorption spectrum of alanine and that this shoulder is not present in the other three absorption spectra in Figure 2 where this group is not present.

The intensity per ligand of the methine VCD in the Cu and Co complexes is ~17 × 10^{-4} and 12 × 10^{-4} L cm^{-1} mol^{-1}, respectively, which is approximately in the range of the value in alanine-*N*-d_3 as determined by curve fitting.[5b] In the alanine complexes, however, there is likely less enhancement of the methine VCD intensity from interaction with the antisymmetric methyl modes due to its shift to lower frequency. Compared to the more isolated methine mode in alanine-C_β-d_3-*N*-d_3, the methine modes in the complexes are very strong indeed. We also note that the CO_2 group in the metal complexes is held in an orientation that is both restricted and comparable to that for our proposed intramolecular hydrogen-bonding mechanism for methine VCD intensity in the free amino acids.[7]

From Table II we note that the VCD bias is 1400 L cm^{-2} mol^{-1} in the Cu(II) complex and 1625 L cm^{-2} mol^{-1} in Co(III) complex. Normalizing to unit ligand bias we have ~700 and ~540 L cm^{-2} mol^{-1} in the two complexes, respectively. This implies a stronger intrinsic methine VCD intensity per alanine in the complexes compared to free alanine. This in turn is evidence for stronger vibrationally induced current in a ring that is now closed by the two transition-metal bonds rather than a hydrogen bond. There appear to be three factors that could contribute to enhanced VCD bias due to the intramolecular rings formed by the metal complexes compared to the free amino acids: (1) increased ring size, (2) increased strength of the bonds that close the ring, and (3) the increased numbers of electrons, including d electrons, that could contribute to a ring current.

Serine and Cysteine. Although both serine and cysteine have only three CH stretching modes compared to four for alanine and lactic acid, their spectra are more complex to analyze due to the existence of rotameric forms about the C_α–C_β bond. Three different rotameric forms need to be considered as having distinct stereochemical contributions to the observed vibrational spectra. Following earlier conventions,[1d] we denote these rotameric structures as



I II III

The rotameric distribution of serine (R = OD) has been determined by NMR[13,14] where it is found that rotamer III is 60% populated, I is 30% populated, and II is 10% populated in D_2O at pD 5.0 and room temperature. In the DL crystal only rotamer III is populated as determined by X-ray diffraction.[30] The

(30) Shoemaker, D. P.; Barieau, R. E.; Donohue, J.; Lu, C.-S. *Acta Crystallogr.* **1953**, *6*, 241.

