Analysis of Vibrational Structure in the Near-Ultraviolet Circular Dichroism and Absorption Spectra of Phenylalanine and Its Derivatives[1]

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Abstract: The circular dichroism (CD) and absorption of L-phenylalanine, N-acetyl-L-phenylalanine amide, and N-acetyl-L-phenylalanine methyl and ethyl esters were recorded at 298 and 77°K. The signs and intensities of the CD bands varied greatly among these compounds. However, all CD spectra revealed similar vibrational perturbations arising from the benzyl moiety. 0–0 and 0 + 520 cm^{-1} transitions occurred in both CD and absorption spectra, whereas a 0 + 180 cm^{-1} band appeared only in CD. These three transitions started progressions with 930-cm^{-1} spacing to shorter wavelengths. The 930-cm^{-1} vibration did not affect either the sign or intensity of CD. In contrast, the 180- and 520-cm^{-1} vibrations always altered the CD intensity relative to that of the 0–0 band. In many cases, these vibrations also reversed the CD sign. By using toluene as a model for the benzyl chromophore and assuming a local symmetry of C_{2v}, the symmetry species of the phenylalanine vibrational modes were identified: 930 cm^{-1}, A_1; 520 cm^{-1}, B_2; 180 cm^{-1}, either A_2 or B_1. Thus only the nontotally symmetrical vibrations appear to alter the CD of phenylalanine compounds. The total rotatory strength increased three- to eightfold when the temperature was reduced from 298 to 77°K. This finding suggests that a conformational equilibrium exists at 298°K.

The near-ultraviolet Cotton effects of L-phenylalanine (PhAla) were first described by Moscowitz, Rosenberg, and Hansen[2] on the basis of rotatory dispersion measurements. Recent improvements in circular dichroism (CD) instrumentation have permitted a more complete investigation of the optically active absorption bands in phenylalanine compounds. Vibrational fine structure has been observed in the CD spectra of PhAla,[3] N-acetyl-L-phenylalanine amide[4] (NAcPhAlaA), and phenylalanyl moieties in proteins.[5] In PhAla the prominent absorption bands at 264 and 258 mμ were found to be dichroic, but the minor absorption bands at 268 and 262 mμ were not.[3] On the other hand, in NAcPhAlaA[4] and in phenylalanyl moieties of proteins[5] only the weak absorption bands at 268 and 262 mμ were reported to be optically active.

This communication gives an analysis of the vibrational fine structure in the near-ultraviolet CD and absorption spectra of phenylalanine compounds. The relationship between CD and absorption bands is examined by using high-resolution spectra recorded at 77°K. The vibrational fine structure observed in these spectra is described in group theoretical terms. In addition, the influence of temperature upon rotatory strength is considered.

Experimental Section

Instrumentation. CD was recorded on a prototype of the Beckman Far UV-CD spectrophotometer using a computer of average transients.[6] Each memory unit in the computer stored the CD signal for a spectral band of 0.03 mμ. A relatively slow scanning speed (18 mμ/min) was used for the low-temperature CD measurements so that the photomultiplier servo mechanism could maintain a constant reference energy[6] while scanning through the very sharp phenylalanine absorption bands. The room temperature spectra also had to be recorded using the 18 mμ/min speed, because most of our xenon lamps contained a Hg impurity which produced a strong emission line at 253.7 mμ. This speed was sufficiently slow to permit maintaining a constant reference energy even while scanning through the Hg lines.

Absorption spectra were measured on a Cary Model 15 spectrophotometer.

The wavelength settings of both instruments were calibrated at 253.7 and 296.8 mμ by using a Hg lamp. The positions of sharp bands were accurate to within ±0.1 mμ for absorption and ±0.3 mμ for CD.

Low-Temperature CD and Absorption. Spectra of samples at 77°K were readily obtained in a specially constructed dewar having strain-free, flat, parallel, Suprasil windows.[7] Two precautions were necessary to obtain reliable low-temperature CD spectra. First the dewar had to be aligned with its windows perpendicular to the light beam. Secondly, all solutions had to be frozen without any significant increase in light scattering, because excessive scattering depolarized the light.

Two solvent systems were used. EPA (ethyl ether–isopentane–ethyl alcohol, 5:5:2, v:v:v) solutions gave completely transparent glasses for paths as long as 1 mm.[8] Although this solvent permitted excellent resolution of bands at 77°K, the rapid evaporation made quantitative measurements difficult. Similar resolution was obtained in 9 to 1 (v:v) methanol–glycerol glasses at 77°K and quantitative measurements were possible. All the compounds except PhAla readily dissolved in either EPA or 9 to 1 methanol–glycerol. PhAla spectra were obtained in 1 to 1 (v:v) methanol–glycerol. Methanol–glycerol solutions having 0.1–0.4-mm paths could be frozen without any significant increase in light scattering. Although some cracks formed in the methanol–glycerol glasses, the fractured surfaces were parallel to the direction of light and caused no significant depolarization.

The methanol–glycerol solutions had to be frozen slowly to minimize cracking. First liquid nitrogen was added to the reservoir in the bottom of the dewar. Next the holder containing the cuvette was positioned just above the liquid nitrogen for about 5 min to cool the sample. Then the bottom of the cuvette holder was placed in contact with the liquid nitrogen for about 10 min. Finally the sample was immersed in liquid nitrogen to ensure complete freezing. After another 5 min the liquid nitrogen was poured

(1) This work was supported by Contract AT (04-1) GEN-12 between the Atomic Energy Commission and the University of California.