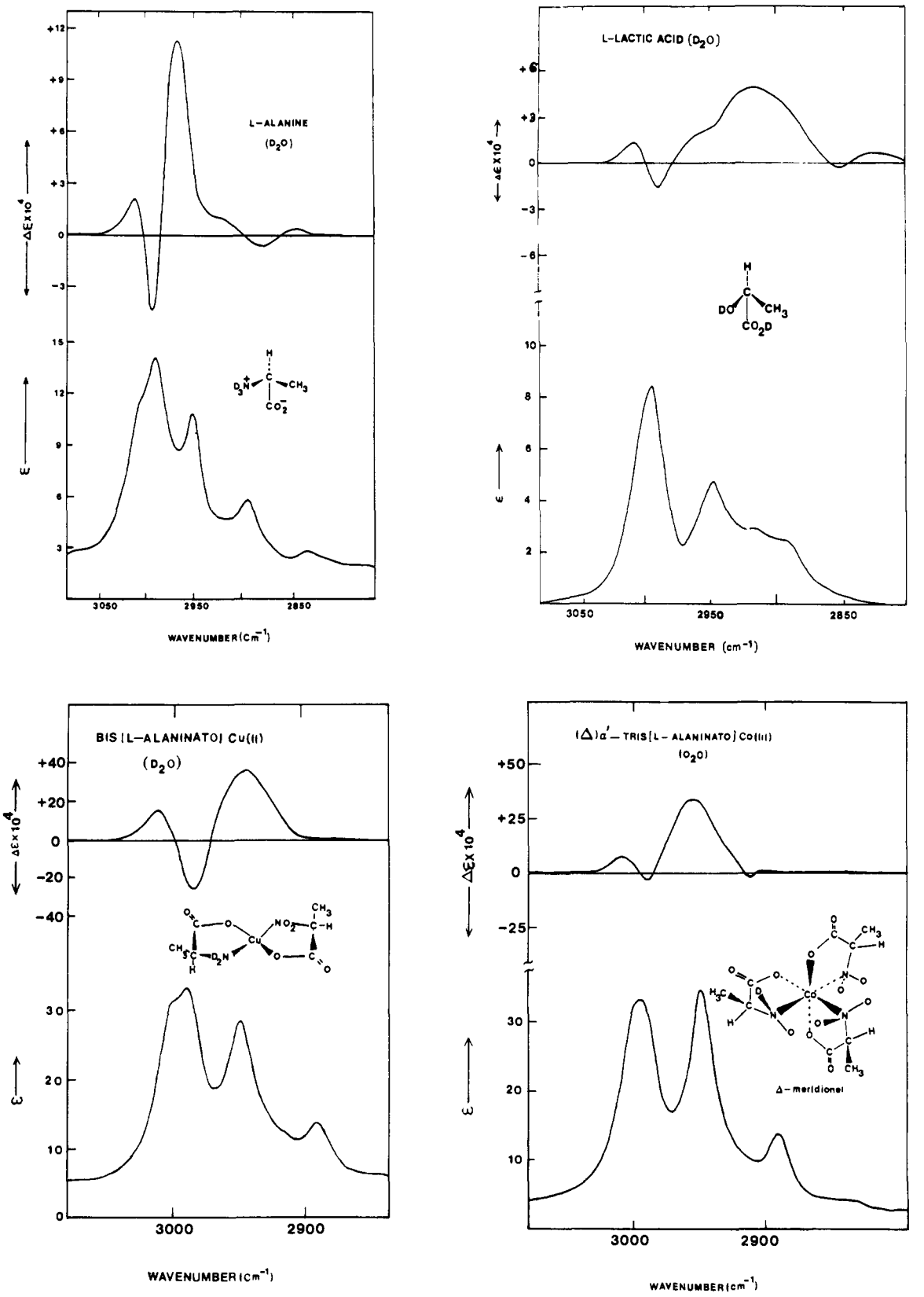


Figure 2. VCD and absorbance spectra of L-alanine, L-lactic acid, bis(L-alaninato)copper(II), and tris(L-alaninato)cobalt(III) in D_2O. Spectral conditions: alanine, concentration 1.7 M, path length 200 μm, Δε uncertainty ±0.08 × 10^{-4}; lactic acid, concentration 5.5 M, path length 150 μm, Δε uncertainty ±0.1 × 10^{-4}; bis(L-alaninato)copper(II), concentration 0.13 M, path length 300 μm, Δε uncertainty ±0.3 × 10^{-4}; tris(L-alaninato)cobalt(III), concentration 0.25 M, path length 250 μm, Δε uncertainty ±0.2 × 10^{-4}.

distribution for cysteine, measured in basic solution, pD 14, has been found to be[15] 45% I, 38% III, and 17% II. The most common conformational preference of the amino acids is rotamer I, where the side group is trans to the bulky CO_2^- group; however, other factors besides steric hindrance may play a role, such as electrostatic attraction and solvent interactions. The preference for rotamer III in serine indicates an electrostatic or hydrogen-bonding attraction of the OD group for the ND_3^+ group or the CO_2^- group or both. On the other hand, the SD group is bulkier and less capable of forming strong hydrogen bonds.

The VCD and absorption spectra thus represent composites of three stereochemically distinct rotamers. Nevertheless, the absence

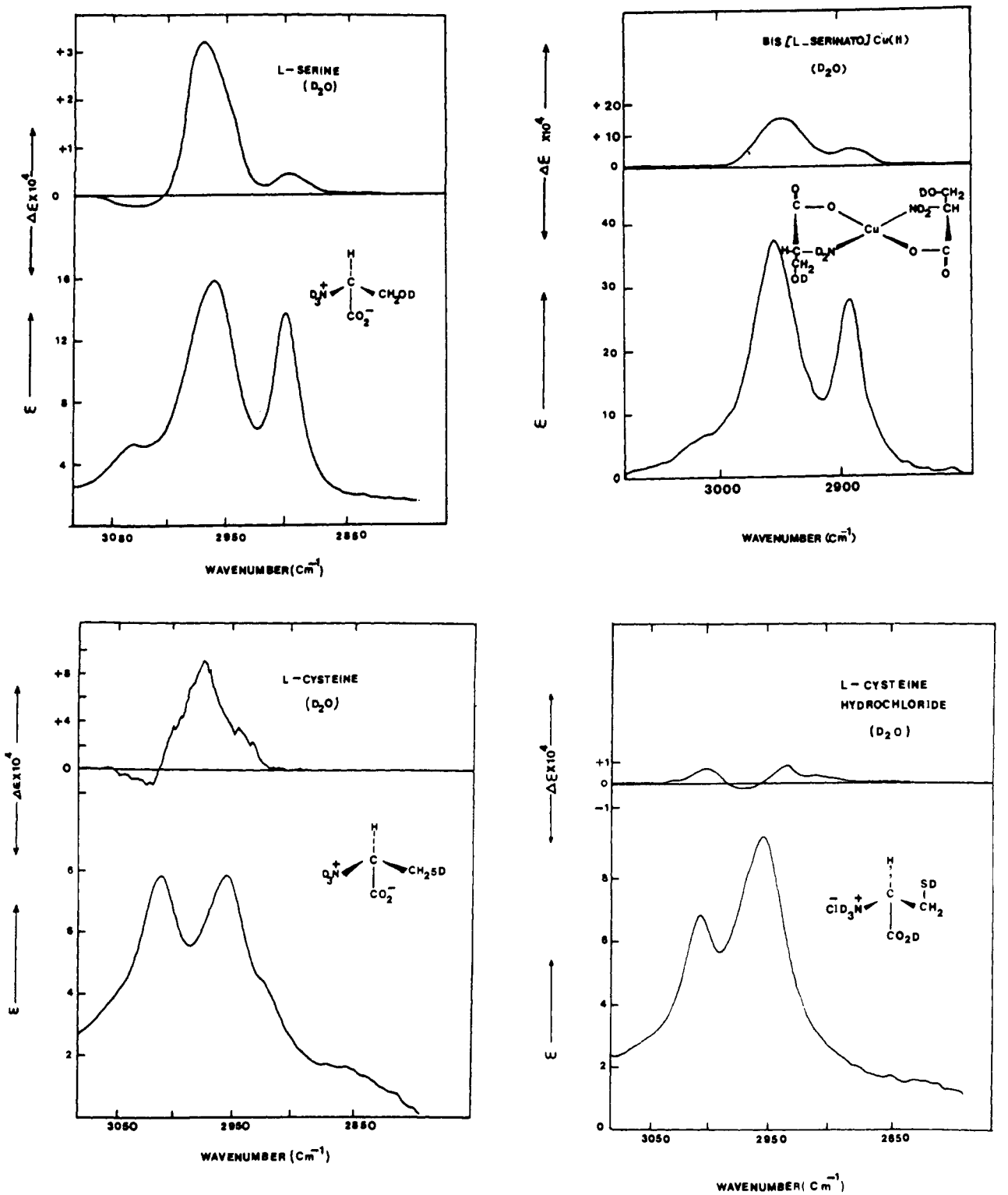


Figure 3. VCD and absorbance spectra of L-serine, L-cysteine, L-cysteine hydrochloride, and bis(L-serinato)copper(II) in D_2O. Spectral conditions: serine, concentration 3.3 M, path length 200 μm, $\Delta\epsilon$ uncertainty $\pm 0.1 \times 10^{-4}$; cysteine, concentration 0.83 M, path length 200 μm, $\Delta\epsilon$ uncertainty $\pm 0.8 \times 10^{-4}$; cysteine hydrochloride, concentration 0.8 M, path length 200 μm, $\Delta\epsilon$ uncertainty $\pm 0.8 \times 10^{-4}$; bis(L-serinato)copper(II), concentration 0.16 M, path length 200 μm, $\Delta\epsilon$ uncertainty $\pm 1.0 \times 10^{-4}$.

of splitting or band broadening indicates that the *frequencies* of the vibrational modes are relatively unaffected by the rotameric variation. In Figure 3 we assign the transition in serine at 3030 cm^{-1} to the CO_2^- antisymmetric combination band. The intensity of this band is the same as in alanine-C_β-d_3-N-d_3 for both absorption and VCD. The $C_\alpha H$ stretching mode is not resolved but most likely is associated with the peak VCD frequency at 2970 cm^{-1}. The band at 2895 cm^{-1} is assigned as a Fermi-enhanced overtone of the 1475-cm^{-1} CH_2 bending vibration that interacts with the symmetric CH_2 stretching mode. The unperturbed overtone would occur near 2930 cm^{-1} but is lowered in frequency, while the methylene frequency is raised beyond 2950 cm^{-1} by the Fermi interaction. Due to the strength of the 2895-cm^{-1} band, it appears that the unperturbed methylene frequency is close to 2930 cm^{-1} and that a strong interaction has occurred. The positive VCD shoulder at 2950 cm^{-1} and the positive intensity at 2895 cm^{-1} represent the distributed VCD intensity of this Fermi diad. Finally, the antisymmetric CH_2 stretching mode is assigned as the shoulder in the absorption spectrum near 2990 cm^{-1} and contributes a small amount of positive VCD intensity.

The assignments for cysteine are similar, but here no Fermi resonance occurs due to the absence of a CH_2 bending band near 1475 cm^{-1} in the mid-infrared. The CO_2^- combination can be seen as a high-frequency shoulder in the absorption, and negative VCD intensity of the appropriate magnitude and frequency ($\sim$ 3025 cm^{-1}) is present. The absorption band at 3010 cm^{-1} is assigned to the antisymmetric CH_2 stretch, while the symmetric stretch appears at 2955 cm^{-1}. Both appear to contribute small positive VCD intensity, as in serine. The methine stretch is taken to be located at 2975 cm^{-1}, corresponding to the maximum of the VCD spectrum and the absorption intensity filling the gap between the methylene modes.

The magnitudes of absorption and VCD intensities for serine and cysteine are strikingly different. The VCD intensity of serine is only one-third that of cysteine even though the absorption intensities of serine are twice that of cysteine. The intensities thus may be quite sensitive to conformational preferences and intramolecular interactions since otherwise serine and cysteine would be expected to be chemically similar. Both VCD spectra are due predominantly to $C_\alpha H$ stretching VCD. The factor of 3 increase in magnitude of peak VCD intensity and VCD bias in cysteine may be related to the strength of the intramolecular ring current. It is likely that hydrogen bonding of the OD group within rotamer III alters the nature of the ND_3^+–CO_2^- association in a manner that reduces or delocalizes the vibrational ring current. This does not occur in cysteine where an above-average methine VCD is present; the methine VCD intensity in serine is well below average.