(2) A. Moscowitz, A. Rosenberg, and A. E. Hansen, *J. Am. Chem. Soc.*, 87, 1813 (1965).

(3) M. Legrand and R. Viennet, *Bull. Soc. Chim. France*, 2798 (1966).

(4) N. S. Simmons, A. O. Barel, and A. N. Glazer, *Biopolymers*, in press.

(5) E. H. Strickland, E. Kay, L. M. Shannon, and J. Horwitz, *J. Biol. Chem.*, 243, 3560 (1968).

(6) J. Horwitz, E. H. Strickland, and E. Kay, *Anal. Biochem.*, 23, 363 (1968).

(7) Constructed by the J. F. Scanlon Co., Whittier, Calif.

(8) R. L. Sinsheimer, J. F. Scott, and J. R. Loofbourow, *J. Biol. Chem.*, 187, 299 (1950).

Table I. Absorption Bands of NAcPhAlaME in EPA at 77°K

λ, mμ	1/λ, cm^{-1}	Assignment, cm^{-1}	λ, mμ	1/λ, cm^{-1}	Assignment, cm^{-1}
267.7	37,360	0–0	264.0	37,880	0 + 520
261.1	38,290	0 + 930	259.0	38,610	0 + 520 + 750
256.2	39,030	0 + 750 + 930	257.7	38,800	0 + 520 + 930
254.9	39,230	0 + 2(930)	252.7	39,570	0 + 520 + 750 + 930
250.2	39,970	0 + 750 + 2(930)	251.8	39,710	0 + 520 + 2(930)
245.7	40,700	0 + 2(750) + 2(930)	247.5	40,400	0 + 520 + 750 + 2(930)
244.6	40,880	0 + 750 + 3(930)	242.5	41,240	0 + 520 + 2(750) + 2(930)
			241.8	41,360	0 + 520 + 750 + 3(930)

off until none was in the light path, but enough remained in the reservoir to contact the bottom of the cuvette holder. Spectra could be recorded for 6 to 8 min before all the nitrogen evaporated. By refilling the reservoir at 6- to 8-min intervals, the sample could be kept at 77°K for several hours.

In view of the complexity of the low-temperature spectra, all data are presented as photographs of the actual instrument tracings. Each record, which represents the average of 25 to 100 CD scans, was verified by at least one additional experiment.

Materials. PhAla, N-acetyl-L-phenylalanine (NAcPhAla), and N-acetyl-L-phenylalanine ethyl ester (NAcPhAlaEE) were Grade A from Sigma Chemical Co., St. Louis, Mo. NAcPhAlaA and N-acetyl-L-phenylalanine methyl ester (NAcPhAlaME) were Grade I from Cyclo Chemical Co., Los Angeles, Calif. EPA was obtained from American Instrument Co., Silver Spring, Md.

Results

Absorption Spectra. NAcPhAlaA, NAcPhAlaME, and NAcPhAlaEE were selected for high-resolution studies since these compounds dissolved readily in solvents which gave the greatest band sharpening at 77°K. Figure 1 shows the absorption spectrum of NAcPhAlaA in methanol–glycerol before and after freezing with liquid nitrogen. At 77°K the absorption bands are far better resolved than at 298°K and are also shifted to shorter wavelengths by about 0.5 mμ. The increased resolution of the 267.5- and 261.0-mμ absorption bands is especially striking. In addition, shoulders can be seen at 258.8, 255.8, 254.8, and 250.0 mμ; and a double peak is evident at 251.5 and 252.5 mμ.

The effect of temperature upon the intensity of the NAcPhAlaA absorption was evaluated by measuring the areas under the spectra in Figure 1. No difference in area was detected, implying that the intensity was unaffected by the temperature decrease.

The spectra of both NAcPhAlaME and NAcPhAlaEE in EPA at 77°K are identical with the curve for NAcPhAlaA (Figure 1), except for a 0.2-mμ red shift. NAcPhAlaME was selected to illustrate the assignment of the vibrational fine structure in the absorption spectra of phenylalanine compounds. Table I shows that the vibrational bands can be divided into two series. The first is based upon the lowest energy transition, the 0–0 band. The second series begins with 0 + 520 cm^{-1} band. By adding multiples of 930 and 750 cm^{-1} to either the 0–0 or the 0 + 520 cm^{-1} band, two progressions can be obtained. The 0–0, 0 + 930, 0 + 520, and 0 + 520 + 930 bands are relatively sharp and may represent essentially single transitions. However, at higher energies the bands are broader and less well resolved, which suggests the overlapping of several transitions.

Circular Dichroism Spectra. At 298°K in aqueous solutions the various phenylalanine compounds have different CD spectra—both in terms of the signs and positions of the individual bands. PhAla has positive CD bands (Figure 2). NAcPhAla (Figure 2), NAcPhAlaME, and NAcPhAlaEE have positive and negative bands. NAcPhAlaA has only negative bands.[4] More complete information about the transitions giving rise to these CD bands was obtained from high-resolution CD spectra at 77°K.

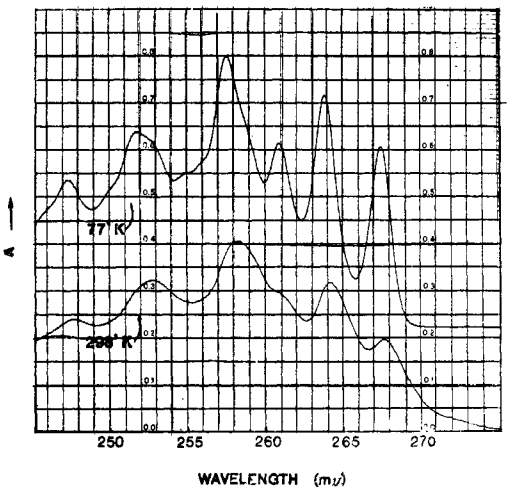

Figure 1. Instrument trace of absorption spectra of 180 m*M* NAcPhAlaA in methanol–glycerol (9:1, v:v) at 298 and 77°K. Path length, 0.1 mm; spectral half-band width (SHBW), less than 0.15 mμ. Base line for 77°K spectrum was offset 0.22 units to separate the two spectra. Both solvent base lines for these spectra were flat. The areas under the two curves were the same, within our experimental accuracy of ±15%.

N-Acetyl-L-phenylalanine amide in methanol–glycerol illustrates the increased resolution of CD bands at low temperature. At 298°K, NAcPhAlaA has negative bands at 267.5, 261, and 254.5 mμ (Figure 3). Freezing the solution sharpened the negative CD bands and revealed other bands not previously observed (Figure 3). Positive bands are evident at 263.7, 257.5, and 251.5 mμ. Shoulders can be seen at 259.5 and 253 mμ. The sharp change in curvature at 266 mμ suggests another band.

In the 77°K spectrum the major CD bands form two obvious progressions—an intense one with negative bands and a weak one with positive bands. The first two members of the negative progression coincide with the corresponding members of the 0–0 progression observed in the absorption spectrum (Table II). Positive CD bands coincide with the 0 + 520 and 0 + 520 + 930 cm^{-1} absorption bands. Table II further indicates the possible occurrence of a 0 + 180 cm^{-1} progression, because other CD spectra revealed that under some conditions this progression has intense

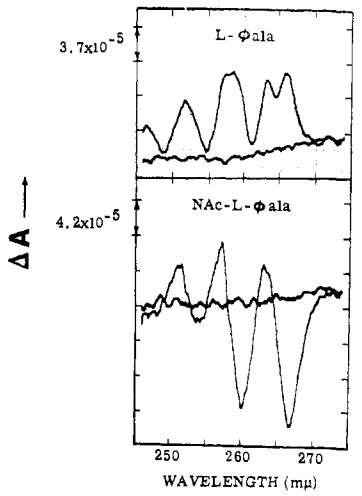

Figure 2. CD records of PhAla (top) and of NAcPhAla (bottom) in water (pH 6) at 298°K. Path length, 1.0 cm; SHBW, less than 0.8 mμ; time constant, 1 sec; concentrations, PhAla was 5.8 m*M* and NAcPhAla was 4.6 m*M*. The splitting of the 263- and 266-mμ CD bands in PhAla was established by averaging 100 scans so that the peak-to-peak noise was reduced to less than 1×10^{-5} ΔA. The NAcPhAla spectrum is the average of 25 scans.

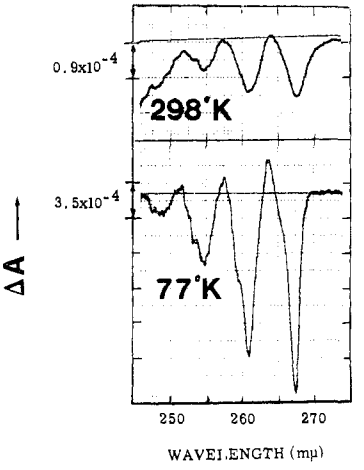

Figure 3. CD records of 220 m*M* NAcPhAlaA in methanol–glycerol (9:1, v:v) at 298 and 77°K. Path length, 0.2 mm; SHBW, less than 0.4 mμ; 25 scans; time constant, 1 sec for 298°K curve and 0.3 sec for 77°K curve. The base lines are indicated by straight lines. The area under the 77° spectrum was 8.2 times greater than that under the 298°K spectrum (from 275 to 257 mμ).

CD bands (see below). The CD shoulders at 266 and 259.5 mμ suggest that a very weak 0 + 180 cm^{-1} progression may also exist in NAcPhAlaA.

Table II. Resolved CD and Absorption Bands of NAcPhAlaA in Methanol–Glycerol (9:1, v:v) at 77°K

λ, mμ	1/λ, cm^{-1}	Assignment, cm^{-1}	CD sign intens[a]	Absptn intens[a]
267.5	37,380	0–0	− S	S
266.0	37,590	0 + 180	+ VW	ND
263.7	37,920	0 + 520	+ W	S
261.0	38,310	0 + 930	− S	S
259.5	38,530	0 + 180 + 930	+(?) VW[b]	ND
257.5	38,830	0 + 520 + 930	+ W	S

[a] VW, very weak; W, weak; S, strong; ND not detected. [b] The sign of this band is difficult to determine, owing to the sharpness and intensity of the neighboring band at 261 mμ.