The hydrochloride of cysteine exhibits a remarkably altered VCD spectrum compared to that of cysteine. The protonation of the CO_2^- group and association of the ND_3^+ and Cl^- apparently disrupt the intramolecular bond between the CO_2^- and ND_3^+ groups in the free base and lead to the loss of positive methine VCD intensity. In addition the CO_2^- combination band and negative VCD near 3030 cm^{-1} are absent in the hydrochloride. The remaining VCD intensity has only a small VCD bias and can be regarded as arising from the coupling of CH stretching motions of the α-hydrogen and the two β-hydrogens. Here the $C_\alpha H$ stretching mode contributes negative VCD, and the methylene modes give positive VCD. Although we have not yet carried out detailed measurements of VCD as a function of pD, by obtaining spectra for cysteine and its hydrochloride, we have an indication that the positive VCD intensity of the $C_\alpha H$ mode and the overall strong positive VCD bias may be lost in the amino acids at low pD values.

The Cu(II) complex of serine also shown in Figure 3 exhibits very similar features to those of free serine, except for the absence of the negative VCD absorption band at 3030 cm^{-1} due to the CO_2^- group. There is an approximate doubling of the absorption intensity in keeping with two serine ligands per complex; however, the VCD has increased by a factor of 5. Again, the enhancement of VCD intensity may be due to an enhanced intramolecular ring current, as pointed out for the alanine complexes. The methylene modes and the Fermi overtone are also enhanced, indicating methine stretching character or induced vibrational ring current for these modes. The increase in the absorption intensity of the broad band at 2955 cm^{-1} relative to the Fermi band at 2895 cm^{-1} appears to be due to the shift of the methine from 2970 cm^{-1} in free serine to 2950 cm^{-1} in the complex, again as was observed in alanine. The VCD maximum accordingly shifts to 2950 cm^{-1} in the complex. Finally we note that the VCD bias is 900 L cm^{-2} mol^{-1} for the complex or 450 L cm^{-2} mol^{-1} per ligand. The latter is nearly 4 times the value for free serine. It is also the lowest bias value of the metal complexes investigated and implicates a rotameric influence that also results in serine having the smallest VCD maximum (except glycine) of the free base amino acids investigated.

Remaining Amino Acids. Spectra of the remaining amino acids and Cu complexes listed in Tables I and II have been obtained[6] but will not be presented here. Due to their increased complexity, assignments and analyses comparable in detail to those presented above are not yet possible. Nevertheless, a number of interesting correlations with structure and conformational preference have been noted and these will be the subject of future publications. The conformations I′, II′, and III′ in Table I correspond to amino acids having only one β-hydrogen which in turn is trans to ND_3^+, H_α, and CO_2^-, respectively. The rotameric preferences of these amino acids are given in parentheses in Table I.

Briefly, the VCD spectra of asparagine, glutamine, phenylalanine, histidine, valine,[7] methionine, lysine deuteriochloride, and arginine deuteriochloride are broad and largely positive. The hydrochloride component of arginine and lysine is associated with the amine function on the side chain only and does not disrupt the VCD bias, as in the case of cysteine deuteriochloride. The VCD spectra of leucine, isoleucine, threonine, *allo*-threonine, pencillamine, proline, hydroxyproline, and *allo*-hydroxyproline are in general stronger and more varied in their VCD intensity patterns.

IV. VCD Calculations

The results of the previous section support the view that the VCD spectra of the amino acids in the CH stretching region can be understood in terms of three kinds of contributions: (1) VCD from the methine $C_\alpha H$ stretching vibration alone, (2) VCD from coupling among the aliphatic CH stretching modes, and (3) VCD from the combination and overtone vibrations. The first contribution results in the large positive VCD bias observed in all of these spectra at neutral pD, except glycine, and is proposed to arise from the ring current mechanism involving an intramolecular hydrogen bond, as discussed previously[7] and above. The second VCD contribution arises from the coupling of the stretching motions in the various CH bonds among themselves. We assume that for this contribution, the CH stretching motion in the side chain does not activate the ring current mechanism and that the bias of this contribution to the VCD spectrum is near zero. When the motion of the $C_\alpha H$ bond is included, without its ring current contribution, one obtains a perfect nuclear following description for this part of the VCD intensity which can be described by the FPC model.[24] This model has been shown generally to work well in the absence of electronic contributions to the vibrational motion that are distinct from the nuclear motion. The third contribution to the VCD spectra cannot be accurately calculated by any VCD model developed to date.[22] Reliable correlations between intensities for overtones and combination bands and theoretical predictions have only been made in the context of intensity borrowing from fundamental transitions due to Fermi resonance. In the case of glycine-C_α-d_1 in Figure 1, however, the VCD appears to arise directly from the combination bands without the borrowing of VCD intensity from a more intense fundamental. In fact, the negative VCD bias for glycine arises primarily from these combination bands, and the VCD is close to zero for the lone, fundamental $C_\alpha H$ stretching mode.

In order to understand the loss of VCD bias in glycine, as well as the patterns of VCD superimposed on the $C_\alpha H$ methine contributions, that results from coupled CH side-chain motions in the other amino acids, we have carried out two sets of VCD calculations. The observed VCD spectra result from the superposition of bands of varying half-widths and sign. A direct numerical comparison with calculated rotational strengths, which are proportional to the integrated intensities of individual vibrations, requires a knowledge of band shapes, widths, and positions, which lies beyond the goal of the present analysis. The calculated rotational strengths reported here can, however, be qualitatively compared with the observed sign patterns and sense and degree of VCD bias.