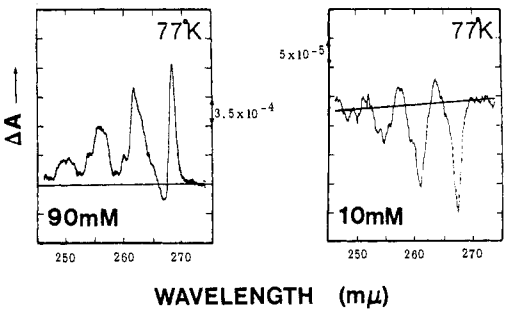

Figure 4. Two types of CD records given by NAcPhAlaA in EPA at 77°K. The spectrum of aggregated NAcPhAlaA (left) was obtained at concentrations of 45, 50, and 90 m*M*. The right-hand spectrum was obtained at concentrations of 10 and 25 m*M*. Path length, 0.4 mm; SHBW, less than 0.5 mμ; 25 scans; time constant, 0.3 sec for 90 m*M* and 0.6 sec for 10 m*M*. Base lines are indicated by straight lines.

The CD bands occurring below 257.5 mμ were not assigned to any progressions, since their broadness and reduced intensity may indicate that several transitions contribute to these bands. Furthermore, these CD bands lie about 1 mμ toward shorter wavelengths than the nearest absorption bands (Figure 1).

The CD spectra in Figure 3 demonstrate that the rotatory strength of the near-ultraviolet CD bands of NAcPhAlaA increases greatly at low temperature. The area of the CD curve at 77°K is eight times greater than that of the curve at 298°K.

Studies of NAcPhAlaA dissolved in EPA instead of methanol–glycerol revealed that not only the intensity but also the signs of the CD bands can change. Low concentrations of NAcPhAlaA in EPA gave the same CD spectrum as was obtained in methanol–glycerol at 77°K. However, after freezing EPA solutions which were nearly saturated with NAcPhAlaA at 298°K, the major CD bands had their signs reversed and were shifted toward the red (Figure 4). Four observations suggest that this abnormal CD spectrum resulted from aggregation (perhaps microcrystallization) of NAcPhAlaA during the cooling and freezing. (1) A 0.5-mμ red shift occurred in the absorption spectrum relative to that for low concentrations of NAcPhAlaA in EPA at 77°K. (2) The need for a high concentration suggests an intermolecular interaction. Furthermore, since NAcPhAlaA is not very soluble in EPA, the solutions may become supersaturated at low temperature. (3) Even though these EPA glasses were transparent to the eye, the absorption spectra revealed an unusually large amount of light scattering. (4) The CD spectrum of nearly saturated solutions of NAcPhAlaA in EPA at 298°K had the typical negative bands.

The aggregated NAcPhAlaA CD spectrum in Figure 4 shows positive bands at 268.2 and 261.6 mμ, a negative band at 266.7 mμ, positive shoulders at 264 and 263 mμ, and other bands at shorter wavelengths. Comparison of the CD and absorption spectra revealed that the 268.2- and 261.6-mμ bands belong to the 0–0 progression, which is red shifted (Table III). The 266.7-mμ CD band is approximately a 0 + 180 cm^{-1} transition. Apparently none of these CD bands results from contamination by unaggregated NAcPhAlA, because the absorption bands which are characteristic of the unaggregated state were not detected.

Table III. Resolved CD and Absorption Bands of Aggregated NAcPhAlaA in EPA at 77°K

λ, mμ	1/λ, cm⁻¹	Assignment, cm⁻¹	CD sign intens[a]	Absptn intens[a]
268.2	37,290	0–0	+ S	S
266.7	37,500	0 + 180	− W	ND
264.5	37,810	0 + 520	+ W	S
263	38,020	0 + 730	+ W	ND
261.6	38,220	0 + 930	+ S	S

[a] S, strong; W, weak; ND, not detected in absorption spectrum.

NAcPhAla, NAcPhAlaME, and NAcPhAlaEE in EPA at 77°K possess fine-structure CD bands similar to those described in Table II for NAcPhAlaA *in methanol–glycerol.*

L-Phenylalanine CD spectra recorded in glycerol–methanol (Figure 5) reveal, in addition to the positive bands observed in water (Figure 2), a series of negative bands. The 298°K spectrum in Figure 5 suggests three progressions: 0–0, 0 + 180, and 0 + 520 cm⁻¹ (Table IV). Freezing the glycerol–methanol solution markedly enhanced the positive CD bands at 266.4 and 260.2 mμ (Figure 5). The positive bands at 264 and 258 mμ were sharpened to a lesser extent. At 77°K no negative bands are evident. Thus the most intense CD bands of PhAla at 77°K belong to the 0 + 180 cm⁻¹ progression (Table IV). This progression of CD bands does not coincide with any progression observed in the absorption spectrum.

Table IV. Resolved CD and Absorption Bands of PhAla in Methanol–Glycerol (1:1, v:v)

Temp,[a] °K	λ, mμ	1/λ, cm⁻¹	Assignment, cm⁻¹	CD sign intens[b]	Absptn intens[b]
298	268	37,310	0–0[c]	− S	W
	266.7	37,500	0 + 180	+ W	ND
	264.4	37,820	0 + 520	+ S	S
	261.7	38,210	0 + 930[c]	− S	ND
	260	38,460	0 + 180 + 930	+ W	ND
	258.6	38,670	0 + 520 + 930	+ S	S
77	267.7	37,360	0–0[c]	ND	S
	266.4	37,540	0 + 180	+ S	ND
	264.0	37,880	0 + 520	+ W	S
	261.1	38,300	0 + 930[c]	ND	W
	260.2	38,430	0 + 180 + 930	+ S	ND
	257.7	38,800	0 + 520 + 930	+ W	S

[a] Temperature. [b] W, weak; S, strong; ND, not detected. [c] The loss of these negative CD bands after cooling PhAla raises the possibility of a contribution from hot bands. However, temperature was not the only factor which caused these bands to be weak; they were not observed in aqueous solutions at 298°K. A more likely explanation appears to be that all phenylalanine compounds have a 0–0 CD progression whose intensity varies.

Discussion

An analysis of the vibronic transitions in the absorption spectrum of phenylalanine compounds permits identifying the transitions giving rise to most of the CD bands. In evaluating the near-ultraviolet absorption, we consider phenylalanine to be a derivative of toluene, as was first proposed by Sponer.[9] Our investigation extends Sponer's earlier study in two significant respects. First our analysis correlates both high-resolution absorption and CD spectra, whereas

(9) H. Sponer, *J. Chem. Phys.*, 10, 672 (1942).

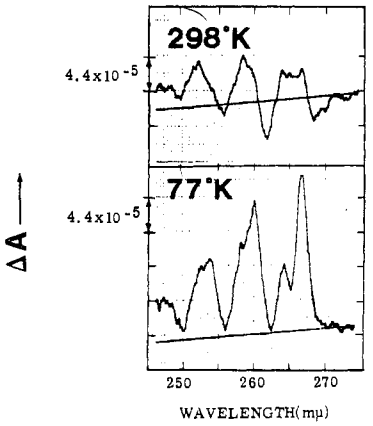

Figure 5. CD records of 190 m*M* PhAla dissolved in glycerol–methanol (1:1, v:v) at 298 and 77°K. Path length, 0.2 mm; SHBW, less than 0.5 mμ; 25 scans; time constant, 1 sec for 298°K and 0.6 sec for 77°K. The area under the 77°K curve was three times greater than that under the 298°K curve. Base lines are indicated by straight lines. The negative CD bands observed in the 298°K spectrum were also obtained using 5 m*M* PhAla dissolved in glycerol–methanol (1:1, v:v).

Sponer had access only to a low-resolution absorption spectrum. Secondly, in accordance with other investigators,[10,11] we take toluene to have C_{2v} symmetry instead of the C_s originally assigned by Sponer.

The low-temperature absorption spectra of PhAla and its derivatives revealed identical vibrational fine structure, as was expected since the near-ultraviolet absorption is determined almost entirely by the benzyl moiety.[9] Better resolution at 77°K was obtained for derivatives which dissolved readily in methanol–glycerol or EPA.

Most vibrational assignments in phenylalanine were based on an analogy with those which have been worked out for toluene vapor.[11] This procedure seems justified because the wavelength maxima of toluene frozen in EPA have about the same spacing as NAcPhAlaME. However, the intensity distribution in the toluene spectrum is different (see below).

The transitions which cause the two progressions observed in the phenylalanine absorption spectrum can be described in terms of group theory.[12] The longest wavelength absorption band at 77°K corresponds to the 0–0 transition. It is an $A_1 \rightarrow B_2$ electronic transition.[11,13] A progression occurs because changes in totally symmetrical vibrations (A_1 symmetry species) are superimposed on the electronic transition. The dominant A_1 vibrations observed in the phenylalanine absorption spectrum were 930 and 750 cm⁻¹. These wave numbers match excited-state vibrations 1 and 12, respectively, in the toluene vapor spectrum (see Table II in ref 11). The excellent agreement between the assignments (Table I) and the absorption spectrum of phenylalanine is partly fortuitous. For example, toluene vapor has additional fairly intense A_1 vibrations at 964 and 1189 cm⁻¹ (ref 11). Since bands as close as

(10) K. S. Pitzer and D. W. Scott, *J. Am. Chem. Soc.*, **65**, 803 (1943).
(11) N. Ginsburg, W. W. Robertson, and F. A. Matsen, *J. Chem. Phys.*, **14**, 511 (1946).
(12) A more detailed description is given in the Appendix.
(13) Since the 1940's the conventions for assigning symmetry species in the C_{2v} point group has been modified. The present B_2 state corresponds to the B_1 state in these references; our B_1 corresponds to their B_2. See H. H. Jaffé and M. Orchin, "Symmetry in Chemistry," John Wiley & Sons, Inc., New York, N. Y., 1965, p 160.

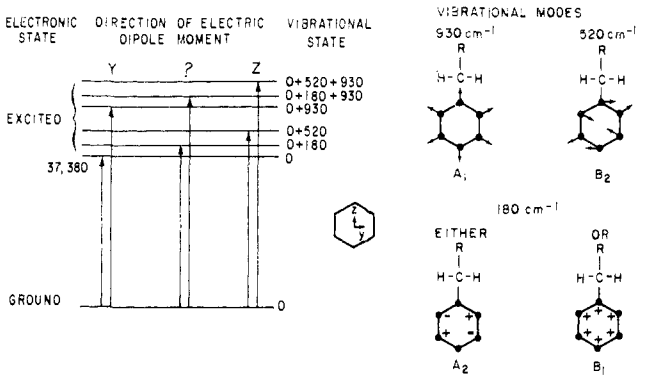

Figure 6. Well-resolved transitions occurring in the CD spectra of phenylalanine compounds. Weak and overlapping transitions have been omitted. The y and z axes lie in the plane of the phenyl ring, as indicated in diagram. The x axis is perpendicular to the ring. The vibrational modes are taken from Pitzer and Scott.[10,13] Plus and minus signs for A_2 and B_1 vibrations refer to motion along the x axis; arrows are in the yz plane.