In the first set of calculations we have extended the LMO–VCD calculations of alanine that successfully accounted for the observed VCD bias in L-alanine-N-d_3 and L-alanine-C_β-d_3-N-d_3 to the $C_\alpha H$ stretching mode of (*S*)-(−)-glycine-C_α-d_1-N-d_3. After modifying the alanine force field to fit the observed $C_\alpha H$ stretching frequency in glycine, we carried out LMO–VCD calculations as a function of the CO_2^- torsion angle. The results of these calculations are given in Table III for three torsion angles. The angle listed as 0° corresponds to an orientation in which one of the oxygens of CO_2^- is directed midway between two opposing amine deuteriums

Table III. Calculated Infrared Absorption and VCD Intensities for the $C_\alpha H$ Stretching Mode of (*S*)-(−)-Glycine-C_α-d_1-*N*-d_3 vs. CO_2^- Torsion Angle

torsion angle of CO_2^-, deg	rot. strength × 10^{44}, esu^2 cm^2	dipole strength × 10^{39}, esu^2 cm^2
0	0.275	2.58
20	0.505	3.00
90	−3.39	3.41

as in the glycine crystal structure,[31] thereby forming a bifurcated hydrogen bond. In reference to **1** with R equal to deuterium, the plane of the CO_2^- group bisects the $DC_\alpha H$ angle as well as the DND angle of the amine. If the CO_2^- group is rotated clockwise about the C_α–CO_2^- bond by 20° as viewed in **1**, a more direct hydrogen bond is formed with the lower of the hydrogen-bonding deuteriums in ND_3^+. This orientation corresponds to that found in the alanine crystal structure and is that used in the LMO–VCD calculations for alanine referred to above.[5c] We notice in Table III that the rotational strength for glycine increases from a small positive value at 0° to a somewhat larger positive value at 20°. These values are approximately an order of magnitude smaller than those calculated for alanine. If the torsion angle is increased to 90° so that hydrogen–bonding interactions with the amino are minimized, a rather large negative rotational strength is calculated.

Since little if any VCD is observed in the $C_\alpha H$ stretching band of glycine in Figure 1, these calculations are consistent with a torsion angle near 0°, where the rotational strength is calculated to be the smallest of the three angles. The torsion angle of 20°, where hydrogen bonding to a single deuterium is dominant, yields the larger positive rotational strength, but this value is still small relative to that for alanine and is also consistent with the experimental results for glycine. The minimal hydrogen-bonding orientation at 90° is the least probable orientation according to these calculations.

As a final point concerning the LMO–VCD calculations of (*S*)-(−)-glycine-C_α-d_1-*N*-d_3, we note that for the same conformation a major difference between this calculation and the LMO–VCD calculation of L-alanine-C_β-d_3-*N*-d_3 is the presence of LMO displacements in the β-carbon bonds of alanine. We thus attribute the stronger calculated and observed VCD bias in alanine compared to glycine to be due to the presence of a β-carbon in the former. In glycine there is only vibrational or nuclear chirality, whereas in alanine and all the other amino acids in Table I the presence of a β-carbon provides a chirality in electronic structure as well. Apparently, electronic chirality due to the β-carbon is an important factor in the large magnitude of the positive VCD bias of the L-amino acids.

In the second set of calculations we have used the FPC model to determine a perfect following contribution for lactic acid, serine, and cysteine. As discussed above, these contributions are to be combined with the positively biased lone methine contribution (and modified by considerations involving overtones and combination bands) to obtain a VCD spectrum to be compared with experiment. In a previous publication[5b] we showed that for alanine the FPC model predicts a (+−+−) sign pattern from high to low frequency for the two $\nu_{CH_3}^{as}$ modes, the $\nu_{C_\alpha H}$ mode, and the $\nu_{CH_3}^{s}$ mode. The FPC result gives nearly zero bias but the observed VCD spectrum can be understood as a superposition of the perfect following pattern (FPC) and the strong, positive $C_\alpha H$ ring current contribution.

A similar VCD result is obtained for lactic acid. In Table IV, the perfect following pattern determined from an FPC calculation is seen to yield a (+−+−) contribution; however, in lactic acid the $C_\alpha H$ stretch becomes the lowest frequency mode. Even though the $\nu_{C_\alpha H}$ and $\nu_{CH_3}^{s}$ frequencies are reversed compared to those for alanine, the pattern is still the same. When the positive methine ring current is included the pattern becomes (+−++), as observed, and the spectrum is now positively biased. Note that by itself the perfect following contribution possesses nearly zero VCD bias.

Table IV. Perfect Following VCD Patterns for Coupled CH Stretching Motions

molecule	freq, cm^{-1} obsd VCD[a]	freq, cm^{-1} calcd	rot. strength × 10^{44}, esu^2 cm^2
lactic acid	3010 ($\nu_{CH_3}^{as}$)	3005	+0.17
	2992 ($\nu_{CH_3}^{as}$)	2992	−0.45
	2948 ($\nu_{CH_3}^{s}$)	2933	+0.76
	2920 ($\nu_{C_\alpha H}$)	2914	−0.42
	2890 ($2\delta_{CH_3}$)		
serine	2990 ($\nu_{CH_2}^{as}$)	2993	+0.81
	2970 ($\nu_{C_\alpha H}$)	2969	−3.12
	2950 ($\nu_{CH_2}^{s}$)	2948	+2.30
	2895 ($2\delta_{CH_2}$)		
cysteine	3010 ($\nu_{CH_2}^{as}$)	3007	+0.54
	2975 ($\nu_{C_\alpha H}$)	2975	−3.61
	2955 ($\nu_{CH_2}^{s}$)	2960	+3.12

[a] Vibrational assignments given in parentheses.