34 cm^{-1} cannot be resolved in EPA at 77°K, the 930-cm^{-1} spacing in phenylalanine probably includes some contribution from a 964-cm^{-1} vibration. This overlapping of absorption bands is relatively minor in the 0 + 930 band and is entirely absent in the 0-0 band. Consequently, these absorption bands are sharp. However, at shorter wavelengths the number of possible combinations of totally symmetric vibrations increases rapidly.[14] This leads to considerable overlapping of different transitions and broadens the absorption bands of the higher members of the progression.

The 0 + 520 cm^{-1} transition in phenylalanine compounds gives rise to a second progression, having the same spacing as the 0–0 progression (Figure 6). The energy of the 520-cm^{-1} vibration in phenylalanine matches that of the strong vibration 6b (B_2 symmetry species) in the spectrum of toluene vapor.[11] This assignment in phenylalanine, however, cannot be made without considering the 0 + 456 cm^{-1} band, which is fairly intense in toluene vapor. In our spectrum of toluene frozen in EPA, the 0 + 456 cm^{-1} transition was not resolved from the 0 + 528 cm^{-1} transition. For these particular bands, however, toluene is not a suitable model for phenylalanine, because the intensity of the 0 + 456 cm^{-1} band is influenced by substitution in the methyl moiety. In both ethylbenzene and isopropylbenzene vapor the 0 + 528 cm^{-1} band is much more intense than the 0 + 456 cm^{-1} band.[15] Since phenylalanine is also a substituted toluene, the 0 + 456 cm^{-1} band should be relatively weak. Thus the 520 cm^{-1} vibration observed in phenylalanine belongs principally to the nontotally symmetric symmetry species B_2.

Group theoretical considerations indicate that a third progression of electric dipole allowed absorption bands may arise from a transition to an excited vibronic state containing a single A_2 nontotally symmetric vibration.[11] This progression must be extremely weak, however, because it has not been resolved either in the phenylalanine absorption spectrum at 77°K or in the toluene vapor spectrum.[11] Nevertheless, the 0 + 180 cm^{-1} progression of CD bands may indicate the positions of a third progression of electric dipole allowed absorption bands which are too weak to be resolved. Vibration 16a, which belongs to A_2 symmetry species, occurs at 240 cm^{-1} in the excited state of benzene[16] and would be expected to have about the same value in a monosubstituted benzene.[14] On the other hand, vibration 11 (B_1 symmetry species) probably also occurs near 200 cm^{-1} in the excited state.[11] This latter vibration is forbidden by symmetry rules from having a zero-order electric dipole transition, which could account for the absence of a 0 + 180 cm^{-1} progression in the absorption spectrum. Thus the 180-cm^{-1} vibration in phenylalanine may belong to either A_2 or B_1 symmetry species.

There are differences in the intensities of the two progressions observed in the phenylalanine absorption spectra. The 0 + 520 cm^{-1} progression is more intense than the 0–0 progression, which is well resolved only at 77°K. The relative intensities of these two progressions is reversed in toluene. The 0–0 progression is absent in benzene and occurs in monosubstituted benzenes only because the side chain hyperconjugates with the aromatic ring.[15] Therefore, the spectral distribution indicates that phenylalanine hyperconjugates less than toluene. This finding supports the assignment of C_{2v} symmetry to the benzyl moiety of phenylalanine.[2]

Symmetry properties are also reflected in the three progressions of CD bands observed in phenylalanine compounds. Each CD progression contained either all positive or all negative bands. The first two members of a progression had approximately the same CD intensity in relation to their absorption intensity. This finding suggests that the 930-cm^{-1} vibration did not affect either the sign or intensity of CD bands. In contrast, a single 180- or 520-cm^{-1} vibration frequently reversed the sign of a CD band and always changed its intensity relative to that of the 0–0 band. Therefore, only the nontotally symmetrical vibrations appear to alter the CD of phenylalanine compounds.

The relative intensities of the three progressions varied with the solvent, temperature, and any chemical substitutions on PhAla. In any given circumstance, one, two, or even three CD progressions could be fairly intense. These experimental results are in accord with Weigang's theoretical description of vibrational fine structure in CD bands.[17]

The progressions of CD bands could not be followed beyond the first two members of each progression. The other CD bands were located about 1 mμ on the short-wavelength side of the absorption bands that were resolved. This appears to be due to an inability to resolve single transitions at shorter wavelengths. The broadness of the absorption bands in the third and higher members of the absorption progressions indicates that more than one transition contributes to these bands. Effects of this type are especially destructive in CD progressions because overlapping of a positive and a negative transition tends to cancel circular dichroism. An overlapping of CD bands could arise from totally symmetrical vibrations other than 930

(14) H. Sponer and S. H. Wollman, *J. Chem. Phys.*, 9, 816 (1941).
(15) F. A. Matsen, W. W. Robertson, and R. L. Chuoke, *Chem. Rev.*, 41, 273 (1947).
(16) H. Sponer, G. Nordheim, A. L. Sklar, and E. Teller, *J. Chem. Phys.*, 7, 207 (1939).
(17) O. E. Weigang in "Developments in Applied Spectroscopy," Vol. 5, L. R. Pearson and E. L. Grove, Ed., Plenum Press, New York, N. Y., 1966, pp 259–281.

cm^{-1} being added to members of another progression, *e.g.*, 0 + 2(1189) = 0 + 520 + 2(930). Effects of this type may account for the weakness observed in the short-wavelength CD bands.

Group theoretical analysis indicates that the 0 + 520 cm^{-1} progression of CD bands must arise from an electric dipole allowed transition, because the zero-order magnetic dipole transition is forbidden by symmetry considerations.[12] Thus this progression of CD bands had to occur at the same wavelengths as a progression observed in the absorption spectrum. Group theoretical arguments allow the 0–0 progression of CD bands to be either the electric or magnetic dipole type. Two experimental results suggest that the 0–0 progression is an electric dipole allowed transition. First, in a number of cases the first two members of the 0–0 progression have CD and absorption bands of comparable intensity to those in the 0 + 520 progression. Secondly, the 0–0 CD bands coincide with well-resolved absorption bands. On the other hand, the 0 + 180 cm^{-1} progression of CD bands may be a magnetic dipole allowed transition, since no corresponding bands were resolved in the absorption spectra. If the 180-cm^{-1} vibration belongs to the B_1 symmetry species, then symmetry considerations indicate that the transition must be the magnetic type.[12] If this vibration belongs to A_2 symmetry species, either the electric or magnetic type is allowed.

The signs of the CD progressions are explained qualitatively by the static perturbation method, described by Schellman.[18] The circular dichroism of phenylalanine arises because the wave functions of the benzyl chromophore are perturbed by the amino acid moiety. Those perturbations which have the property of a pseudo scalar in the C_{2v} point group can induce circular dichroism. Acylation, esterification, or amidation of phenylalanine alters the electrostatic perturbation experienced by the benzyl moiety. This perturbation is also influenced by the orientation of the amino acid moiety relative to the benzyl group. Therefore, the sign of a CD progression is not necessarily an intrinsic property of each compound. This is illustrated by the sign reversal of the 0–0 CD progression in aggregates of NAcPhAlaA (Figure 4).

The conformational dependence of phenylalanine circular dichroism is further exemplified by the increase in intensity at low temperature. The total rotatory strength of phenylalanine compounds increased three- to eightfold at 77 °K relative to that at 298 °K. Since the total absorption intensity did not vary, the increased CD intensity must represent a change in conformation. Similar effects have been reported for some carbonyl compounds which exhibit a conformational equilibrium at 298 °K.[19] In a similar way each phenylalanine compound may have a number of conformations which are interconvertible by rotations about single bonds in the amino acid moiety. The room-temperature CD spectrum appears to represent the weighted average of all these conformations. For some, the CD bands of a given progression may be negative; for others the same CD bands may be positive. Thus at 298 °K the CD strength from some conformations would cancel that of others. When the temperature is lowered, the minimum energy conformation(s) would become more heavily populated, in accordance with the Boltzmann factor. The average CD intensity would increase because a much larger fraction of the phenylalanine molecules would have the same CD spectrum.

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Appendix

Symmetry considerations can be used to determine which electronic and magnetic dipole transition moments may be nonzero[20] in phenylalanine. In accord with the approach outlined by Sponer,[9] we treat phenylalanine as a benzyl radical perturbed by an amino acid moiety. The symmetry species of the electronic states in the benzyl radical can be determined from the symmetry species in benzene.[11,13,14] The substitution of a methyl group lowers the benzene symmetry from D_{6h} to C_{2v}.[10,11] In benzene the weak band near 260 mμ is due to an $A_{1g} \rightarrow B_{2u}$ electronic transition.[21] The symmetry species of the corresponding transition in the benzyl moiety can be obtained by comparing the properties of each symmetry operation in C_{2v} with the properties of the same symmetry operations in the D_{6h} point group. In C_{2v} the identity operation (I), the twofold rotation axis ($C_2(z)$), and the reflection planes $\sigma_v(xz)$ and $\sigma_v(yz)$ correspond to the symmetry operations designated I, C_2, σ_v, and σ_h, respectively, in D_{6h}. (Note that the coordinate systems are assigned differently in D_{6h} and C_{2v} point groups.[22]) For symmetry species B_{2u} in D_{6h}, I = +1, C_2 = −1, σ_v = −1, σ_h = +1. In C_{2v}, then I = +1, $C_2(z)$ = −1, $\sigma_v(xz)$ = −1, and $\sigma_v(yz)$ = +1, which corresponds to symmetry species B_2 (see Table V). Similarly, A_{1g} symmetry species in D_{6h} corresponds to A_1 species in C_{2v}. Thus the near-ultraviolet absorption band in the benzyl group is an $A_1 \rightarrow B_2$ electronic transition.[11,13]

A transition may occur if the excited-state wave function is of the same symmetry species as at least one component of the dipole moment operator.[20] For a purely electronic transition from ground-state A_1 to excited-state B_2, Table V shows that an electric dipole transition may occur (polarized in the y direction), since the y component of the electric dipole moment operator also belongs to symmetry species B_2. However, polarizations in the x or z direction are not possible for transitions to an excited state with symmetry B_2.