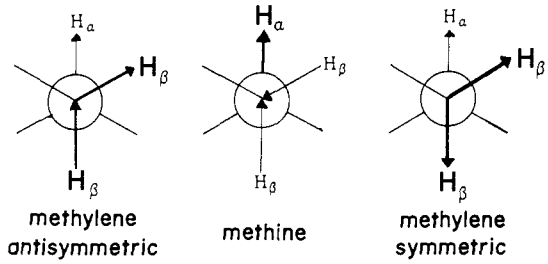


Figure 4. Results of model calculations of the $C_\alpha HC_\beta H_2$ fragment for the rotamer I conformation. The relative phasing of the local CH stretching moments results from the frequency ordering of the three normal modes. The projections show for the ordering ($\nu_{CH_2}^{as}$, $\nu_{C_\alpha H}$, $\nu_{C_\beta H_2}^{s}$) from high to low frequency that the VCD patterin is (+−+). Principal motions are depicted by heavier arrows than secondary coupled motions.

By further modification of the alanine (lactic acid) force field, perfect following simulations have been carried out for serine and cysteine. The results are provided in Table IV. The calculations were carried out by substitution of one of the β-hydrogens in alanine with a deuterium. The resulting structure possesses two β-hydrogens and an α-hydrogen, in correspondence to serine and cysteine. Consideration of the three possible conformers of these amino acids (I, II, and III as discussed earlier) reveals that I and II possess $C_\alpha HC_\beta H_2$ fragments with opposite chirality, while conformer III possesses a $C_\alpha HC_\beta H_2$ fragment that is achiral and is expected to have only a small perfect following contribution. Referring to Table I, we see that both serine and cysteine have a preference for conformer I over II, and hence the observed VCD pattern should contain a perfect following contribution corresponding to that of conformer I.

Vibrational analysis yields the relative CH stretching motion depicted in Figure 4 for both the serine and cysteine simulations. The FPC VCD results in Table IV can be understood directly from this figure since only the gauche hydrogens possess a chiral disposition. Employing the simple coupled oscillator-like expression $\mathbf{R}_{\alpha\beta}\cdot\boldsymbol{\mu}_\alpha \times \boldsymbol{\mu}_\beta$ for the chiral pairs of hydrogens yields the qualitative sign pattern (+−+) by inspection. When the methine ring current contribution is added to this result, the observed VCD pattern is obtained, namely (+++). As a final step, the negative CO_2^- combination band near 3025 cm^{-1} needs to be included.

The VCD spectrum of cysteine deuteriochloride in Figure 3 gives a clear glimpse of the perfect following contribution devoid of interference from the methine ring current contribution. The bias is negligible and the (+−+) sign pattern is quite evident. Even a small negative VCD intensity due to the methine $C_\alpha H$ stretching mode (opposite in sign to the ring current contribution) is present.

V. Discussion

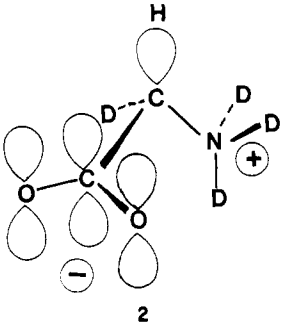
The experimental and theoretical results presented above demonstrate the importance of considering an intramolecular ring current as a unique source of VCD intensity for the interpretation

(31) Lehmann, M. S.; Koetzl, T. F.; Hamilton, W. C. *J. Am. Chem. Soc.* **1972**, *94*, 2657.

of amino acid spectra in the CH stretching region. Such a ring current is induced by the motion of the $C_\alpha H$ stretching mode and is sustained by an intramolecular association (hydrogen bond or transition-metal bridge) between the carboxylic and amine functional groups, both of which are attached to the α-carbon. The remaining VCD features due to fundamentals can be explained qualitatively in terms of a perfect following contribution of CH stretching modes involving the side-chain and α-carbon hydrogens.

A variety of specific points can be enumerated that collectively imply that the conformation of the amino acids in neutral pH (pD) must allow an intramolecular hydrogen bond to form between the zwitterionic CO_2^- and ND_3^+ groups. The evidence for such a bond consists of the following: (1) a positive VCD bias for all of the amino acids, except glycine-*C*-d_1, at neutral pD, (2) the strengthening of VCD bias for the transition-metal complexes, (3) the similarity of VCD patterns for the Cu complexes and the free amino acids and the stereochemical similarity of the amino acid conformations in the complexes to the proposed hydrogen-bonded conformation, (4) LMO–VCD calculations of alanine-*N*-d_3, alanine-C_α-d_1-*N*-d_3, and glycine-C_α-d_1-*N*-d_3 that are consistent with a hydrogen-bonding orientation of the CO_2^- group, (5) the loss of VCD bias for cysteine deuteriochloride where intramolecular hydrogen bonding may be disturbed at low pD, (6) the strength of absorption and VCD intensity for combination bands involving the CO_2^- stretching modes and $C_\alpha H$ bending modes (vide infra), and (7) NMR rotameric preferences about the C_α–C_β bond that show small populations for conformer II, where the bulky substituent occupies space that would be sterically hindered by the non-hydrogen-bonding oxygen in CO_2^- when the entire group is in a hydrogen-bonding orientation.

The first five points in this list have already received extensive discussion, whereas the last two points merit further consideration. We have noted earlier that strong combination bands contribute to the VCD spectra of glycine-*C*-d_1-*N*-d_3 and alanine-C_β-d_3-*N*-d_3 that arise from the antisymmetric CO_2^- stretch and the $C_\alpha H$ bending modes. With the CO_2^- group in a position to form a hydrogen bond with the amine, a degree of π-overlap will occur between the $C_\alpha H$ bond and the π-orbital on the CO_2^- carbon atom, as depicted in **2**.



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Rotation of the CO_2^- group away from this orientation sharply reduces this overlap and its source of electronic and vibrational coupling. It can be seen that CO stretching motion that causes the carboxylic carbon to move with respect to the $C_\alpha H$ bond changes the π-overlap and allows coupling with the $C_\alpha H$ bending motions.

The sources of absorption and VCD intensity of the combination bands in the 3000-cm^{-1} region can be further understood from studies of the mid-infrared fundamentals in alanine from which the combination bands arise. The antisymmetric CO_2^- stretching mode, which contributes to all three combination bands, is by far the most intense infrared fundamental. The normal coordinate analysis for alanine, referred to above, closely fits the frequencies of five alanine isotopomers. The calculated nuclear trajectories for alanine-*N*-d_3 show a 10–20% contribution of methine bending to the carboxylate modes at 1607 and 1409 cm^{-1} and a corresponding 10–20% contribution of carboxylate stretching to the methine bending modes at 1337 and 1291 cm^{-1}. Due to this mixing, it is more probable that large anharmonic terms in the potential are present to provide significant intrinsic intensity for combination bands involving these four modes.[32] We have further observed[33] that the sign of the VCD of the methine bending modes in alanine-*N*-d_3, 1337 (−) and 1291 cm^{-1} (+), is the same as that of the corresponding combination band VCD in glycine, 2940 (−) and 2888 cm^{-1} (+). Most probably, the negative VCD intensity of the $\nu_{CO_2^-}{}^{as} + \nu_{CO_2^-}{}^{s}$ band derives from the contribution to this combination mode of methine bending, rather than the motion of planar CO_2^-.

The glycine VCD spectrum provides the first observation of overtone or combination band VCD in the CH-stretching region that clearly does not derive from Fermi resonance borrowing from a fundamental. Such intrinsic VCD contributions can occur in this region in other molecules as well and should be considered when interpreting CH-stretching VCD spectra.

For the last point in the list we note from Table I that NMR data support the following general conclusions. The rotameric populations of the amino acids display a consistency to place, whenever possible, β-hydrogens trans to the ND_3^+ and to avoid the placement of bulky substituents in that position. It is interesting to note that this preference is consistent with a CO_2^- torsion angle in which one oxygen points toward the ND_3^+ group (forming a hydrogen bond) and the other by necessity points back in a manner that sterically interferes with substituents on the β-carbon that are trans to the ND_3^+ group.

While the terms intramolecular hydrogen bond and vibrationally induced ring current are rather specific in their description of the VCD mechanism that we have proposed, a more quantitative measure of their strength and spatial extent has not been presented. Clearly such a refinement would be of considerable interest in relation to this new VCD intensity mechanism as well as a more detailed understanding of the conformational details of amino acids in solution. A major question of theoretical interest that needs to be resolved is the extent to which electronic current density moves around the ring during the vibrational motion. Due to the circular nature of such motion, is there a component of the current that travels around the ring without changing the electron density anywhere in the ring? Since the LMO model describes electronic current in terms of displacements of centroids of charge, how completely can the LMO describe ring currents? Recently we developed a new approach to the calculation of VCD intensities that involves the application of non-Born–Oppenheimer vibronic coupling expressions for the calculation of VCD intensities.[34] It is our expectation that these new theoretical expressions will provide the means to answer the provocative questions posed above regarding the nature of electronic ring currents in amino acids and related molecules.

In order to further resolve remaining conformational and mechanistic details in the amino acids additional experimental and theoretical work is necessary. In particular VCD spectra as a function of pD and other transition metals will reveal useful data relating to conformation and to the ring current mechanism. Direct calculations of vibrationally induced currents[34] will answer the questions posed above regarding the nature of ring currents. And finally, selective deuteration of side-chain hydrogens will simplify the VCD spectra of the more complex amino acids and lead to an improved relation between VCD and rotameric populations about the C_α–C_β bond.