If the excited state is both electronically and vibrationally excited, the symmetry species of the excited-state wave function may be found by multiplying in the appropriate way the characters of the symmetry species of the electronic state by those of the symmetry species of the vibrational state.[23] If the B_2 electronic state is excited with a totally symmetrical vibration (A_1), the symmetry species of the excited state is $B_2 \times A_1$. This product can be evaluated by multiplying the

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(20) H. Suzuki, "Electronic Absorption Spectra and Geometry of Organic Molecules," Academic Press, New York, N. Y., 1967, p 70.
(21) Reference 20, p 71.
(22) H. H. Jaffé and M. Orchin, "Symmetry in Chemistry," John Wiley & Sons, Inc., New York, N. Y., 1965, pp 9–10.
(23) Reference 20, pp 50–52, 72–73.

Table V. Symmetry Properties of the Benzyl Moiety in Phenylalanine and Its Derivatives

Symmetry species	Properties of symmetry species of C_{2v} point group[a]: Under symmetry operations[b] I	$C_2(z)$	$\sigma_v(xz)$	$\sigma_v(yz)$	Under translation (electric dipole moment operator)	Under rotation (magnetic dipole moment operator)	Symmetry species of: Electronic states	Vibrational states: Wave no., cm^{-1}	Mode no.[c]
A_1	+1	+1	+1	+1	z		Ground state	930	1
								750	12
A_2	+1	+1	−1	−1		R_z		~200 (?)	16a
B_1	+1	−1	+1	−1	x	R_y		~200 (?)	11
B_2	+1	−1	−1	+1	y	R_x	Near-uv excited state	520	6b

[a] Reference 13. [b] I, identity operation; $C_2(z)$, 180° rotation about z axis; $\sigma_v(xz)$, reflection in xz plane (see Figure 6 for orientation of coordinate system); $\sigma_v(yz)$, reflection in yz plane. +1 indicates that a symmetry operation leaves the wave functions or vibrations unchanged; −1 indicates that a symmetry operation changes the sign of the wave function or reverses the direction of a vibration. [c] Reference 10.

values of B_2 for each symmetry operation times the values of A_1 for the corresponding symmetry operation. The values of the symmetry species $B_2 \times A_1$ are: for operation I, $(+1)(+1) = +1$; for $C_2(z)$, $(-1)(+1) = -1$; for $\sigma_v(xz)$, $(-1)(+1) = -1$; for $\sigma_v(yz)$, $(+1)(+1) = +1$. The result is that $B_2 \times A_1$ has the same properties as B_2, *i.e.*, $B_2 \times A_1 = B_2$. Thus a totally symmetrical vibration (A_1) does not affect the symmetry of the excited state. Indeed, any number of A_1 vibrations would not change the symmetry species. Therefore, a progression of absorption bands is possible, because transitions may occur to a number of excited states which differ only in the quantum number for the symmetrical vibrations.[14]

In addition to the transitions which are polarized in the y direction, x or z polarizations are possible if the excited state includes a nontotally symmetrical vibration. For an A_2 vibration, the symmetry species of the excited state is $B_2 \times A_2$, which equals B_1. This transition would be polarized in the x direction. For a B_1 vibration, no electric dipole transition is possible since $B_2 \times B_1 = A_2$; and there is no component of the electric dipole operator belonging to symmetry species A_2. For a B_2 vibration, an electric dipole transition would be polarized along the z axis ($B_2 \times B_2 = A_1$). These vibronic states may be combined with any number of symmetrical vibrations (A_1) without changing the symmetry properties of the excited state. Thus progressions of absorption bands may also be built up from transitions to excited states having either one A_2 or one B_2 vibration plus a variable number of A_1 vibrations.[11]

Symmetry properties can also be used to examine the origins of circular dichroism. In chromophores which contain a plane of symmetry, *e.g.*, the benzyl moiety (C_{2v}), it is well known that circular dichroism does not occur because the vector dot product of the electric dipole moment and the magnetic dipole moment is zero.[18] The benzyl chromophore in phenylalanine exhibits circular dichroism due to the perturbation from the amino acid moiety. In general, such circular dichroism may arise either from an electric dipole transition (zero-order electric dipole moment times first-order magnetic dipole moment) or from magnetic dipole transition (zero-order magnetic dipole moment times first-order electric dipole moment).[18] Symmetry considerations indicate that the progression of circular dichroism bands containing the $0 + 520\ cm^{-1}$ transition must be of the electric dipole type. The excited state of the $0 + 520\ cm^{-1}$ transition belongs to the A_1 symmetry species, because both the electronic state and the vibrational state are B_2 ($B_2 \times B_2 = A_1$). But since no component of the magnetic dipole moment operator belongs to symmetry species A_1, the zero-order magnetic dipole moment must be zero. In contrast, the zero-order electric dipole moment is allowed in the z direction (see above). Thus the circular dichroism bands of the $0 + 520\ cm^{-1}$ progression must result from the zero-order electric dipole moment term.

For the 0–0 progression of CD bands, symmetry properties do not permit distinguishing between electric and magnetic dipole types, because both the zero-order electric and zero-order magnetic dipole moments may be nonzero. Both the y component of the electric dipole moment operator and the x component of the magnetic dipole moment operator belong to the same symmetry species (B_2) as the excited state of the 0–0 progression (Table V).

A progression of CD bands based upon transitions to an A_2 excited state must be of the magnetic dipole type. As described above, the zero-order electric dipole moment is forbidden. However, the zero-order magnetic dipole moment may occur, since R_z belongs to A_2. This state can be obtained by including a single B_1 vibration in the excited state of phenylalanine ($B_2 \times B_1 = A_2$).