Although the hypothesis of a ring current mechanism as an explanation for the VCD intensity bias observed in the CH stretching region of the amino acids and related molecules is so far consistent with experiment, it must be pointed out that we have not yet proven that ring currents are the actual source of the effect. Until theoretical calculations have been performed which demonstrate that ring currents are present and responsible for the VCD bias and until other possible VCD mechanisms, such as the dipole-induced dipole mechanism,[35] are ruled out, the concept of

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vibrationally induced ring currents must remain a working hypothesis and a conceptual model. It can be stated, however, that more recent data obtained in our laboratory continue to be consistent with the ring current hypothesis.

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Registry No. Bis(L-alaninato)copper(II), 14263-10-6; (Δ)α'-tris(L-alaninato)cobalt(III), 28167-90-0; bis(L-serinato)copper(II), 14221-45-5; bis(L-valinato)copper(II), 14267-13-1; bis(L-threoninato)copper(II), 15491-47-1; bis(L-prolinato)copper(II), 30955-20-5; glycine-C_α-d_1, 62061-52-3; alanine-C_β-d_3, 63546-27-0; alanine, 56-41-7; lactic acid, 79-33-4; serine, 56-45-1; cysteine, 52-90-4; cysteine deuteriochloride, 94706-34-0; asparagine, 70-47-3; glytamine, 56-85-9; phenylalanine, 63-91-2; histidine, 71-00-1; valine, 72-18-4; leucine, 61-90-5; isoleucine, 73-32-5; threonine, 72-19-5; *allo*-threonine, 2676-21-3; penicillamine, 52-67-5; methionine, 63-68-3; lysine deuteriochloride, 94706-35-1; arginine deuteriochloride, 94706-36-2; proline, 147-85-3; 4-hydroxyproline, 51-35-4; *allo*-4-hydroxyproline, 618-27-9.

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Intramolecular Selectivity of the Alkylation of Substituted Anilines by Gaseous Cations

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Abstract: The intramolecular selectivity of the electrophilic reactions of Et^+, i-Pr^+, and Me_2F^+ cations with substituted anilines, including *m*-toluidine, *m*-anisidine, and *m*- and *p*-fluoroaniline, has been investigated in the dilute gas state at pressures ranging from 100 to 720 torr by a radiolytic technique, complemented by chemical ionization mass spectrometry. The results indicate an appreciable kinetic bias for the nitrogen atom, leading to predominant N-methylation by Me_2F^+. The reactivity of the carbenium ions is complicated by the simultaneous occurrence of proton transfer, in particular to the NH_2 group, which increases the relative extent of ring alkylation. The positional selectivity is characterized, aside from the usual orienting effects of the substituents, by the enhanced reactivity of the ring positions ortho to an n-type substituent, irrespective of its activating or deactivating properties. The effect is traced to the preliminary formation of an electrostatic adduct between the aniline and the gaseous electrophile.

The current interest in gas-phase ionic reactions is largely associated with their recognized value as simplified and generalized models of the corresponding processes occurring in solution. In particular cases, experiments carried out in the dilute gas state may even represent the only practicable approach to the mechanistic study of specific reactions. A case in point is the attack of electrophilic carbon on aromatic amines, whose conventional study in solution is complicated by the ionization of the NH_2 group promoted by the acids used as reactants or catalysts in Friedel-Crafts reactions. As a consequence, the very nature of the substrate is affected, which largely precludes the mechanistic study of alkylation of *free* anilines.[1]

A recent paper from this laboratory has reported a radiolytic and mass spectrometric study of the alkylation of the model substrate, $C_6H_5NH_2$, with simple cations in the dilute gas state.[2] The radiolytic approach proved particularly useful, providing direct information on the positional selectivity of the substitution measured under conditions of kinetic control, a feature of paramount relevance to allow meaningful comparison with solution-chemistry studies. It should be noted in this connection that the results obtained with structurally diagnostic mass spectrometric techniques, e.g., MS/MS, have been found to reflect thermodynamic, rather than kinetic, control.[3]

The present paper reports extension of the study to substituted anilines, namely *m*-toluidine, *m*-anisidine, and *m*- and *p*-fluoroaniline, to focus the role of n-type substituents in dictating the positional selectivity of the gas-phase alkylation and to gather additional evidence on the direct partecipation mechanism suggested by previous results.[2,4]

The reagents used, i.e., Et^+, i-Pr^+, and Me_2F^+ ions, have been obtained respectively from the radiolysis of CH_4 (or C_2H_6), C_3H_8, and CH_3F, according to a technique extensively applied to the study of gas-phase aromatic substitution, whose principles and results have been recently reviewed.[5]

Experimental Section

Materials. The gases uses were research grade products from Matheson Gas Products Inc., with a stated purity of 99.99 mol %. Fluka AG and Merk Co. were the source of the amines. *m*-Toluidine, *m*-anisidine, and *m*- and *p*-fluoroaniline were research-grade chemicals and were freshly redistilled before use, *m*-anisidine being further purified by preparative GLC. The alkylated anilines used as reference standards in the analysis of the products by GLC and GLC/MS were obtained from Fluka AG or synthesized according to unexceptional procedures.

Procedure. The gaseous samples were prepared by conventional vacuum techniques in a greaseless system as described elsewhere[5] and enclosed in sealed 250-mL Pyrex ampules. The γ irradiation was carried out to a total dose of 2.5 Mrad, delivered at the rate of 1.06 Mrad h^{-1}, in a 220 Gammacell from Nuclear Canada Ltd., within a thermostat set

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